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23-26 NOVEMBER 2023

ANTALYA, TURKEY

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İlgili makama;

5th International Conference on Food, Agriculture and Animal Sciences (ICOFAAS 2023) kongresi 23-26 Kasım 2023 tarihleri arasında Porto Bello Hotel Resort & Spa Antalya'da 18 farklı ülkenin (Türkiye:44, Diğer Ülkelerden:94) akademisyen/araştırmacılarının katılımıyla gerçekleştirilmiştir. Kongre 16 Ocak 2020 Akademik Teşvik Ödeneği Yönetmeliğine getirilen "Tebliğlerin sunulduğu yurt içinde veya yurt dışındaki etkinliğin uluslararası olarak nitelendirilmesi için Türkiye dışından en az beş farklı ülkeden sözlü tebliğ sunan konuşmacının katılım sağlaması ve tebliğlerin yarıdan fazlasının Türkiye dışından katılımcılar tarafından sunulması esastır." değişikliğine uygun düzenlenmiştir.

Bilgilerinize arz edilir,

Saygılarımla

Prof. Dr. Fatih DADAŞOĞLU
Chair of ICOFAAS 2023

Dear Conference Participants,
Welcome to 5th International Conference on Food, Agriculture and Animal Sciences.
On behalf of the Organizing Committee, I am very happy to open 5th International Conference on Food, Agriculture and Animal Sciences. I believe that this event, which is the fruit of an intensive and devoted teamwork, will have an invaluable contribution to the scientific world. At the end of busy schedule of nearly one year, we have now achieved to organize this conference.
However, due to pandemics like COVID-19 affecting our daily lives, we wanted to add our conference to the online meeting, as we do with many events. This is because of the fact that, we, the organizers, are concerned about your health and we want to do our part to keep everyone safe. Presentations will be online or face to face. The conference will be organized with the bigbluebutton platform for online participants. We, organizing committee members, wish you all good health and high spirits.
The aim of the International Conference on Food, Agriculture and Animal Sciences is to bring together experts and young researchers from all over the world working in Food, Agriculture and Animal Sciences to present their researches, exchange new ideas, discuss challenging issues, foster future collaborations and interact with each other. In this sense, we are happy to bring together world mathematicians and exchange information with them.
The main objective of our conference is to discuss recent results in Food, Agriculture and Animal Sciences and applications and their relationship with other disciplines. We expect the participation of many prominent researchers from different countries who will present high quality papers. The conference brings together about over 281 participants from 18 countries (Algeria, Jordan, France, India, Iran, United Arab Emirates, Pakistan, Azerbaijan, Spain, Malaysia, Morocco, Moldova, Tunisia, Russia, Ukraine, Egypt, Germany, Turkey), out of which 99 are contributing to the meeting with oral presentations and with 78 poster presentations, including eight keynote talks.
It is also a purpose of the conference to promote collaborative and networking opportunities among senior scholars and graduate students in order to advance new perspectives. The papers presented in this conference will be considered in the journals listed on the conference websites.
I'd like to express my gratitude to all our authors, members of scientific committee, keynote speakers and contributing reviewers. I believe we will see the best papers of scholars in this event. My sincere thanks go to Prof. Dr. Mehmet KUL, the rector of Sivas University of Science and Technology, and Prof. Dr. Ömer ÇOMAKLI, the rector of Atatürk University, sets the goal of being also a top-ranking university in scientific sense, for supporting and motivating us in every respect. Special thanks are also due to the organizing committee members, for completing all preparations that are necessary to organize this conference. I express my gratitude to the members of technical committee of the conference for the design and proofreading of the articles.
We wish everyone a fruitful conference and pleasant memories in our online event.
Thank you.

Prof. Dr. Fatih DADAŞOĞLU
Chair of ICAFOAS 2023

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Regenerative Effects of Boron-Based Hydrogel Formulations Used in Human and Animal Health on Radiation-Induced Dermatitis and Wound Healing

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ABSTRACT: The destruction of skin integrity or tissue by biological, physical or chemical causes is the most common and destructive forms of wounds. Acute wounds usually heal within 3-6 weeks without the need for professional treatment modalities. However, chronic wounds are mainly associated with infection and prolonged inflammation, healing impairment and continuous tissue degradation. Radiation dermatitis is a common side effect of radiotherapy, which is one of the most frequently used treatments for cancer. Some people will experience mild redness and itchiness, while others may suffer painful, broken skin that is prone to infection. Any deformation that can occur in skin integrity can leave the human body vulnerable to many pathological conditions such as infection, excessive fluid loss and electrolyte imbalance. Although a vast number of products have been introduced in the market, claiming to provide a better optimization of local and systemic conditions of patients, they do not meet the expectations of clinicians and patients. Therefore, developing new, safe, self-applicable, effective, and cheap wound care products with broad-range antimicrobial activity has always been an attractive area for scientists. In the present study, effects of boron compounds [boric acid and sodium pentaborate pentahydrate (NaB)] pluronics (Plu; F68 and F127), and their combinations on the differentiation of mesenchymal stem cells (MSCs) isolated from different sources, and their antimicrobial activities, proliferation, migratory, angiogenesis, gene, and growth factor expression promoting effects on dermal cells *in vitro* were investigated. In addition, the preclinical and clinical studies were conducted to determine the effect of a novel hydrogel formulation containing NaB on the radiation-induced dermatitis in breast cancer and chronic wounds healing. The results revealed that while boron compounds significantly increased MSCs differentiation, and proliferation, migration, vital growth factor, and gene expression levels of dermal cells along with displaying remarkable antimicrobial effects against bacteria, yeast, and fungi, NaB displayed greater antimicrobial properties as well as gene and growth factor expression inductive effects. Preclinical and clinical studies proved that NaB-containing gel formulation enhanced healing rate of chronic wounds, and completely prevent the radiation-induced dermatitis in breast cancer tested. Therefore, our results suggested that NaB, and its pluronics combination, could be used in dermatological clinics and be a future solution for chronic wounds and dermatitis.

Keywords: Boron, Chronic wounds, Cancer treatment, Dermatitis, Radiation, Hydrogel

Growth Parameters of Clone R5 Cabernet Sauvignon in the Southern Region of the Republic of Moldova

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ABSTRACT: At present, in the Republic of Moldova, clones of classic European grape varieties are actively being introduced. This process is driven by the need to adapt the clones to the local growing conditions. It has been identified that the climate in the southern part of the Republic of Moldova is warm, with temperatures reaching 10°C and above for 179-187 days. The sum of active temperatures is 3300°C, and the annual amount of atmospheric precipitation ranges from 295.3 mm to 569.3 mm. The soil cover consists mainly of carbonated chernozem and southern soils. During the conducted research, key aspects of the growth and development of Clone R5 of the Cabernet Sauvignon Italian selection were identified in the agroecological conditions of the Southern region of the Republic of Moldova. We found that in the conditions of the Southern region of the Republic of Moldova, the Clone R5 Cabernet Sauvignon, when grown onto the rootstock BxR Kober 5BB, develops 40.1 shoots per vine; onto the rootstock RxR 101-14, it is 39.2 shoots per vine. When grafted onto BxR Kober 5BB, the length of One-year Growth is 125.5 cm, and the Growth Volume is 2.73 dm^3/vine; when grafted onto RxR 101-14, the length Growth is 117.1 cm, and the Growth Volume is 2.41 dm^3/vine. Analyzing these characteristics, we identified the individual features of the Clone R5 Cabernet Sauvignon and assessed their impact on the overall efficiency of grape production in this region. The obtained data provide valuable information that can serve as a basis for further improvements in the practices of cultivating introduced clones, aimed at optimizing the processes of viticulture in the Southern region of Moldova.

Keywords: Clone, R5, Republic of Moldova, One-year Growth, Growth Volume, Parameters

Implementation of an Agrisharing GIS Platform on The Basis of Bioeconomic Approach to Overcome Global Food Problems Due to The War in Ukraine

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ABSTRACT: The study emphasizes Ukraine's role in the world food system and the need to implement urgent measures to improve the state of its agro-industrial complex to meet the world's food needs. The key factor in restoring Ukraine's position in the global food system is how quickly and extensively Ukraine will be able to restore its production and logistics infrastructure. A quick solution to the above-mentioned problems after the end of the war in Ukraine can become a reality if the agrisharing GIS platform will be implemented on the basis of the bio-economic approach. The purpose of the study was to determine the place of the bioeconomic approach and GIS-platform in the agrisharing model. The global challenges, global opportunities and goals of the implementation of the above model have been identified and the closed cycle of the agrisharing platform based on the bio-economic approach has been determined. The indisputable argument that makes GIS the only possible platform for the development of agrisharing is that almost all data have a spatial (geographic) reference and certain information about the corresponding object. The study presents the functions and tasks of the agrisharing GIS platform and defines the advantages of the corresponding system for each sphere of cooperation.

Keywords: Agrisharing, GIS-platform, Bioeconomy, Global food problem

Improving Seed Germination and Seedling Growth of Wheat and Flax Seeds Using Seven Biogenic Metal-Based Nanoparticles

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ABSTRACT: Recently, large-scale agriculture has led to increasing crop production. To increase crop productivity in large-scale cropping systems, attempts have been made to make nano-fertilizers and deliver them to the crops by extension of nanotechnology. Hence, nano-fertilizers might be defined as nanoparticles that may directly assist in supplying essential nutrients for crop productivity. Seed germination is the first and the most susceptible stage in the plant's growing phases, so could be considered as an index to evaluate the effect of newly developed materials such as nanoparticles (NPs), providing useful information for researchers. In our experiments, germination tests have been carried out in Petri dishes containing wet filter paper and nano-primed seeds. We had biosynthesized seven nanoparticles in our previous studies including calcinated and non-calcinated zinc oxide, zinc, magnesium oxide, silver, copper, and iron nanoparticles. The effect of these biogenic nanoparticles and their counterpart metallic salts including zinc acetate, magnesium sulfate, silver nitrate, copper sulfate, and iron (III) chloride was studied on two popularly grown plants, wheat and flax, in laboratory conditions to obtain preliminary information for future field experiments. Germination percentage, shoot length, root length, seedlings length, root–shoot ratio, seedling vigor index (SVI), shoot length stress tolerance index (SLSI), and root length stress tolerance index (RLSI) were calculated on the second and seventh days of the experiment. According to the results, the response of the plants to metal containing nanoparticles and metal salts mainly depend on the type of the metal, plant species, concentration of the NP suspension or salt solution, condition of the exposure, and the stage of growth.

Keywords: Metal-based nanoparticles, Biosynthesis, Wheat, Flax, Seed germination, Seedling growth, Phytotoxicity

ACE and Covid-19: The Key and the Lock Blocking the ACE2 Active Site will Prevent the Virus Entering to the Human Cells

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ABSTRACT: Angiotensin-Converting Enzyme 2 ACE-2 is a receptor that has been confirmed as the entry point for the SARS-CoV-2 virus to infect human cells. It suggests that a strong interaction exists between human ACE-2 molecules and SARS-CoV-2. Therefore, the SARS-CoV-2 receptor-binding domain (RBD) successfully interacts human ACE2-expressing cells but not with any other receptors, ensuring that this interaction allows the virus to penetrate cells inside the epithelial oropharyngeal. Infection normally begins with respiratory mucosal cells and then spreads to alveolar epithelial cells in the lungs, leading to acute respiratory distress syndrome (ARDS) in patients. There are currently no successful therapies that target SARS-CoV-2 and it takes months and years for these drugs to develop. Researchers are working now *in silico* to identify promising treatments to save lives and develop potential preventive vaccines. Targeting virus entry by inhibiting ACE2 with suitable ligand has a greater advantage than inhibiting subsequent steps of the viral life cycle. In this study, we propose that a plant compounds targeting ACE2 and spike protein of SARS-CoV-2 *in silico* will be the most effective strategy of inhibiting SARS-CoV-2 entry and limiting its spread in the general population.

Keywords: ACE2, Inhibition, Plants, SARS-CoV-2, Spike protein

Plant Endophyte Interaction Under Extreme Environment: Benefits for Sustainable Agriculture

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ABSTRACT: Plant growth and nutrition are adversely affected by various factors such as water stress, high temperature, and plant pathogens. Soil and rhizospheres are complex environments with high carbon concentrations, oxygen, nutrients, and microorganisms. Rhizosphere-inhabiting microbes such as beneficial bacteria and pathogenic fungi compete for nutrients and niches Plant-associated microbes play a vital role in the growth and development of their hosts under biotic and abiotic stresses. Plant-beneficial microorganisms belong to several genera and are able to modulate plant physiological process, helping them to survive in their environment. The use of a rhizosphere microbiome for plant growth stimulation and the biological control of fungal disease can lead to improved crop productivity. Mechanisms used by plant-growth-promoting rhizobacteria (PGPR) to protect plants from soilborne pathogens include antibiosis, the production of lytic enzymes, indole-3 acetic acid production, decreasing ethylene levels by secreting 1-aminocyclopropane-1-carboxylate deaminase, competition for nutrients and niches, parasitism and induced systemic resistance. The use of secondary metabolites produced by endophytic microorganisms for biological control and induced resistance to plant pathogens shows great promise for sustainable agriculture, as it offers an environmentally friendly alternative to synthetic fungicides. However, the performance of biocontrol microbes depends on their environment and interactions among plants and pathogens as well. Thus, the physiological properties of biological control microbes, their interactions with other microorganisms, including pathogens, and the mechanisms involved in the plant-beneficial effect under hostile climatic conditions still need to be researched. The future prospects of the biological control of plant disease and plant stress tolerance by microbiome are promising.

Keywords: Plant microbiome, Abiotic, Biotic, Stresses, Plant benefits, Biocontrol

Wild Edible Fruit Diversity in the World: Opportunities and Challenges

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ABSTRACT: Wild edible fruit species are found throughout the world, especially in rural, mountainous and forestry areas, either as populations or as single trees/shrubs, and constitute an indispensable part of rural life. They are plants that have morphologically and physiologically very different characteristics compared to cultivated fruit species and constitute the most important source of food, medicine and livelihood, especially among local tribes where agriculture is not developed. In recent years, there has been an increased interest in these plants, which are an important source of nutrition and shelter for wildlife. One of the most important reasons for this interest is the extremely important biologically active substances available on these fruits. Another factor that makes these fruits very valuable is that they are more resistant than cultivated plants to climate change, which has become increasingly important in the world in recent years. For this reason, they are also seen as the food insurance of the future for humans and wildlife. These nutrient-rich species can enhance food security and alleviate poverty in particular rural areas. However, recent developments like the introduction of improved fruit varieties, changes in dietary choices, and infrastructure development are expected to influence indigenous knowledge and consumption of wild edible fruits (WEFs).

Keywords: Wild edible fruits, Nutrition, Diversity, Climate change

INTRODUCTION

Fruits are important in human health and nutrition, especially as they are rich in vitamins and minerals. The importance of fruits on human nutrition and health are;

1. They provide high levels of human health promoting substances
2. They contain minerals
3. They are rich in various vitamins
4. The have attractive appearance and appetite
5. They are rich for fibers

They can prevent human health against microorganisms as well (Figure 1). Fruits can be divided in general 2 groups including cultivated (cultivars) fruits and their wild relatives.

Wild edible fruits (WEFs)

Wild edible fruits (WEFs) refer to edible fruit species which are not cultivated but are collected from their natural habitats including rural mountains, forests, field edges etc. (Deshmukh and Waghmode, 2011). They are widely found areas where tribes or rural peoples are living and are mainly consumed during off-season periods of cultivated fruits (Rymbai et al., 2023). Currently most of the parts of the world agricultural communities rely mostly on improved cultivated fruit cultivars due to their higher productivity but the habit of consuming wild fruitshas not been entirely abandoned (Shan et al., 2019).

Opportunities

It is estimated that the world population will be increased to 9 billion by 2050, boosting global food demand by 50% compared to 2013. Thus, to meet the global food demand, the domestication of other food-producing species and intensifying the use of underutilized and neglected species, including wild food resources, may become more important. Wild food resources comprise a variety of edibles, including WEF, vegetables, mushrooms, orchids, canes, and herbal plants; and WEFs contribute the most to the total number of wild edible resources. These nutrient-dense fruits have been discovered to be good sources of vitamins, minerals, bioactive substances including phenolic acids, anthocyanins, carotenoids etc. and also serve as antioxidants. As a result, in most of the under developed countries, WEFs constitute a vital source of food, healthcare, and material subsistence and are linked to human survival (Chalise et al., 2010; Li et al., 2016; Chauhan et al., 2018).

Wild fruits are exotic or underutilized and shows great biodiversity. Majority of them contain many bioactive compounds, such as anthocyanins and flavonoids. Many studies have shown that wild edible fruits possess various bioactivities and health benefits, such as free radical scavenging, antioxidant, anti-inflammatory, antimicrobial, and anticancer activity. Therefore, wild edible fruits have the potential to be developed into functional foods or pharmaceuticals to prevent and treat several chronic diseases (Rawat et al., 2011; Yildiz et al., 2014; Ramos et al., 2015; Blando et al., 2016).

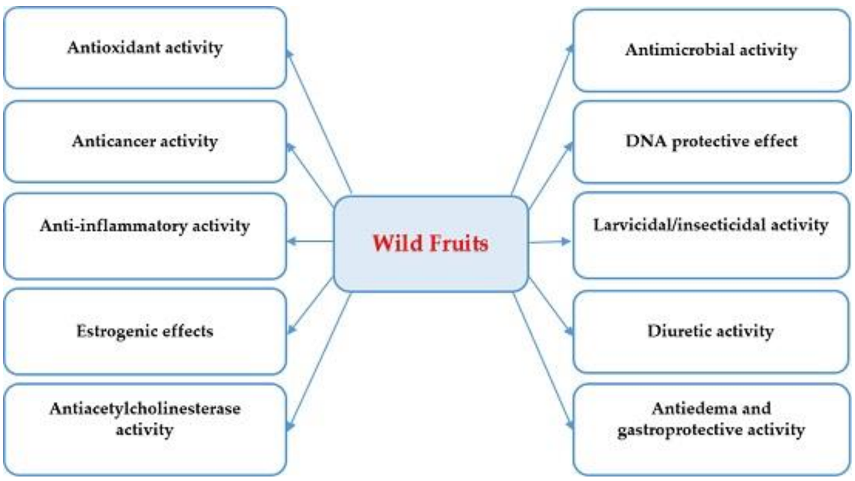

Figure 1. Wild edible fruits and their importance for human health

The most of the rural areas where found in mainly Asia, Africa, Pacific and South and Middle America has mountain areas with various altitudes, dense forests and different agro-ecological zones favor the growth of a wide range of wild edible fruits. Those continents also one of the world's biodiversity hotspots, housing numerous plant species.

It estimated that these continents have over 5000 wild edible fruit species and these species are excellent sources of food, medicine, fuel, wildlife feed, and timber and have various household and ritual applications. Similarly, numerous WEFs are employed in oil extraction, dyeing, and traditional medicine in these countries. As a result, it has significantly contributed to the food and nutritional well-being of rural areas of these continents (Rymbai et al., 2023).

In addition, the countries found in these continents and in general rich for wild edible fruits suffered from food insecurity in their rural areas, with nearly 30% of the population facing malnourishment and related health issues such as stunting. Hence, with its high nutrient content and potential for income generation through value addition, WEFs species can considerably contribute to food security and poverty alleviation in remote areas of these continents.

A good example of Amazon forest

Amazon forests located in South America is very rich for wild edible fruits (Figure 2). Camu camu, also known as rumberry, grows in the Peruvian and Brazilian Amazon on a bushy riverside tree. While the fruit itself is rather sour, it is used in many ways and served often as ice cream and in juice form. The fruit harvests during the rainy season so are collected by canoe. It has a very high level of vitamin C, between 2-3% of its fresh weight, and is extremely popular throughout Iquitos and the Amazonas. This fruit is becoming increasingly popular as a health supplement for its high antioxidant and anti-viral properties. Camu camu is used for medicinal purposes to fight off cold sores, herpes, and your common cold. This superfruit has been used to fight inflammation and because it contains valine (an amino acid), Camu camu prevents muscle breakdown and is great for nervous system’s cognitive function (Akter et al., 2011; Bataglion et al., 2015; Neri-Numa et al., 2018)

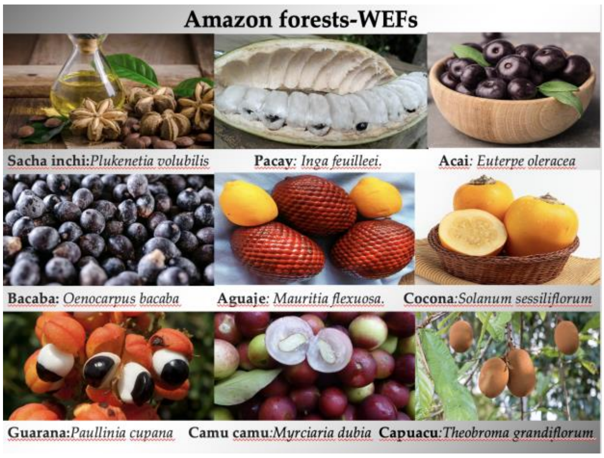

Figure 2. Amazon forests wild edible fruits

Native to Central and South America, tiny purple berries of Acai (similar in appearance to grapes) are found on palms that grow as tall as 25 meters. Acai has recently started to become extremely popular internationally owing to its antioxidant, high fiber, and healthy fat properties (omega fatty acids). The berries help support healthy hair, skin, and nails and are also known to aid with digestive problems by keeping digestive system in optimal function. Their high vitamin C content protects human from cardiovascular diseases and increases overall energy (Bataglion et al., 2015; Barbosa et al., 2016; Neri-Numa et al., 2018).

The bacaba palm, also known as kumbu, is another native to the Amazon. It actually produces more fruits than any other palm in South America. They have a dark red to purple colored shell and have a high nutritional value thanks to their rich fiber content (Bataglion et al., 2015; Neri-Numa et al., 2018).

Cupuacu is related to cacao, and has been cultivated by indigenous peoples in Colombia, Peru, Brazil, and Bolivia for centuries, but only now it is in the North American spotlight because of its extremely potent vitamins, antioxidants, and essential nutrients. The primary health benefit of this amazon superfood is heightening the body's immune system and lowering blood pressure. It has a caffeine-like effect but does not actually contain any caffeine. It is known for lowering the body's overall cholesterol level through lipid inhibition. Amazon tribes use Cupuacu for pain relief, especially during child birth (Bataglion et al., 2015; Neri-Numa et al., 2018).

Aguaje, or the moriche palm fruit, has three times more vitamin A than a carrot and is packed with protein, vitamins, and oils. It's also very high in vitamin C content and often used to make jam, juice, and ice cream, and is even fermented into fruit wine. It is found in tropical wet areas of South America. Its oil contains high concentrations oleic acid, tocopherols, carotenoids, and vitamin A, and is known as a miracle fruit because it's used to treat burns and various skin conditions like psoriasis and eczema. When applied directly to a burn, the high vitamin A, vitamin E and oil content soothe the wound, its natural anti-inflammatory ingredients taming redness and calming the skin. Its rich beta-carotene component is also known to protect from sun damage and makes for a great natural sunblock. Despite its miracle healing properties, the Aguaje fruit is becoming more famous globally for its phytoestrogens (plant-based estrogens) (Bataglion et al., 2015; Neri-Numa et al., 2018).

Cocona is a red, yellow, or orange edible berry from the cocona plant, a small shrub with sturdy branches and huge, hairy leaves. Coconas have a similar appearance to tomatoes and taste somewhere in between a tomato and a lemon. They are native to the Andean regions of South America and are known to be very rich in iron and vitamin B5. Although they can be eaten raw, they can be quite bitter, so are more commonly made into a sweetened juice or salsa served in Amazonian restaurants as a popular accompaniment to banana plantain chips (Bataglion et al., 2015; Neri-Numa et al., 2018).

Famed for their seeds which are commonly referred to as the jungle or Inca peanut, Sacha Inchi are in fact star-shaped fruits that grow on the *Plukenetia volubilis* plant native to much of tropical South America. The seeds packing a whopping 75% protein and 25% Omega-3's by size, and contain all of the essential amino acids. Sacha Inchi has been cultivated in the Peruvian Amazon Rainforest for centuries and is now becoming widely used to promote weight loss. Seeds help burn excess abdominal fat. Sacha Inchi seeds are also used to fight off depression, high cholesterol and prevent heart disease. It is widely consumed as a snack, but can also be bought in tablet form or as an oil (Bataglion et al., 2015; Neri-Numa et al., 2018).

This unusual fruit resembles a humongous green bean, stuffed with cotton candy. Pacay is more commonly known to travelers as the ice-cream bean thanks to the smooth texture of its white flesh that has a flavor not too dissimilar to that of vanilla ice cream (and indeed it is often used to make ice cream and other desserts). This fruit is full of dietary fiber which supports the body's removal of cholesterol and fat, its dried stems are used to control digestion and alleviate stomach pains, and the seeds (which need to be cooked to remove their toxic compounds) are used to fight diarrhea and rheumatism. In the Colombian Amazon, the pulp is used to prepare an alcoholic beverage called *cachiri* during a festival of the same name (Bataglion et al., 2015; Neri-Numa et al., 2018).

Challenges

However, the government's push for commercialization and the promotion of high-yielding cultivars in recent decades threatens to erode traditional WEFs use in most of the countries in those continents. Moreover, the reliance on wild edibles is likely to diminish over time because of the easy accessibility of improved varieties, the decline in species diversity owing to habitat destruction through deforestation and infrastructure development. As a result, indigenous knowledge and the consumption of WEFs are rapidly declining among the younger generations. The extinction of indigenous knowledge is also found to be linked to the reduction of plant diversity. With the increasing erosion of indigenous knowledge on WEFs and increasing reliance on improved fruit varieties, there is a risk of complete substitution of wild fruits with imported fruit types, resulting in the disruption of the coexistence of people and forest, and loss of traditional knowledge sooner (Uprety et al., 2012; Bhatia et al., 2018; Chauhan et al., 2018).

Thus, it is crucial to document the diversity of wild species and their indigenous potential for sustainable management of wild resources before the extinction of indigenous species and their traditional knowledge.

REFERENCES

Akter, S., Oh, S., Eun, J.B., Ahmed, M. (2011). Nutritional compositions and health promoting phytochemicals of camu-camu (*Myrciaria dubia*) fruit: A review. Food Res. Int. 44:1728-1732.

Barbosa, P.O., Pala, D., Silva, C.T., Souza, M.O. (2016). Açaí (*Euterpe oleracea* Mart.) pulp dietary intake improves cellular antioxidant enzymes and biomarkers of serum in healthy women. Nutrition, 32:674-680.

Blando, F., Albano, C., Liu, Y.Z., Nicoletti, I., Corradini, D., Tommasi, N., Gerardi, C., Mita, G., Kitts, D.D. (2016). Polyphenolic composition and antioxidant activity of the under-utilised *Prunus mahaleb* L. fruit. J. Sci. Food Agric. 96:2641-2649.

Bhatia, H., Sharma, Y.P., Manhas, R.K., Kumar, K. (2018). Traditionally used wild edible plants of district Udhampur, J&K, India. J. Ethnobiol. Ethnomed. 14:73.

Chalise, J.P., Acharya, K., Gurung, N., Bhusal, R.P., Gurung, R., Skalko-Basnet, N., Basnet, P. (2010). Antioxidant activity and polyphenol content in edible wild fruits from Nepal. Int. J. Food Sci. Nutr. 61(4):425-432.

Chauhan, S.H., Yadav, S., Takahashi, T., Łuczaj, Ł., D’Cruz, L., Okada, K. (2018). Consumption patterns of wild edibles by the Vasavas: a case study from Gujarat, India. J. Ethnobiol. Ethnomed. 14:57.

Deshmukh, B.S., Waghmode, A. (2011). Role of wild edible fruits as a food resource: Traditional knowledge. Int. J. Pharm. Life Sci., 2 (7):919-924.

Li Y, Zhang J-J, Xu D-P, Zhou T, Zhou Y, Li S, Li H-B. Bioactivities and Health Benefits of Wild Fruits. *International Journal of Molecular Sciences*. 2016; 17(8):1258.

Neri-Numa, I.A., Soriano Sancho, R.A., Pereira, A.P.A., Pastore, G.M. (2018). Small Brazilian wild fruits: Nutrients, bioactive compounds, health-promotion properties and commercial interest. Food Res. Int. 103:345-360.

Ramos, A.S., Souza, R.O.S., Boleti, A.P.D.A., Bruginski, E.R.D., Lima, E.S., Campos, F.R., Machado, M.B. (2015). Chemical characterization and antioxidant capacity of the araca-pera (*Psidium acutangulum*): An exotic Amazon fruit. Food Res. Int. 75:315-327.

Rawat, S., Jugran, A., Giri, L., Bhatt, I.D., Rawal, R.S. (2011). Assessment of antioxidant properties in fruits of *Myrica esculenta*: A popular wild edible species in Indian Himalayan Region. Evid. Based Complement. Altern. 1-8.

Rymbai, H., Verma, V.K., Talang, H., Assumi, S.R., Devi, M.B., Vanlalruati, Sangma R.H.C.H., Biam, K.P., Chanu, L.J., Makdoh, B., Singh, A.R., Mawleiñ, J., Hazarika, S., Mishra, V.K. (2023) Biochemical and antioxidant activity of wild edible fruits of the eastern Himalaya, India. Front. Nutr. 10:1039965

Shan, S., Huang, X., Shah, M.H., Abbasi, A.M. (2019). Evaluation of polyphenolics content and antioxidant activity in edible wild fruits. Biomed Res Int. 11:1381989.

Yildiz, H., Ercisli, S., Hegedus, A., Akbulut, M., Topdas, E.F., Aliman, J. (2014). Bioactive content and antioxidant characteristics of wild (*Fragaria vesca* L.) and cultivated strawberry (*Fragaria ananassa* Duch.) fruits from Turkey. J. Appl. Bot. Food. Qual. 87:274-278.

Uprety, Y., Poudel, R.C., Shrestha, K.K., Rajbhandary, S., Tiwari, N.N., Shrestha, U.B., Asselin, H. (2012). Diversity of use and local knowledge of wild edible plant resources in Nepal. J. Ethnobiol. Ethnomed. 8:16

FACE TO FACE

ABSTRACT

ORAL PRESENTATIONS

Determination of the Total Phenolic and Flavonoid Contents of *Alcea hohenackeri* Boiss. Flower in n-Hexane, Ethyl Acetate, Methanol and Water Extracts*

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ABSTRACT: Medicinal plants are rich sources for fenolics and flavonoids. The genus Alcea (Malvaceae) consists of around 70 species worldwide. The aim of this study was to determine the total phenolic and flavonoid contents of Alcea hohenackeri Boiss. Flower in different fractions ; n-hexane, ethyl acetate, methanol and water. For this purpose, dried flowers were cut into small pieces and fractions were extracted in different fractions. Fractions were evaporated and lyophilized extracts were obtained by a dry freezer. Total phenolic and flavonoid contents were quantified using an UV-Vis spectrophotometer. According to obtained results, the highest phenolic content was found in methanol extract (107.60±2.55). The total phenolic content in water, ethyl acetate and n-hexane was determined as 62.96±0.85, 32.87±1.40 and 17.88±0.42, respectively. The highest total flavonoid content was in the methanol extract (43.04±0.73). It was quantified in water extract as 16.09±0.20. On the other hand, total flavonoid content of n-hexane and ethyl acetate was not determined. In conclusion, the methanol extract of Alcea hohenackeri flower had the highest total phenolic and flavonoid contents. The investigation of the pharmaceutical effects of methanol extract in in vitro and in vivo models is being considered as further studies.

Keywords: *Alcea hohenackeri*, Medicinal plant, Phenolic content, Flavonoid content

Sustainable Management of Soil and Water Resources in Arid Regions by the Application of Date Palm Derived Biochar

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ABSTRACT: Arid and semi-arid regions are more vulnerable to the land degradation owing to water scarcity and lower soil organic carbon contents. Therefore, sustainable management of soil and water resources in such areas is critical in enhancing crop productivity and soil health. Biochar has recently came up as an ideal candidate to improve soil conditions and water conservation in arid and semi-arid areas due to large surface area, higher porosity, abundant functional groups, and high cation exchange capacity. Thus, the efficiency of date palm waste-derived biochar to improve soil hydro-physical properties and subsequent increase in tomato growth was studied under lab-scale and greenhouse trials. Biochar was produced by at 300°C, 500°C, and 700°C using date palm waste and separated into various size fractions (<0.5 mm, 0.5–1 mm, and 1–2 mm). Columns experiments were conducted by amending the calcareous sandy soils at 1%, 2.5%, and 5% of the produced biochar along with a control (without biochar). The results of column trials suggested that biochar applications resulted in decreased saturated hydraulic conductivity while increased cumulative evaporation. Lower pyrolysis temperature (300°C and 500°C) resulted in higher cumulative evaporation, while higher pyrolysis temperature (700°C) resulted in reduced cumulative evaporation. Likewise, smaller particle size resulted in higher cumulative evaporation. Greenhouse trials demonstrated that biochar application resulted in 5.48%–8.11% improvement in soil moisture content than control and substantially increased tomato growth. Thus, biochar application enhanced soil health and water conservation in sandy soil, consequently increasing tomato plant growth.

Keywords: Water conservation; Cumulative infiltration; Sustainable soil management

Treatment of Olive Mill Wastewater by Adsorption Using Natural Clay

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ABSTRACT: The Olive Oil Mill Wastewater (OMW) is one of the by-products of the olive oil industry. The discharge of these effluents without any prior treatment poses serious problems because they are harmful to the environment due to their richness of organic matter and phenolic compounds. Hence this work aims to treat the phenolic compounds of this OMW with the adsorption process and to seek out the best conditions to treat it using the clay as an adsorbent. The experiences show that the olive oil mill wastewaters are characterized by a high acidity (pH= 4.5), a very high organic load (COD= 93. 6 g of O_2/l), and they are rich in polyphenols (4.2 g/l). The treatment of these effluents by adsorption can eliminate 60% of polyphénole after 30 minutes using 5g of the clays. Moreover, the pseudo-second-order is the suitable model for the kinetics of the adsorption of phenolic compounds in olive oil mill wastewater.

Keywords: Olive Oil Mill Wastewater, Clay, Adsorption, Polyphenols, Treatment, Langmuir kinetics

Effect of Essential Oil of *Cinnamomum zeylanicum and Chamaemellum nobile* against *Chryseobacterium indologenes,* which Cause of Soft Rot on Some Plants

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ABSTRACT: In this study, it is aimmed to be determined the antimicrobial effects of the essential oil *in vitro* conditions, extracted from wild forms of plant which is known as *Cinnamomum zeylanicum* and *Chamaemellum nobile* around the world against *Chryseobacterium indologenes* isolates, which are the agent of Soft Rot for some fruits and vegetables. For this purpose, 7 strains of *C. indologenes* which have been determined as the agent of Soft Rot in previous studies performed in plants such as cucumber, pepper, tomato, and pumpkin. As the positive control, Streptomycin antibiotics sold as ready produce were used. According to the obtained results, the essential oil of *Cinnamomum zeylanicum* have the antibactericidal effect of 15-24 mm against 7 isolates of *C. indologenes.* the essential oil of *Chamaemellum nobile* have the antibactericidal effect of 0,6-15 mm against 7 isolates of *C. indologenes*. It has been observed that the antibiotics used as the positive control has the antibacterial effect of 0,9-11 mm. In conclusion, the essential oil has the lethal effect against 7 *C. indologenes* isolates which are agents of Soft Rot. It is assesed that this essential oil extracted from *Cinnamomum zeylanicum* and *Chamaemellum nobile* can be used against these Soft Rot pathogens.

Keywords: Antibacterial activity, *Cinnamomum zeylanicum* and *Chamaemellum nobile*, Soft rot

Investigation of the Efficacy of Bioagent Bacteria Against *Penicillium digitatum* (Pers.) in Lemon in *In-Vitro* Conditions*

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ABSTRACT: Citrus fruits are among the most widely produced fruit types globally. In Turkey, at least 25% of fresh fruits produced go to waste before reaching consumers. The primary reasons for this are inadequate storage facilities, poor determination of harvesting time, mishandling during harvesting, failure to provide proper storage conditions, and inappropriate packaging techniques. In addition to these factors, citrus fruits are susceptible to infection by fungal pathogens, which can cause significant losses during storage and transportation. worldwide through the application of synthetic fungicides such as imazalil, sodium ortho-phenylphenate, thiabendazole, pyrimethanil, azoxystrobin, and fludioxonil. However, growing concerns about the health risks and environmental pollution resulting from the use of chemicals have led to the need for the development of new strategies that can replace synthetic fungicides. In this context, this study aimed to investigate the effects of some bacterial biocontrol agents in the management of P. digitatum in lemons. To achieve this, the effectiveness of 29 bacterial biocontrol agents against P. digitatum was tested under in vitro conditions through dual culture trials. In dual culture tests, the inhibitory zones of bacterial biocontrol agents ranged from 3.00 to 23.67 mm, and inhibition rates varied between 18.99% and 79.75%. As a result, under in vitro conditions, the highest impact on the development of the green mold pathogen P. digitatum was observed in the TV-3D isolate (79.75%), followed by the ES-6 (76.37%) and TV-85D (75.95%) isolates.

Keywords: Biological control, *Penicillium digitatum*, Lemon

*This study is a part of the Deniz EKEN's master thesis.

Determination of Some Biological Properties of *Talaromyces Funiculosus* as a Basis for Mass Production*

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ABSTRACT: By 2050, the world population is expected to reach approximately 10 billion and the need for agricultural production is expected to increase by ~50%. In recent years, about 5.2 billion hectares of land where agricultural production activities can be carried out has been gradually decreasing day by day due to climate change-related drought, salinization, and conversion to tourism and residential areas. In agricultural production, excessive use of chemical fertilizers and pesticides are used with the principle of obtaining more productive and high-quality products per unit area. The current agricultural system is facing major challenges due to the increasing dependence on the intensive use of agrochemicals, global climate change, population growth and the increasing economic and environmental costs of non-renewable resources. For this reason, there has been a need to search for an effective alternative to chemical fertilizers in agricultural practices, including organic wastes and plant growth-promoting applications for purposes such as increasing productivity, improving the chemical and physical structure of soils, protecting human and environmental health, and supplementing the soil with ecological nutrients. As an alternative to chemical fertilizers and pesticides in agriculture, the use of beneficial microorganisms existing in the existing natural balance as microbial fertilizers and biopesticides in agriculture is of great importance. The main material of the study is *Talaromyces funiculosus* (ST976) isolate, which has the ability to dissolve phosphate bound in the soil and has the ability to stimulate plant growth in some plants, isolated from Şanlıurfa province agricultural areas and microscopically and molecularly identified. In this study, it was aimed to determine the maximum values of the development curves of *T. funiculosus* (ST-976) isolate as a microbial fertilizer by using the methodology determined by Pitt and Hocking (1977) and Gekas et al. (1998). *T. funiculosus* ST976 showed the highest spore production at 99.5% and 99% water activity values at 25 °C and statistically these two water activity values were in the same group (a) at both temperature values. At the remaining water activity values (98 and 96%), relatively less spore production was realized and these groups were statistically in the same group (b). At 98 and 96% water activity ratio, relatively little spore production was realized and spore production did not develop at low water activity ratio, 92 and 94. Again, in the study where the effects of temperature on spore production were tested, statistically 2 groups were formed (A and B), all growth values at 25 °C were in group A, while all growth values at 30 °C were in group B. As a result, ST976 showed the highest spore production at 99.5% and 99% water activity values at 25 °C, indicating that water activity and temperature are very important factors in spore formation of the fungus.

Keywords: Fungi, *Talaromyces funiculosus* (ST-976), Biological control, Biofertilizer

Autoecological Aspect and Monitoring of Rapeseed Cultivation in The Tlemcen Region (West Algeria)

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ABSTRACT: Rapeseed oil is an important natural source of unsaturated fatty acids, which makes it a very beneficial food for a balanced diet. It also plays a very important role in green chemistry and in the creation of renewable energy. It is an annual herbaceous plant of the eudicot class, resulting from a natural cross between cabbage. Its importance lies in the production of a large quantity of cereals whose yield reaches 34 quintals per hectare per year. She adapted to the state's environment and studied morphological changes based on previous cultures. This species belongs to the Brassicaceae family (formerly called cruciferous), such as mustard. A family name which can be explained by a simple reason: the corolla of its flowers is made up of four petals arranged in a cross. Establishment is a key stage of cultivation, particularly to combat pests (flea beetles, weevils). It also helps feed bees whose flowering period is longer than six weeks. It helps improve soil quality with a depth of 1.2 meters and also provides a fertilizer rich in olive oil which is mixed immediately after harvest. It can also be easily introduced into the agricultural cycle and replace wastelands. Rapeseed contains 40 percent oil and 60 percent bagasse, rich in protein, supporting the livestock sector. In this context, and the benefits of this plant, it was tested in several regions of the country, and during the year 2020-2021 this program was adopted in the Wilayat of Tlemcen within the following experimental farms: Hamdouche farm , commune of Shatwan, Crepe farm, commune of Sabra, and farm of Colonel Lotfi, commune of Sayed Abdali, and from there this study is based on the multipoint test e Iron diseases and pests related to the rapeseed plant, all the natural and unnatural factors that affect it, as well as ways to combat and prevent them.

Key words: Tlemcen, Rapeseed, Autoecology, Soils, İrrigation, Pests, Prevention

The Reasonable Use of Nitrogen in Cereal Cultivation in The Wilaya of Tlemcen (Case of Durum Wheat)

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ABSTRACT: Wheat, like all cereals, requires environmental conditions necessary for good development and therefore, obtaining the best yield is impossible to achieve. Just as the symptoms of nitrogen deficiency on the plant are serious damage, its increase also causes many problems. This can lead to loss of the harvest. Among the most important problems of increasing nitrogen fertilization are: undesirable increase in plant growth with plant drooping, double flowering, delay in maturity phase, deformation and cracking of plants. fruits reduce the storage capacity of fruits, leading to the problem of lodging. This work was carried out in the commune of Bensekrane, wilaya of Tlemcen. It aims to study the effective use of nitrogen in the cultivation of durum wheat, variety (Ouarsenis). Three fractionation modes were tested on this variety according to a block experimental model. The results obtained showed significant effects on all the parameters studied: plant height, grain yield, number of ears/m², number of grains per ear, thousand grain peas. This study confirms that a dose of 1.80 qx/ha of nitrogen (Urea 46%), applied in three fractions (12U at sowing, 36U at tillering and 36U during bolting) is more effective. We improved both the yield components compared to other treatments.

Keywords: Bensekrane, Durum wheat, Nitrogen, Fractionations, Components, Yield

Antiviral Effect of Essential Oils on Tobacco Mosaic Virus in Plant Tissue Culture Model

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ABSTRACT: Tobacco Mosaic Virus (TMV) can infect 150 different plant species, including tomato, pepper, eggplant, tobacco, spinach, petunia and marigold. t can cause serious damage in agriculture and negatively affect the food sector in the long term. The annually agricultural lost cost attributable to plant viruses are estimated more than $30 billion in worldwide. For this reason, there is a need to develop antiviral substances for plant viruses, which will protect plant viral contamination, reduce the virulence effect or inhibit the viral replication in infected plants. In order to develop such products, it is thought that it should be in controlled areas such as plant tissue culture in order to protect the health of other plants. Therefore, two types of plant tissue culture were developed, suspension plant culture and agar plant culture. AFter indefction period, TMV were detected by Transmission Electron Microscopy (TEM). In our ongoing project, the antiviral activity of lavender and tea tree essential oils will be tested against TMV in callus agar and/or suspension culture. Antiviral activity analysis was performed by RT-PCR method. According to our results it was shown that both lavender and tea tree essential oil applications were effectively inhibited TMV in both callus culture.

Keywords: TMV, Antiviral, Lavandula, Lavender oil, Tea tree oil, Callus culture, Plant viruses

The Biological Control of *Fusarium proliferatum*, the Causal Agent of Onion Basal Rot Disease*

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ABSTRACT: Onion is a significant export product with great importance for the country's economy. However, the production and yield of onions can be negatively affected by various biotic (living) and abiotic (non-living) factors both before and after harvest. Particularly in the post-harvest period, fungal and bacterial pathogens can cause serious product losses in onions. These losses are a significant problem for both producers and the country's economy. In areas where onion production is carried out in Turkey, the problem of bulb rot caused by Fusarium proliferatum is quite important. This disease causes serious damage in the root area of onions, leading to product losses. Various methods are recommended to combat onion bulb rot. These include the use of resistant onion varieties, crop rotation, solarization, sanitation, biological control, and fungicide applications. In addition, various fungicides such as benomyl, benomyl + mancozeb, carbendazim, carboxin, maneb, methoxyethyl mercury chloride, procymidone, penconazole, tebuconazole, thiram, and vinclozolin have been shown to reduce root and bulb rot damage in onions when applied to seeds and seedlings. However, the potential of these fungicides to harm human health and the environment has led to their ban worldwide and in our country. Moreover, other fungicides can also lead to residue problems in directly consumed products. Therefore, new strategies are needed to combat this pathogen. In this context, this study aimed to investigate the effects of some bacterial biocontrol agents in the management of onion bulb rot. To achieve this, the effectiveness of 24 bacterial biocontrol agents against F. proliferatum was tested under in vitro conditions through dual culture trials. In dual culture tests, the inhibitory zones of bacterial biocontrol agents ranged from 0.25 to 0.62 cm, and inhibition rates varied between 18.85% and 66.67%. As a result, under in vitro conditions, the highest impact on the development of bulb rot pathogen *F. proliferatum* was observed in the PM-18 isolate (66.67%), followed by the TV-17C (66.53%) and A-16 (65.26%) isolates.

Keywords: Biological control, *Fusarium proliferatum*, Onion basal rot

*This study is a part of the Fırat Gürkan AKSOY's master thesis.

Phenolic Content and Antioxidant Activity of the Aqueous Extract of *Syzygium aromaticum* Flower Buds

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ABSTRACT: *Syzygium aromaticum* is an aromatic plant, belongs to the *Myrtaceae* family. The aim of this study was to determine the phenolic content and to evaluate the antioxidant activity of the aqueous extract of cloves. The yield of the aqueous extract was 13,14%. The total polyphenol was estimated as 304,74±0,01 mg gallic acid equivalent/g of extract, while the content of flavonoids was 41,36±0,00 mg quercetin equivalent/g of extract. This extract also contains 392,71±0,002 mg tannic acid equivalent/g of extract. The antioxidant activity was evaluated by three tests: 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, chelation of ferrous ions and beta-carotene bleaching tests. The extract demonstrated a good DPPH radical scavenging activity with a value of IC_{50} of 0,01±0,00 mg/ml compared to the quercetin (IC_{50}=0,002±0,00 mg/ml). For the iron chelation test and in comparison, with the reference antioxidant, ethylene diamine tetra-acetic acid, the value of IC_{50} of the aqueous extract was higher (extract: IC_{50}=0,16±0,01 mg/ml, EDTA: IC_{50}=0,01±0,00 mg/ml). The beta-carotene bleaching test showed that BHT has a better profile than the extract and vitamin C with values of inhibition of 90,28±1,76%, 63,75±1,57% and 63,125±1,60% respectively. The antioxidant activity of the extract may be partially due to the presence of polyphenols like flavonoids and tannins.

Keywords: Aqueous extract, Antioxidant activity, DPPH, Polyphénols, Syzygium aromaticum

Phenolic Contents, DPPH Radical Scavenging and β-carotene Bleaching Activities of Aqueous Extract from *Magydaris tomentosa*

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ABSTRACT: *Magydaris tomentosa* is a vegetal species belonging to the Apiaceae family. It is widely used in traditional medicine. This study aims to estimate the content of phenolic compounds and to evaluate the antioxidant activity of the aqueous extract of *Magydaris tomentosa*. The extraction yield was estimated at 14,64%. The quantity of phenolic compounds was 386,86±0,001 mg gallic acid equivalent/g of extract. The content of flavonoids was 10,97±0,0002 mg quercetin equivalent/g of extract. The extract also contains 357,31±0,0005 mg tannic acid equivalent/g of extract. The antioxidant activity of the aqueous extract of *Magydaris tomentosa* was evaluated using the DPPH radical scavenging and the beta-carotene bleaching tests. The results revealed that the extract showed a weak activity against the DPPH radical, with an IC_{50} value of 0,45±0,026 mg/ml compared to the quercetin, with an IC_{50} value of 0,002±0.00 mg/ml. In the beta-carotene test, the 2 mg/ml extract demonstrated a very low inhibitory activity against lipid peroxidation, achieving an inhibition percentage of 33,57±6,16%. The reference antioxidants, butylhydroxytoluene (BHT) and vitamin C, proved to be more effective with inhibitions of 90,28±1,76% and 63,12±1,60%, respectively. The richness of the extract of *Magydaris tomentosa* in polyphenols may be partially responsible for its antioxidant activity.

Keywords: Antioxidant activity, Beta-carotene, DPPH, *Magydaris tomentosa*, Polyphenols

Prevalence and Risk Factors of *Eimeria spp.* and *Giardia spp.* in Rabbits of Local Algerian Population

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ABSTRACT: The objective of this study was to determine the prevalence and to identify the risk factors of *Eimeria spp.* and *Giardia spp.* infection in rabbits from the local population of four localities in northern Algeria. Dung samples were collected from 16 farms, totalling 111 rabbits, and were analysed by the flotation method. Additional, data regarding the farms and management practices were obtained by means of a questionnaire used in the surveys and interviews. The results revealed that the prevalence of *Eimerias pp.* contamination was 68.75% (11/16) for farms and 58.56% (65/111) for rabbits, respectively. The prevalence of *Giardia spp*. was respectively 56.25% (9/16) for farms and 11.7% (13/111) for rabbits. The analyses showed that the prevalence of *Eimeria spp*. was significantly higher in the farms that did not comply with hygiene and non-conventional feeding and watering. However, the prevalence of *Giardia spp*. was significant in rabbits kept in poor conditions of rearing. In conclusion, this study showed that the prevalence of these two parasites in rabbits from the local population is relevant and may have important implications for the rabbit industry and public health, especially in rural areas.

Keywords: Algeria, DDigestive parasites, Prevalence, Rabbits, Risk factors

Artificial Intelligent Optimize Agricultural Technology, Economy and Food Quality

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ABSTRACT: Knowledge Technology for progressive Quality in Agricultural economy recently belong to the most development issues in the agricultural economics also in food industry. Based on that it has been introduced numerous challenges for novel marketing strategies, efficient management in practical approaches for food consumption. There are integrated technologies addressing these multifaceted challenges and requires a heightened emphasis on improving the quality of various aspects within this dynamic process. AI progressive aspect such as machine learning, Decision making and Recommender systems navigating and collecting Data, information and Knowledge through a large among of production data, environmental insights, extensive existing knowledge, and accumulated experiential wisdom poses a significant challenge for human resources. We applied AI resources and them necessitates for effective strategies to optimize kind of drug, environment, water and air in agricultural science. Of course, cyber-technologies due to their ability can prove instrumental, utilize metadata for data fusion, and offer lot more possibilities to improve qualities in Food production. Artificial Intelligence (AI) algorithms has the abilities in tackling complex tasks, but their solutions often demand extended time or remain unattainable due to time-sensitive data availability. Developing AI algorithms becomes paramount in enhancing our ability to comprehend the intricacies inherent in the challenges we seek to address. This, in turn, disrupts the agricultural processes, creating a cyclical production issue. AI's core components encompass knowledge acquisition, meta-knowledge utilization, and metadata integration, forming the cornerstone of its technological capabilities. By leveraging these resources, we aim to uncover innovative solutions that transcend the limitations of traditional approaches.

Prevalence and Risk Factors of *Eimeria spp.* and *Giardia spp.* in Rabbits of Local Algerian Population

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ABSTRACT: The objective of this study was to determine the prevalence and to identify the risk factors of *Eimeria spp.* and *Giardia spp.* infection in rabbits from the local population of four localities in northern Algeria. Dung samples were collected from 16 farms, totalling 111 rabbits, and were analysed by the flotation method. Additional, data regarding the farms and management practices were obtained by means of a questionnaire used in the surveys and interviews. The results revealed that the prevalence of *Eimerias pp.* contamination was 68.75% (11/16) for farms and 58.56% (65/111) for rabbits, respectively. The prevalence of *Giardia spp*. was respectively 56.25% (9/16) for farms and 11.7% (13/111) for rabbits. The analyses showed that the prevalence of *Eimeria spp*. was significantly higher in the farms that did not comply with hygiene and non-conventional feeding and watering. However, the prevalence of *Giardia spp*. was significant in rabbits kept in poor conditions of rearing. In conclusion, this study showed that the prevalence of these two parasites in rabbits from the local population is relevant and may have important implications for the rabbit industry and public health, especially in rural areas.

Keywords: Algeria, Digestive parasites, Prevalence, Rabbits, Risk factors

Nano-Enhanced Bay Laurel Essential Oil: A Promising Solution for Tilapia Health Enhancement*

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ABSTRACT: Aquaculture is a key source of protein for populations in the face of a growing human population and the threat of ocean overfishing. However, it faces risks such as the spread of disease and environmental issues that need to be addressed to ensure sustainability and reliable protein production. Medicinal plants, especially their essential oils, play an important role in improving fish health, strengthening their immunity and resistance to disease, offering an alternative to chemicals and antibiotics in aquaculture. Essential oil nanoparticles demonstrate unique properties to improve fish health by effectively delivering active ingredients and enhancing immunity. In this context, Bay laurel (BL), a long-established medicinal plant, was used to investigate the effects of its nano-essential oil (NE) on antioxidant and immunological responses in Nile tilapia. Eight weeks of feeding fish with supplements at dosages of 0, 25, 50 and 100 mg/kg BL-NE resulted in improved immunological and antioxidant parameters of plasma compared to the control group. Based on these results, dietary supplementation of BL-NE at a dose of 50 mg/kg is recommended as a natural immunostimulant in Nile tilapia aquaculture. This study highlights the importance of using natural resources to improve the sustainability and quality of fish production.

Keywords: Medicinal plants, Aquaculture, Nanotechnology, Antioxidant, Immunity, Nutrition

Anti-Inflammatory Activity of Total Alkaloid Extract from *Fumaria capreolata* on Epithelial Cell Line

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ABSTRACT: Different species from genus *Fumaria* have been traditionally used against several disorders. *Fumaria capreolata* is used in traditional medicine in North Africa for its gastrointestinal and anti-inflammatory activities. The present study investigates the effects of total alkaloids extracted from the aerial parts of *Fumaria capreolata* (AFC) *in vitro*. Different doses of AFC (1, 10, and 100 μg/ml) were assayed on the mouse intestinal epithelial cell line CMT93 stimulated with LPS. AFC significantly reduced the inflammatory response inhibiting the production of nitric oxide (NO), TNF-α, and IL-6 in a dose-dependent manner, without affecting the viability of cells, and down-regulated mRNA expression of pro-inflammatory key players: IL-6, IL-1β, iNOS, TNF-α, and ICAM-1. The beneficial effect of AFC was associated with the normalization of the expression of MUC-2 and ZO-1 involved in the epithelial integrity. The present study suggests that total alkaloid fraction from *Fumaria capreolata* significantly exerted intestinal anti-inflammatory effects in experimental colitis model in mice, which AFC inhibits the expression and/or release of different anti-inflammatory mediators.

Keywords: Inflammation, Cytokine, Chemokines, Alkaloid, *Fumaria capreolata*

Varietal Behaviour of Some Chickpea Genotypes to Wilt Disease Induced by *Fusarium oxysporum* f. sp. *ciceris*

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ABSTRACT: The behavior study of forty-two varieties and genotypes of chickpeas regarding to root wilt disease induced by *Fusarium oxysporum* under the natural conditions of infection was conducted at the ITGC experimental station in Sétif. The infected plants of the different Chickpea genotypes have shown a multiple symptom in the field caused by the local strain of *Fusarium oxysporum f.sp. cecris* belonging to race II of the pathogen. These symptoms ranged from lateral or partial wilting of some ramifications to total desiccation of the plant; sometimes combined with very slow growth of symptomatic plants. The results of the search for sources of resistance to Fusarium wilt of chickpeas in the 42 genotypes tested revealed that in terms of infection rate, the presence of 7 groups and no genotype showed absolute resistance. While in terms of severity, the results revealed the presence of three homogeneous groups. The first group formed by the most resistant genotypes in this case Flip10-368C; Flip11-77C; Flip11-186C; Flip11-124C; Flip11-142C, Flip11-152C; Flip11-69C; Ghab 05; Flip11-159C; Flip11-90C; Flip10-357C and Flip11-37C while the second group is the FLIP genotype 10-382C which was found to be the most sensitive for the natural infection test. Thus, the genotypes of *Cicer arietinum* L. which have shown significant levels of resistance to Fusarium wilt can be integrated into breeding and improvement programs.

Keywords: Chickpea, *Fusarium oxysporum*, Genotype resistance

A Research on The Determination of Household Food Consumption Habits during the Covid-19 Pandemic in the Center of Van Province*

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ABSTRACT: The aim of this study is to examine the changes in the food consumption habits of consumers living in the central districts of Van (Edremit, İpekyolu, Tusba) during the Covid-19 pandemic. In addition, it is aimed to determine the food consumption habits of consumers during the Covid-19 pandemic according to their social, economic and demographic structures. The main material of the study consists of the data obtained from the questionnaires made with individuals living in the Van Province Center. In the study, crosstab analysis was used to determine the change in food consumption habits of consumers during the covid-19 pandemic, and CART analysis in SPSS program and LIMDEP statistical program and Binominal Probit Regression Model were used to determine the factors affecting food consumption change levels. Male consumers who have a green card or other insurance system, whose education level is primary school, associate degree and doctorate, and who are married, have increased their expenditures for food products during the Covid-19 pandemic, have private insurance and SGK, have secondary school graduates or have completed a master's degree and have an income. It has been determined that consumers with 1000-3000 and above 1000 do not increase their expenditures for food products during the Covid-19 pandemic. According to the results of econometric analysis; Status of passing the Covid-19 disease, the amount paid per person for each meal eaten outside the home during the pandemic, the change in the number of meals during the pandemic, the change in the eating habits of stress and anxiety during the pandemic, the gender status, the weight change status during the pandemic and during the pandemic. The variables of stocking status in food products were found to be statistically significant.

Keywords: Covid-19, Van Province, Food Consumption Habits

*This study was presented to Atatürk University, Institute of Science and Technology as a master's thesis in 2022.

Evaluation of the Effects of Various Metals on the Production of Pigments in Cultures of *Fusarium oxysporium*

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ABSTRACT: Microorganisms are subject to physical, chemical and biological stresses, as are all living organisms in nature. One of the most effective defense mechanisms against these stress factors is the production of secondary metabolites. One of the secondary metabolites that are produced are pigments with a high antioxidant capacity. In many industrial sectors, pigments are important compounds with high economic value and a wide range of applications. Although they are obtained from many different sources, microbial pigments are more stable and less toxic than other pigments, which is an advantage for the use of these pigments in the food, cosmetics and textile industries. Fungal pigments, in particular, are the most widely used pigments in these sectors. It is known that fungi change their secondary metabolites under stress conditions. In view of this information, the aim was to determine the change in the fungal pigments under the stress of different types of metals. For this purpose, *Fusarium oxysporium* isolate OG15, known to produce different pigments, was used. *F. oxysporium* was inoculated on potato dextrose agar (PDA), incubated for one week at room temperature and used as active culture. For the extraction of pigments, the media were prepared by adding different metals ($CuSO_4$, H_3BO_3, $CoCl_2$, $MnCl_2$) to the potato dextrose broth (PDB) at a concentration of 200 mM. Slices of 1 cm square were taken from the active culture and left to incubate at 150 rpm for 7 days at room temperature. At the end of the incubation, the pigments obtained by extraction of the pigments with ethanol were measured and recorded in a UV spectrophotometer between 300 and 800 abs. The best absorbance of the samples developed as control was measured at about 450 nm, while peak values were measured at 500 nm for $MnCl_2$, 550 nm for H_3BO_3, 600 nm for $CoCl_2$ and 650 nm for $CuSO_4$. The highest absorption value (2.04) among all samples was obtained with boron application. When the pigment yield was evaluated, a higher yield than the control was obtained in all applications. The purified pigments were also subjected to TLC and colour analysis. At the end of the study, it was found that *F. oxysporium* exposed to different metals produced different pigments.

Keywords: Metal stress, Pigmentation, Fungal pigment

Investigation of Fungal Biomass Growth Parameters in İron and Chrome Mineral Processing Wastes

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ABSTRACT: A wide range of technologies have been manufactured and continue to be developed to meet the modern needs of humankind. To manufacture technological equipment and process many minerals, it is necessary to extract usable materials from ores in the correct form and purity. The mining industry is concerned with the extraction and processing of minerals needed by mankind in various fields and forms for exactly these purposes. Unfortunately, in addition to the valuable resources that mining produces, it also creates waste. Depending on the structure of the mine being processed, these wastes can be hazardous, or even if they are considered non-hazardous, they can reach very large quantities and cause problems just by their volume. For this reason, efforts are made to utilize and dispose of all types of waste/by-products generated during the processing of minerals. Iron and chromium, two economically important minerals for our country, which require extensive heat treatment during processing, produce large quantities of slag with varying contents. The alternative use and disposal of these slags is very important. In this study, firstly, an attempt was made to determine the general growth parameters of iron and chromium mining slags using a metal/heavy metal stress resistant fungus (*Fusarium verticillioides* OG8.). The aim was to obtain information for the design of application models using the isolate whose general growth model was determined, and to obtain data for ore recovery from waste or environmental risk reduction applications. The amount of wet and dry biomass in the culture media prepared using 6 slags (electric arc furnace and pot slag) with different contents, obtained from chromium and iron mines operating in our country, was evaluated together by measuring the amount of ash remaining at the end of the high temperature treatment (350 °C). While there was almost no growth (<0.1% culture yield) in the chimney waste samples of the slags used in the application, the growth and biomass formation (>18.0% culture yield) in the iron and chromium first pot slags was remarkable. When the ash content (~77% of total dry biomass) was determined by incineration, the mold mycelia retained high levels of inorganic compounds.

Keywords: Biomass, Slag, Chromium, Iron, *Fusarium* sp.

Tea Tree Oil Plant Repositioning in Cancers Implying Stathmin

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ABSTRACT: Among microtubules (MTs) destabilizing prtoeins, the ubiquitous phosphoprotein Stathmin which controls the dynamics of MTs by sequestering tubulin and inhibits MT spindle. Therefore, it constitutes an interesting therapeutic target in the fight against cancer by blocking mitotic spindle. Tea Tree Oil (TTO) presents antitumor activity in several types of common cancers such as breast and colorectal cancers. This study aims to search for TTO bioactive molecule that could potentially interact with the N-ter and α helix of Stathmin. By molecular modeling, we selected 287 TTO compounds and their isomers. We analyzed their interaction with Stathmin by molecular docking in order to reposition the best ligand with interaction affinities with this protein particularly in N-ter domain. The analysis of data allowed highlight p-cymene as the molecule that can best target N-ter of Stathmin. Although 4-Terpineol showed strong binding liaison at N-ter and α helix of Stathmin at phsosporylaion sites. Finally, further molecular simulation analyzes and an *in vivo* study are necessary to confirm the interaction in N-ter and α-helix of p-cymene and 4-terpineol on Stathmin.

Keywords: Cancer, Docking, Stathmin, TTO

Comparative Study of Bioactive Compounds and Antioxidant Activity of Olive Oils

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ABSTRACT: This study is devoted to a comparative study of the phenolic compound content and the antioxidant activity of olive oils derived from three Algerian varieties (*Limli*, *Azeradj and Chamlal*) harvested in Bejaia location during the season 2020/2021. Primarily, the quality of tested olive oils was determined by measuring physico-chemical parameters. The preparation of extracts was adopted by the liquid-liquid method using the methanol 60% as solvent. Spectrophotometric methods were used to measure the content of the different classes of phenolics (total phenolic compounds, *orthodi*phenols, flavonoids) as well as antioxidant activity which is tested with three methods (antiradical activity, reducing power and iron chelating activity). The results showed that the values for the analyzed oils were in accordance with the standards set by the C.O.I (2019). The statistical analysis indicates that the phenolics contents and antioxidant activity are dependent on the variety; *Azeradj* is the variety which contain the highest levels of total polyphenols, and its extracts exhibit the potent reducing and anti-free radical activity. While, the highest ortho-diphenol concentration was noted in the *Limli* variety. Concerning the iron chelating activity, extracts of the *Chemlal* variety present the best power.

Keywords: Olive oil, Variety, Bioactive compounds, Antioxidant activity

POSTER PRESENTATIONS

Performance of Claudin-4 in The Diagnosis of Malignant Pleural Mesothelioma *versus* Lung Adenocarcinoma

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ABSTRACT: Lung adenocarcinoma (ADC) is difficult to distinguish from epithelioid malignant pleural mesothelioma (MPM), this distinction often requires additional studies and clinical correlation. Some studies have recently revealed that immunohistochemistry (IHC) of claudin-4 (CL-4), a protein of the tight junctions, expressed in most epithelial cells but absent in the mesothelial cells, allowed to distinguish with a high-performance ADC pulmonary from MPM, mostly on surgical specimens, but also on pleural effusions. However, despite its performance, CL-4 is not yet part of the panel of antibodies used in the differential diagnosis of these pathologies. Further studies are needed to validate the integration of this protein in this panel. This study aimed to confirm the performance of CL-4 in the differential diagnosis of epithelioid MPM *versus* lung ADC on human biopsies. To address this issue, 41 human biopsies (13 epithelioid MPM and 28 pulmonary ADC were used in this study by performing histology and immunohistochemistry. Our findings reveal a strong expression of CL-4 in 28 cases of lung ADC (100%) and a total lack of expression of this protein in 13 cases of MPM (0%) thus, confirming the results of previous studies. Claudin-4 appears to be very effective in discriminating between the lung ADC and epithelioid MPM because its expression formally excluded a diagnosis of mesothelioma. Further studies should be performed in the future to validate the use of this protein as a marker in the differential diagnosis of lung ADC versus MPM.

Keywords: Claudin-4 (CL-4), Adenocarcinoma, Malignant pleural mesothelioma (MPM), Differential diagnosis

Barley Wholemeal Bread with Natural Leaven

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ABSTRACT: Despite these nutritional qualities, barley is only marginally used in baking, due to its poor suitability for breadmaking linked to its low gluten content. In our study, we prepared sourdough fermented baguette bread by testing different formulations in which whole barley flour (BF) was used alone or partially substituted by soft wheat flour (WF) and/or with added gum. guar as an improver. An evaluation of the physicochemical and sensory quality of the prepared breads is carried out. Bread made from 100% barley flour shows a low specific volume (Vsp) compared to that of soft wheat flour (1.41±1.18 versus 2.63±2.50 cm3/g, respectively). The incorporation of wheat flour leads to an improvement in the volume of the breads. The 50% Bf and 50% Wf formula (Vsp= 2.1±4.12 cm3/g) was closest to that of the soft wheat control. The specific volume of all formulas increased with the addition of %1 of guar gum, particularly for the 70% Bf-30%Wf formula (the Vsp increased from 1.83±3.64 to 2.07±1.24 cm3/ g). The thickness of the crust of breads containing barley is significantly greater than that of the control. Tasters gave bread made with 100% barley flour significantly higher marks for crispy texture. According to the results of the Friedman rank classification test, among all the formulas tested, the improved formula of 70% Bf and 30% Wf was the most preferred by the jury. The 100% barley flour bread was the least popular. Naturally leavened bread was better appreciated by tasters than barley bread prepared with baker's yeast.

Keywords: Whole barley flour, Soft wheat flour, Sourdough, Bread, Specific volume

“Lmermez” Traditional Algerian Product Made from Immature Barley Grain

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ABSTRACT: Barley occupied a priority place in Algerian culinary practices; these traditions deserve to berehabilitated given the nutritional virtues of barley. The aim of this work is to highlight the traditional preparation diagram for a product derived from immature barley grains called "Lmermez". The laboratory scale products were characterized in terms of their physicochemical and technological properties. The results of the survey indicate that immature barley grains «Lmermez» are scalded, roasted and ground, after which several sieving operations are carried out to extract semolina of different sizes, flour and bran. The products derived from lmermez are used to prepare four dishes which are “Boutchich”; dish similar to couscous, “taminet lmermez”; sweet dish, “bakhbukh”; made from fine lmermez semolina and fermented milk and finally, chorba lmermez (like a soup). These two types of semolina have protein contents of 12.95% and 11.61%, lipid contents of 2.53% and 1.71% and ash contents of 1.79% and 2.01%, a degree of delitescence of 124.24 and 44.26% and swelling indices at 100°C of 125% and 120%, respectively. "Bakhboukh" is prepared by mixing fine "lmermez" semolina with fermented "l'ben" milk (ratio: 0.5/3, w/v). The mixture is left to stand for several hours until the semolina becomes soft before being eaten. "bakhboukh" has a sandy-yellow color, a granular, semi-liquid texture and a torrified, lactic, vegetable odor.

Keywords: Immature barley, Barley semolina, Culinary preparation, Algerian traditions

Impact of Climate on The Biodiversity of Date Palm Cultivar Deglet Nour in the Biskra Region

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ABSTRACT: The date palm is cultivated in vast regions of the world with variable pedoclimatic conditions. However, date palm productivity is seriously affected by ongoing climate change. The objective of this study is to fully understand how air temperature influences date palm cultivation and productivity. To understand this phenomenon, monitoring the phenological stages of the date palm (cv. Deglet-Nour) in the north of the Wilaya of Biskra by calculating the thermal accumulation necessary to reach the tamar stage. A comparison of the results was made with those of the year 2018-2019 in the same region. This year (2023), Deglet Nour dates fruits required 203 days with an estimated thermal accumulation of 3836.4°C to reach their final stage of maturity, while, it required about 3457°C and 3574 in 2018-2019, respectively, in the same experimental station. Climate warming will result in the range shifts; thus, cultivation of future date palm orchards should be planned in the most suitable areas in order to avoid the negative consequences of climate change on date palm production.

Keywords: Climate change, Thermal accumulation, Deglet nour, Tamar stage

Simultaneous Shifting of Both Lighting-Cycle and Feeding-Time as One of The Chrono-Physiological Management Protocols Can Enhance the Production Performance of Heat-Stressed Small Ruminants

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ABSTRACT: Fifteen male goat housed in climatic chambers were allocated into three groups (5 kids/group). Kids in the 1st group were placed under normal un-reversed light: dark (L:D) cycle, fed in the morning, and assigned as the control group (C), kids in the 2nd group (T1) were fed in the morning but placed under a reversed L:D cycle, while kids in the 3rd group (T2) were placed under a reversed L:D cycle and fed in the evening. During the experimental period kids were exposed to a hot condition. Reversing the lighting-cycle alone (T1) and/or the simultaneous shifting of both lighting-cycle and feeding-time protocol (T2) under hot climatic conditions had no ($p > 0.05$) influence on body rectal and skin temperatures and albumin and glucose, while kids in both treatments showed ($p < 0.05$) higher triacylglycerol compared to the C group. Moreover, it was clearly that kids in T2 had ($p < 0.05$) reduced kids DFI, increased ($p < 0.05$) their ADG and urea, ($p < 0.05$) better FCR compared to kids in other groups; thereby, suggesting that such chrono-physiological management protocol could have desynchronize the heat load arising from the combined effects of both thermal stress and postprandial metabolism. The study was supported by King Abdul-Aziz City for Science and Technology (KACST) under the grant 14-AGR819-02.

Keywords: Biophysiology, Circadian, Synchronization, Zeitgeber

Identification and Characterization of Lactobacillus from Breast-Fed Infants Faeces in Bechar Region, South West of Algeria

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ABSTRACT: The isolation of the lactobacillus strains from breast-fed infants feces (less than 6 months old) in the town of Bechar, allowed us to obtain 62 strains (Gram positive, Catalase negative, bacilliform), which were purified and identified on the basis of physiological and biochemical methods (fermentation type, growth test at different temperatures (15 ° C, 30 ° C, 45 ° C), growth at different NaCl concentrations up to 6.5%, study of resistance to temperature (63 ° C.), growth at different acid pH and at different concentrations of bile salts, and finally the use of the API 50 CHL gallery. The identification and phylogenetic analysis of the isolated strains were carried out by sequencing the 16S rRNA gene, followed by the ERIC-PCR technique, which led us to identify four species with a different percentage; *Lb rhamnosus* (52 isolates), *Lb. frementum* (22 isolates), *Lb. mucosae* (52 isolates) and finally *Lb. plantarum* (52 isolats).

Keywords: *Lactobacillus*, İnfants feces, İdentification, Phenotypic characterization, Molecular characterization, Sequencing, 16S rRNA gene

Effect of Aqueous Extract of Coffee Parchment on The Lipid Profile of Obese Male Wistar Rats

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ABSTRACT: Obesity is a complex, multifactorial chronic disease associated with a host of complications: diabetes, cardiovascular diseases, musculoskeletal disorders, cancer. Parchment is a coffee byproduct containing polyphenols that may be effective in preventing obesity-related metabolic disorders. The objective of this study is to determine the effect of the aqueous extract of coffee parchment on male Wistar rats made obese. The aqueous extract of coffee parchment was administered orally (100 mg/kg of weight/day) to Mal Wistar rats made obese by the fructose diet (20% fructose in water). Different metabolic parameters were determined (cholesterol, triglycerides, VLDL, LDL, HDL) at the serum level and at the tissue level (liver, adipose tissue, muscle). Our results show that the aqueous extract of coffee parchment gave a good yield of 16.79% with a content of 79.50 mg GAE/g of total phenolic compounds. Our results show that the fructose diet causes obesity associated with dyslipidemia (hypertriglyceridemia and hypercholesterolemia) in rats. following a 2-month nutritional therapy we noted that the aqueous extract of coffee parchment reduced body weight gain with a reduction in plasma and tissue levels (liver, adipose tissue and muscle) of total cholesterol, triglycerides, with a drop in LDL, VLDL contents, and an increase in HDL contents. We conclude that coffee parchment is not waste and should not be thrown away, and we could constitute it as a good adjuvant to prevent obesity, hypercholesterolemia, hypertriglyceridemia in wistar rats.

Keywords: Parchment, Lipid profile, Obesity

Application of ML in UASB Biogas Production Utilizing Cow Manure and Date Fruits Residues*

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ABSTRACT: In the realm of biotechnological research, particularly focusing on the Up Flow Anaerobic Sludge Blanket (UASB) reactor, there is a burgeoning interest in utilizing computational techniques, notably machine learning (ML) methodologies, as they offer a progressive and forward-thinking avenue to augment the stability of the process. This is achieved by enabling the prediction of performances that exhibit complex, nonlinear associations with a diverse set of operational metrics. An ensemble of five distinct ML architectures, encompassing decision trees, Support Vector Machines (SVM), and algorithms grounded on neural network principles, were meticulously evaluated to forecast the production of methane within the confines of the UASB reactor. An intricate analysis of the outcomes elucidated that by harnessing the intricate data patterns from UASB operational performances, these ML models had the capacity to bolster the precision of predictions. The study showed enhancement in prediction accuracy, presenting an uplift of approximately 21.2% relative to traditional algorithmic methods. This enhancement was especially pronounced in models that were underpinned by decision tree and SVM methodologies. The retraining mechanism, demonstrated profound efficacy in generating accurate predictions when relying solely on pH as the primary input variable. This is significant given the plausible simplicity in monitoring pH.

Keywords: UASB, Date residuals, Machine learning, Biogas, Remains

*This study was funded by King Abdulazizi City for Science and Technology (KACST), Saudi Arabia, grant number 14-ENV528-02.

Lactic Bacteria in Food Bioconservation

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ABSTRACT: In response to consumer demand, most agri-food industries are now seeking to produce fresh foodstuffs with a maximum preservation date, using biological means. These bio-preservatives include lactic acid bacteria and bacteriocins, which are known for their antagonistic effect on harmful bacteria. This study shows the antimicrobial action of an S93 lactic acid bacterium belonging to the species *lactococcus lactis subsp lactis* against certain pathogenic and spoilage bacteria, using the technique of Fleming et al, (1975). This action is due to the production by the lactic bacteria of several metabolites, including acid and a protein bacteriocin. The acid produced by S93 acted on *Listeria monocytogenes* and *Enterococcus faecalis*, while the pepsin-sensitive bacteriocin inhibited *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus and E. coli.* The antagonistic effect tested was bacteriostatic. The S93 lactic acid bacterium belonging to the subspecies *Lactococcus lactis subsp lactis* can therefore be used in the agri-food industry as a starter for food bio-preservation**.**

Key words: Lactic acid bacteria, Harmful bacteria, Inhibition, Bio-preservation, Food

Antioxidant and Anti-Hemolytic Effect of *Centaurea calcitrapa* Extracts

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ABSTRACT: In the present study,aerial part of *Centaurea calcitrapa* L. were extracted with solvent of varying polarity allowed their separation into three main subfractions, the analysis of methanol crud (CrE), chloroform (ChE) and ethyl acetate (EaE) extracts, showed that the EaE contains the highest amount of flavonoids (50,71 ± 0,65 mg Eq Rutin / g dry extract and 31,96 ± 0,39 mg Eq Quercetin / g dry extract), followed by ChE (30,96 ± 0,55 mg ER / g dry extract and 19,38± 0,33 mg EQ / g dry extract), and CrE with 27,29 ± 0,18 mg ER / g dry extract and 17,16 ± 0,11 mg EQ / g dry extract. The β-carotene / linoleic acid bleaching assay revealed that the extracts have a very important antioxidant activity. The results showed that CrE has the highest antioxidant activity, followed by EaE and ChE with95.00 ± 3,48 %, 86,43 ± 2,48 % and 80,44 ± 0,19 %, respectively. Using DPPH assay, the highest activity was observed with EaE (IC_{50} = 0,037 ± 0,0006 mg / ml), followed by CrE and ChE with IC_{50} of 0,109 ± 0,0009 and 0,290 ± 0,0053 mg / ml, respectively. The antioxidant activities of the CrE is confirmed by an *in vivo* assay in mice, using two doses: Cr dose 1 (CrD_1: 50 mg/kg/day), Cr dose 2 (CrD_2: 100 mg/kg/day) during 21 days. Total antioxidant capacity of plasma and red blood cells was measured by using DPPH radical and from the kinetics of hemolysis, respectively. All treated groups compared with native control (GCtl) and the treatedwith vitamin C (GVit C) groups, CrD_2 group showed a strong scavenging activity using DPPH assay (51,64 ± 7,82 %), higher than that of GVit C (47,27 ± 6,78 %) and CrD_1 group (45,95 ± 6,26 %). The half-life (HT_{50}), which corresponds to 50% of cell lysis was calculated from the kinetics of hemolysis obtained, the results showed that both groups treated with plant extract had a protective effect against erythrocytes hemolysis (CrD_2: HT_{50}= 167,3 ± 3,72 min), comparable to GVit C (HT_{50}= 163,4 ± 9,10 min) and largely higher than the native control (HT_{50}= 147,7 ± 0,40 min). All results confirmed that the extracts have a dose dependent effect on the growth of overall antioxidant defenses. These results support the use of this plant against anti-inflammatory diseases in traditional medicine.

Keywords: *Centaurea calcitrapa*, in vivo-antioxidant activity, DPPH, hemolysis, flavonoids

Artificial Neural Networks Applied to the Risks Associated with the Use of Pesticides

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ABSTRACT: Pesticides are substance or mixture of substance, which differ in their physical, chemical properties. Hence, they are classified by their properties into various classes. Consequently, to three modes: according to their targets, their mode or period of action. Pesticides have proven to be an effective means for farmers to increase yield and therefore cover the population's demand for food. However, the risk to human health and the environment. In the medium and long term, pesticides are responsible of many reproductive problems, sterility in men also in women such as ABRT and congenital anomalies. The toxic effects have been observed in reproduction disrupt the process of spermatogenesis which leads to degeneration and atrophy of the tubules (the absence of maturity of the tubes) and oligospermia which can even lead to azoospermia. An epidemiological survey was carried out in the region with a strong use of the pesticide in order to make any corelation and predict well the occurrence of certain diseases. Since the effect of these parameters is characterized by uncertainty, because many other ignored factors can influence the results to different degrees, we found it useful to proceed these data by the artificial intelligence techniques including fuzzy logic inference system. Such as fuzzy systems are dynamic and the ability to read the experimental data of the real environment, therefore they are capable of solving complex systems of natural processes. If the proposed program predicts the effect of pesticides residues on public health, it is extensible and allows the introduction of other variables that are not considered in this study.

Keywords: Risck, Pesticides, Sterility in men, Fuzzy logic

Contamination of Water by Pesticides Using Artificial Neural Networks Applied Analysis

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ABSTRACT: Pesticides with a wide range of uses in the agricultural sector are considered potential contaminants of groundwater. In addition to their agricultural benefits, these products correspond to a wide range of chemical molecules. Their mode of action and toxicity to human health have been demonstrated. The level of contamination is influenced by various factors, including rainfall, soil composition, physicochemical properties, and microbiological makeup of the rhizosphere. These phenomena are difficult to model using traditional mathematical approaches to estimate the public health implications. In this scenario, conventional mathematical techniques are inadequate for modelling the phenomenon under investigation, so determining the impact on public health is a complex task. The proposed study presents a technique of artificial intelligence that incorporates artificial neural networks. As neural networks have the capability to support multiple combined variables, their application in this area seems fitting. Furthermore, our team has created a predictive model through extensive analysis of field data. The model links input parameters to impacts on public health, which are measured as the output variable of the system. Proactive measures will be taken to ensure the quality and quantity of pesticides and chemicals used in agriculture. This will protect the wellbeing of the people living in these regions.

Keywords: Groundwater, Pesticides, Public health

Gonad of Female Mussels Inhabiting Polluted Locations from Annaba Coastline

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ABSTRACT: This work aims to investigate the effects of the pollutants on the female reproductive histology of a sentinel species of bivalves *Mytilus galloprovincialis* from Annaba coastline, exposed to industrial, agricultural, and domestic discharges. The animals were obtained from a non-polluted site and two other locations receiving untreated sewage and different types of pollutants in January, February, and March. Ovarian tissues demonstrated histopathological alterations in mussels subjected to anthropogenic activities of during the three months. Conversely, the study revealed that the tissues of females living in the non-polluted site appeared unaffected during the study period. In conclusion, the reproduction of mussels was affected spatially and temporally, which is likely related to the types of pollution and the changing monthly environmental stressors.

Keywords: Histology, Mussels, *Mytilus galloprovincialis*, Ovary, Reproduction, Sentinel species

* This study is a part of the PhD thesis accepted by University Badji Mokhtar-Annaba

Evaluation of Antihemolitic and Antimicrobial Activities of *Prunus persica* L. Leaves

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ABSTRACT: In order to promote medicinal plants and to consider their impact on health, we undertook a study to evaluate the antihemolitic and antimicrobial activities of extracts of *Prunus persica* L. leaves, a plant renowned in traditional medicine. Our goal is to determine their ability to prevent the destruction of red blood cells and inhibit microbial growth. This research will contribute to a better understanding of the potential health applications of *Prunus persica* L. Selective extraction of polyphenols and tannins determined the following levels (90.28 mg/ml and 14.66 mg/ml respectively). The study evaluated the in vitro antihemolitic activity of *Prunus persica* L. leaves extracts using a human erythrocyte membrane model. The results revealed a powerful antihemolitic activity. The tannins extract showed the highest percentage with 80% at a concentration of 0.25 mg/ml, surpassing the ascorbic acid 74.06% at concentration of 1.5 mg/ml. The antimicrobial activity of the two extracts was studied on six bacterial strains. The results demonstrated a significant inhibition of *Enterobacterococcus faecalis*. In fact, the tannic extract showed promising antimicrobial activity, with CMI of 1.83 mg/ml. This result suggests that the tannins of *Prunus persica* L. leaves had the potential to be used as antimicrobial agents against specific bacterial strains.

Keywords: *Prunus persica* L., Medicinal plants, Phenolic compounds, Antihemolitic activity, Antimicrobial activity

Analysis of Phenolic Compounds, Antioxidant Properties, and Hemolytic Activities in *Juniperus oxycedrus* Needles

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ABSTRACT: Phenolic compounds are bioactive molecules exhibiting a lot of scientific attention due to their multiple biological activities. This study aims investigating the antioxidant and the phenolic content in four different extracts (methanol, water, hexane and dichloromethane) of needles of *Juniperus oxycedrus* subsp. *oxycedrus*. Results showed that the methanol extract was the most concentrated in total phenolics (58.8 ± 1.08 mg GAE.g^{-1} DW), flavonoids (16.2 ± 1.20 mg CE g^{-1} DW) and tannins (19.05 ± 1.81 mg CE g^{-1} DW). HPLC-DAD-ESI-MS^n analysis of this extract led to the identification of 27 molecules, among them hydrolizable tannins (proanthocyanidin oligomers), glycosylated flavonoids, biflavones and a furanone glucoside (psydrin). Moreover, the methanol extract exhibited remarkable antioxidant activity. Incubation of the extracts (20 mg/ml) with human erythrocytes for one hour led to haemolytic activities between 1.95% and 4.71%. Our findings identified the appropriate solvent for extracting phenolics, which might provide a rich source of natural antioxidants as food additives replacing synthetic ones in food industry.

Keywords: *Juniperus oxycedrus* subsp *oxycedrus*, Needles, Antioxidant activity, Haemolytic activity, HPLC-DAD-ESI-MS^n

Hemolytic Activity and Anti-Hemolytic Potential of Two Extracts from *Daucus crinitus* Desf. Roots from Algeria

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ABSTRACT: The experiments in this study aimed to investigate the hemolytic properties and anti-hemolytic activity of extracts from different parts of the Daucus crinitus Desf. "Bouzeffour" root found in Tlemcen, Algeria. The extraction process involved hydro-methanolic extraction using a Soxhlet apparatus, followed by liquid/liquid extraction with hexane to remove non-polar compounds. The hydro-methanolic yield obtained from the inner white part of the root was higher at 18.88% compared to the crust, which yielded 10.1%. However, the yields of oils extracted were 3.25% for the crust and 2.25% for the inner white part. Spectrophotometric analysis of phenolic compounds revealed that the crustal extract had higher levels of polyphenols (49.28 ± 7.16 mg EAG/g ES) and flavonoids (12.82 ± 0.78 mg EC/g ES) compared to the white part extract. Hemolysis percentages, assessed using erythrocyte models, increased proportionally with concentration. The white part exhibited the lowest hemolysis rate, starting to increase significantly at 10 mg/ml and reaching 88.71% at 20 mg/ml after a 30-minute incubation period. For concentrations between 0.0156 and 1.25 mg/ml, both parts of the plant showed an anti-hemolytic effect, with percentages ranging from 56.4% to 80% for the white part and from 44.9% to 80% for the crust. Beyond this concentration range, the protective effect disappeared for both extracts and ascorbic acid. These findings contribute to the understanding of the hemolytic and anti-hemolytic properties of Daucus crinitus Desf. "Bouzeffour" root extracts, which may have implications for potential therapeutic applications.

Keywords*:* *Daucus crinitus Desf.* Roots, Phenolic compounds, Hemolytic power, Anti-hemolytic activity

Formulation of New Kombucha Flavors and Evaluation of The Antioxidant Activity

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ABSTRACT: Probiotics are well known to restore a stable gut microbiota. They help regulate immune system, showing the possibility to prevent the attack of viruses as COVID-19 and to combat others diseases. Thus, suggesting the consumption of Kombucha due to its multiple functional properties such as antioxidant activity and anti-inflammatory potential. This beverage is a sweetened tea fermented with a symbiotic culture of yeasts and bacteria. In this study, a new formulation of Kombucha flavored with fresh fruit was developed, adding a novelty to the previous researches. The Scooby is very sensitive to contamination. For this purpose, the symbiotic culture was fermented during 20 days under rigorous hygienic conditions to remove residual alcohol. The acidity level and the taste were acceptable. Flavored samples show significant antioxidant activities *via* the DPPH test, especially Mojito (91%) and Ginger (86%). The online investigation showed an appreciation for the fruity flavor and digestive benefits of Kombucha, despite the limited knowledge of probiotic drinks among 67.7% of the participants. These results were confirmed during the sensory analysis, where the fruity taste of strawberries was widely acclaimed. These promising results suggest that Kombucha could be touted as a superfood, given its antioxidant properties and potential health benefits.

Keywords: Kombucha, Probiotics, Flavor, Antioxidants, Health benefits

*This study is a part of the Tehami Kawther's and Merabet Khadidja master thesis accepted by Ghanemi Fatima Zahra

Hepatoprotective and Antiulcer Effects of The Mixture of *Eriobotrya Japonica* and *Prunus Cerasus* Leaves

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ABSTRACT: Liver disorders and ulcer discomfort are common in Algeria and around the world. The leaves of *Eriobotrya japonica* and *Prunus cerasus* are commonly used in traditional pharmacopoeia. In this study, we investigated the anti-ulcer and hepatoprotective effects of an aqueous extract obtained from the mixture of leaves of these two plants in *Wistar* rats. The herbal mixture was prepared with 1g / 100 mL of water. Omeprazole was used as a standard antiulcer drug. Pure ethanol has been used to induce gastric ulcer. The animals were therefore treated with the aqueous extract during the following hours. The stomachs were removed and dissected then photographed; the visible erosive lesions were counted. Experimental rats poisoned with CCl_4 were treated with the aqueous leaf extract. The results showed that the aqueous extract exhibited a hepatoprotective effect, with the liver profile altered by CCl_4 toxicity, returning to normal control values. Furthermore, histopathological data from the liver of different group of animals also supported the evaluation of hepatoprotective activities of the aqueous mixture based on biochemical analysis. Our preliminary results support the herbal formulation of *E. japonica* and *P. cerasus* leaves for their protective effect against hepatotoxicity and the prevention or treatment of ulcerative diseases.

Keywords: *Eriobotrya japonica*, *Prunus cerasus*, Leaves, Antiulcer effect, Hepatoprotective effect

Anti-Inflammatory and Analgesic Activity of Aqueous Extract of *Inula viscosa* Leaves

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ABSTRACT: The objective of the present study was to evaluate the anti-inflammatory and the analgesic effects activity of aqueous extract of *Inula viscosa* leaves. The leaves are used as traditional folk medicine in north of Algeria to treat inflammatory and painful diseases. Anti-inflammatory activity was evaluated by using the xylene-induced ear edema induced inflammation, whereas acetic acid-induced abdominal constrictions were used to determine antinociceptive effects. The extract of *Inula viscosa* (300 and 150 mg/kg) produced a significant inhibition of ear edema inflammation, and produced a significant reduction of the number of writhes. These findings suggest the aerial parts of *Inula viscosa* exhibits potent anti-inflammatory and analgesic activities on chemical behavioral models of nociception and inflammation in mice.

Keywords: *Inula viscosa*, Xylene, Analgesic effect, Anti-inflammatory

Gastroprotective, Antinociceptive and Antioxydant Activities of The Aqueous Extract of *Centaurium Erythraea* L.

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ABSTRACT: *Centaurium erythraea* (*C. erythraea*) belongs to the Gentianaceae family, which is largely used in traditional medicine to treat many diseases. The objective of this study was to evaluate the anti-ulcerogenic, analgesic and antioxidant activities of the aqueous extract of *C. erythraea*. The total content of phenolic compounds of the extract was found to be 644.74±0.0019 µg EGA /mg of extract. The flavonoids content was 147.85±0.0007µg EQ /mg of extract. The *in vitro* antioxidant activity was evaluated using the DPPH radical scavenging test, with an IC_{50} of 0.092 ± 0.003 mg/mL. The gastroprotective study shows that the extract (200 and 400 mg/kg) showed a good protection against gastric ulcer induced by ethanol, with a percentage of protection of 38.95 % and 73.01%, respectively. The oral administration of the extract (200 and 400 mg/kg) inhibited the pain caused by the intra-peritoneal injection of acetic acid in mice with inhibition percentages of 44.27% and 36.50%, respectively compared to diclofenac (41.25%). In conclusion, these results show that the extract of *C. erythraea* has anti-ulcerogenic, moderate analgesic properties and a good antioxidant activity which may be due at least partly to the the richness of the plant in polyphenols and flavonoids.

Keywords: *C. erythraea*, Antiulcerogenic activity, Analgesic activity, Antioxidant activity, Polyphénols

Iron Chelating, Free Radical Scavenging and Lipid Peroxidation İnhibition Activities of *Centaurium Erythraea* l. Hydroethanolic Extract

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ABSTRACT: In Algeria, medicinal plants are used in folk medicine to treat various diseases, including "Moraret lhnech", an annual or biannual plant belonging to the "*Gentianacaea*" family, which is found in many Mediterranean countries. The objective of this study is to estimate the polyphenols content and to assess the antioxidant activity of the alcohol extract of *C. erythraea* plant, where the yield was14.16%. The total polyphenol content was 84.1846 ± 0.0013 µg EAG/mg of dry extract, while the amount of flavonoid compounds was 18.87 ± 0.0003 µg EQ/mg of dry extract, and the amount of tannins was estimated at 62.81 ± 0.0022 µg EAT/mg of dry extract. The antioxydant activity was estimated by the following tests: the free radical scavenging test 2,2-diphenyl-1-picrylhydrazyl (DPPH), the iron chelating test and β-carotene bleaching test. The results showed that the hydro-ethanolic extract of *C. erythraea* has the ability to scavenge the free radical DPPH (IC_{50} = 103.1 ± 0.0016) µg/mL and to chelate the iron (IC_{50}= 1079.9 ± 0.65) µg/ mL. The test of β-carotène/ linoleic acid of the plant extract showed an antioxidant activity of 50.1%. These antioxydant activities may be due to the presence of polyphenols such as flavonoids in the plant extract.

Keywords: *C. erythraea*, Phenolic compounds, Flavonoids, Antioxidant activity

Mitigation of NaCl Stress in Wheat By Rhizosphere Engineering Using Salt Habitat Adapted PGPR Halotolerant Bacteria

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ABSTRACT: There is a great interest in mitigating soil salinity that limits plant growth and productivity. In this study, eighty-nine strains were isolated from the rhizosphere and endosphere of two halophyte species (*Suaeda mollis* and *Salsola tetrandra*) collected from three chotts in Algeria. They were screened for diverse plant growth-promoting traits, antifungal activity and tolerance to different physico-chemical conditions (pH, PEG, and NaCl) to evaluate their efficiency in mitigating salt stress and enhancing the growth of Arabidopsis thaliana and durum wheat under NaCl–stress conditions. Three bacterial strains BR5, OR15, and RB13 were finally selected and identified *as Bacillus atropheus*. The Bacterial strains (separately and combined) were then used for inoculating *Arabidopsis thaliana* and durum wheat during the seed germination stage under NaCl stress conditions. Results indicated that inoculation of both plant spp. with the bacterial strains separately or combined considerably improved the growth parameters. Three soils with different salinity levels (S1 = 0.48, S2 = 3.81, and S3 = 2.80 mS/cm) were used to investigate the effects of selected strains (BR5, OR15, and RB13; separately and combined) on several growth parameters of wheat plants. The inoculation (notably the multi-strain consortium) proved a better approach to increase the chlorophyll and carotenoid contents as compared to control plants. However, proline content, lipid peroxidation, and activities of antioxidant enzymes decreased after inoculation with the plant growth-promoting rhizobacteria (PGPR) that can attenuate the adverse effects of salt stress by reducing the reactive oxygen species (ROS) production. These results indicated that under saline soil conditions, halotolerant PGPR strains are promising candidates as biofertilizers under salt stress conditions.

Keywords: PGPR, Salinity tolerance, *Arabidopsis thaliana*, Durum wheat, Rhizosphere engineering

Aphicidal Effect of Essential Oils Extracted from Algerian Medicinal Plants

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ABSTRACT: Chemical insecticides are decisive in controlling insect pests, but they are harmful to the environment. Reducing the use of chemical insecticides has become the main goal for having a healthy produce. Therefore, researchs on using plant-based compounds has increased recently. The present study was carried out to analyze essential oils extracted from Algerian medicinal plants by gas chromatography coupled with mass spectrometry (GC-MS) and to determine the insecticidal activity of essential oils from Juniperus phoenicea and Pinus sylvestris. Main components constituting each essential oil were α-Pinene, β-Pinene 1,8- Cineol, Linalool and Linalyl acetate. On the other hand, toxicity of tested essential oils against Aphis gossypii was carried. Results showed different effect and was dependent on the used concentration (1000, 10000 and 100000 ppm), the duration of exposure (24, 48 and 72 hrs) and on the chemical composition of the oil. The highest toxicity was observed with Juniperus phoenicea which were toxic as Actara insecticide. In conclusion, the studied plants and ist essential oils are effectives to control insect pests safely.

Keywords: *Aphis gossypii*, Biopesticides, Essential oils, Plant extracts, İnsecticide

Effects of Extraction Solvent on the Phenolic Content, Antioxidant and Antibacterial Activities from Algerian *Rosmarinus officinalis*

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ABSTRACT: This work aimed to study the impact of the solvent nature (ethanol and ethyl acetate) on the extraction yield, total phenolic contents (TPC), flavonoids, the antioxidant and antibacterial activities from Algerian *Rosmarinus officinalis*. TPC were determined by colorimetric method (Folin-Ciocalteux) and were calculated as Gallic acid equivalent mg/g dry matter. Flavonoid contents were determined using aluminum chloride in a colorimetric method and expressed in quercetin equivalent (QE). Antiradical potential was approached by the ferric reducing antoxidant power FRAP method. Antibacterial activity was studied on two strains *Escherichia coli* and *Staphylococcus aureus*. Extraction yields were 10.88% for the ethanolic extract and 5.38% for the ethyl acetate extract. TPC in ethanol and ethyl acetate extracts obtained were 70 and 60 mg GA equivalent /g respectively. The flavonoid content is 11 µg QE/g for ethyl acetate extraction and 12 µg QE/g for ethanol extraction. Both ethanolic and ethyl acetate extracts possess strong antioxidant activity, with reduction rates of 82.44% 81.615% respectively. Significant antibacterial activity was noted on *Staphylococcus aureus* with inhibition zones of 18mm ±0.9 for the ethyl acetate extract and 12mm ±0.9 for the ethanolic extract. Very low activity is observed on *Escherichia coli* with an inhibition diameter less than 5mm.

Keywords: *Rosmarinus officinalis*, Ethanolic, Ethyl acetate extraction, Antioxidant, Antibacterial activities

Assessment of Flavonoid Contents, Total Phenolic Contents and The Antioxidant Potential in Ethanolic and Ethyl Acetate Extracts from *Curcuma longa L*

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ABSTRACT: The objective of this work is the evaluation of the total flavonoid contents (TFC), the total phenolic contents (TPC) and the antioxidant potential of ethanolic and ethyl acetate extracts from *Curcuma longa L.* The total flavonoid content was measured by a colorimetric assay using aluminum chloride. TFC was calculated as Quercetin equivalent g/100 g dry matter. Folin Ciocalteu was used for total phenolic contents; results are expressed as mg Gallic acid equivalents/g dry matter. The total antioxidant potential of the extracts was determined using the ferric reducing FRAP assay based on the reducing potential of the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}). The results were expressed as µmol trolox equivalent/g DM. Extraction yields were 3.83% in ethanolic extract and 2.55% in ethyl acetate extract. The TFC of the ethyl acetate extract was 113 µg EQ mg/g DM, while that of the ethanol extraction is 54 µg EQ mg/DM. The TPC in ethanol and ethyl acetate extracts were equivalent to 120 and 140 µg EAG/ g DM respectively. The absence of tannins in all extracts is noted. Both ethanolic and ethyl acetate extracts possess very strong antioxidant activity with reduction rates of 93.19%, 93.26%respectively.

Keywords: *Curcuma longa* L, Ethanolic, Ethyl acetate extraction, Antioxidant activity

Formulation of High-Protein Bars Based on Whey Protein and Oat Flakes

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ABSTRACT: Whey proteins are widely used as ingredients in food industry making not only for their good techno-functional properties but also for their nutritional properties. Whey proteins have a high nutritional value because of their richness in branched essential amino acids (BCAA). In this work we were interested in the valorisation of whey proteins by their incorporation in formulas of high -protein bars as snacks to be proposed for athletes for muscle gain, seniors or persons with pathologies causing muscle loss. Four formulas, using 80%, 60%, 40% and 20% of whey powders, are prepared using whey proteins, oats flacks and enriched with oilseeds to improve the nutritional quality of the formulated bars. The formulas were characterized by determining their physicochemical and sensory properties. The ranking test was used to define the optimal formula. The four formulas showed a protein rate of 71.05%, 51.77%, 31.48%, and 13.19%. A pleasant aroma with a low acid taste characterized the sensory properties of highly appreciated formulas. The optimal formula was prepared with 60% of whey powder and 20% oat flakes. It is characterized with a protein content of 51.77g/100g, a carbohydrate content of 28.68g/100g, a fat content of 1.2%, a total dry matter of 87.32%, and 1.66g/100g ash. It also appreciated because of its colour, a pleasant aroma and absence of the specific and unpleasant odour of whey. The taste was inviting and mildly acid. 40% of the tasters preferred this formula.

Keywords: High-protein bars, Whey protein, Oat flakes, Formulation, Sensory properties

Anti-Inflammatory and Antioxidant Activities of *Cistus salvifolius* Acetonic Extract

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ABSTRACT: This work focuses on the phytochemical study and evaluation of anti-inflammatory and antioxidant activities of acetonic extract of *Cistus salvifolius* leaves. Quantitative analysis showed the richness of this extract in total polyphenols, flavonoids and tannins, with 293µg EAG/mg extract, 16µg EQ/mg extract and 34µg EC/mg extract, respectively. The antioxidant power of acetonic extract was measured *in vitro* using DPPH, AAPH and reductive power tests. Results showed that the extract has a powerful effect against the free radical DPPH with an IC_{50}=4µg/mL and reductive power with an EC_{50}= 2.6µg/mL, respectively. Similarly, *Cistus salvifolius* acetonic extract at concentration 0.05 and 0.1 mg/mL exerted a high anti-hemolytic effect against AAPH-induced hemolysis with a value of HT_{50}=52± 16.395min. The anti-inflammatory activity was evaluated *in vitro* and *in vivo*, using human erythrocyte membrane stability tests and croton oil-induced ear edema in mice. Results showed that the acetonic extract preserved the membrane against erythrocyte membrane lysis with an inhibition percentage of 51-55%, while this extract induced an inhibition of ear edema with a percentage of 75%. In conclusion, the acetonic extract of *Cistus salvifolius* plant has a remarkable anti-inflammatory and antioxidant activity due to its content of bioactive compounds such as polyphenols, flavonoids and tannins.

Keywords: Anti-inflammatory, Antioxidant, Edema, *Cistus salvifolius*, Plant extracts, Polyphenols

Evaluation of Antihemolitic and Antimicrobial Activities of *Rubus ulmifolius* S. Leaves from Algeria

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ABSTRACT: Since the beginning, humanity has drawn on the plant resources present in its environment to treat and cure a wide range of illnesses. plants have played, and continue to play, an essential role in the healing arts throughout the world. In order to promote medicinal plants and to consider their impact on health, we undertook a study to evaluate the antihemolitic and antimicrobial activities of extracts of *Rubus ulmifolius* S. leaves, a plant renowned in traditional medicine. Our goal is to determine their ability to prevent the destruction of red blood cells and inhibit microbial growth. This research will contribute to a better understanding of the potential health applications of *Rubus ulmifolius* S. The study evaluated the in vitro antihemolitic activity of *Rubus ulmifolius* S. leaves extracts using a human erythrocyte membrane model. The results revealed a powerful antihemolitic activity. The phenolic and tannins extract showed the highest percentage with 86.7% and 77.46% respectively at a concentration of 0.125 mg/ml, surpassing the ascorbic acid 74.06% at concentration of 1.5 mg/ml. The antimicrobial activity of the two extracts was studied on six bacterial strains. The results demonstrated a significant inhibition of *Bacillus subtilis*. In fact, the tannic extract showed promising antimicrobial activity, with CMI of 4.33 mg/ml. This result suggests that the tannins of *Rubus ulmifolius* S. leaves had the potential to be used as antimicrobial agents against specific bacterial strains.

Keywords: *Rubus ulmifolius* S., Medicinal plants, Phenolic compounds, Antihemolitic activity, Antimicrobial activity

The Dairy Production of Indigenous Arbia Goat Breed and The Growth Performance of Their Kids in Algeria

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ABSTRACT: Knowledge of dairy potential is essential to procreate products that express their genetic potential fully. This study carried out on a total of 94 Arbia goats and 65 goat kids (n=195) (February - May 2022) in the wilayas of Souk-Ahras and Tebessa, enabled us to estimate dairy potential of this breed on the one hand, and a daily gains (ADG) between birth and 90 days of age on the other hand. The data revealed the daily milk production (n=94) was 0.89±0.85 liter per day. The milk quality is characterized by an average composition of 3.2% of fat, 13.46% of total protein, 17.28 % of total dry extract, 0.78% of salt, pH of 7.08±0.01, an acidity of 17.7 °D a density of 1032 and protein/fat ratio of 4.29. Regarding growth performances, the average birth weight was W0 = 1.80±0.61kg, while the average at 90 days was W90 = 10.05±2.02 kg, which corresponds to an overall daily weight gain of ADG0-90 = 91.66 g/day. The study showed that birth weight was not affected by sex ($P>0.05$), though males grew faster after weaning ($P<0.05$), and the performance aspect carried out by some physicochemical characteristics of milk was affected by area ($P<0.05$). Our study provided us that the local breed goat had a good production dairy performance and goat kids demonstrated an acceptable potential for meat production under semi-arid Algerian environmental conditions Therefore, it would be suitable to check lactogenic plants present on the pastures of these breeders to enhance production.

Keywords: Algeria, Goat, Milk, Production, Weaning

Evaluation of the Anti-inflammatory and Antioxidant Activity of *Sedum sediforme* Extracts

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ABSTRACT: The present study was undertaken to evaluate the *in vivo* anti-inflammatory effect and *in vitro* antioxidant activity of *Sedum sediforme* extracts. The plant was extracted with solvent of varying polarity allowed its separation into three sub-fractions: crude extract (CrE), chloroform extract (ChE) and ethyl acetate extract (EaE). Total polyphenol contents of the extracts were determined. The anti-inflammatory activity was evaluated using ear edema induced by phorbolmyristate acetate (PMA) method. The *in vitro* scavenging activity was evaluated using enzymatic and non-enzymatic methods. The results showed that the highest content of phenolics compound was found in the EaE. The administration of CrE (12.5 and 25 mg/kg body wt.), reduced ear edema induced by PMA (% I = 35.81 ± 3.18 % and 38.57 ± 2.80 %, respectively), the effect was comparable with that of diclofenac used as a reference drug (% I = 38.84 ± 1.87 %). The *in vitro* scavenging activity of *S. sediforme* extracts confirmed that the CrE has the highest enzymatic and non-enzymatic activity with an IC_{50} = 0.063 ± 0.005 mg/ml and 0.178 ± 0.006 mg/ml, respectively. These results support the use of this plant in traditional medicine as anti-inflammation and it could be used where scavenging of free radicals is warranted.

Keywords: *Sedum sediforme*, Anti-inflammatory, Superoxide scavenger, Cytochrome c, Metal chelating

The effect of Drying on the Mechanical Properties of Sukkari Date Powder

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ABSTRACT: The aim of the current research was to find out the effect of drying conditions on the mechanical properties of Sukkari date powder. The study was done by vacuum drying (VD) the date paste to check the effect of drying conditions on the mean cake strength, bulk density, cohesion index, compressibility, and flow stability of the date powder. The conditions of drying consisted of three levels of drying temperature (50, 60, and 70 ºC), three levels of drying pressure (10, 25, and 40 kPa), three levels of maltodextrin ratio (0, 20, and 40% (w/w)), and four levels of grinding time (10, 20, 30, and 40 s). The mean cake strength and cohesion decreased with increasing maltodextrin ratio, drying temperature, and drying pressure. The cohesion index increased with increasing grinding time, while mean cake strength decreased to its lowest value of 0.334 N m^{-2} with increasing grinding time to 28 s. Mean cake strength increased with increasing grinding time to reach 38.6 N m^{-2} at grinding time 40 s. Bulk density and flow stability decreased with increasing maltodextrin ratio, drying pressure, and grinding time, while they increased with increasing drying temperature. Compressibility increased with increasing maltodextrin ratio, drying temperature, and drying pressure, while decreasing with increasing grinding time. The compressibility was in the range of 1.24 to 20.29, and the bulk density was in the range of 0.5872 to 0.7312 g/cm^3.

Keywords: Sukkari Date, Powder, Dry, Mechanical

Antioxidant Activities of Algerian *Arctium minus* (Hill) Bernh. subsp. *atlanticum* (Pomel) Maire

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ABSTRACT: The local traditional pharmacopoeia is a rich source of potential resources for molecules with therapeutic effects. This study aimed to evaluate the antioxidant effect of extracts obtained from water, water/methanol, and ethyl acetate of the endemic plant *Arctium minus* (Hill) Bernh. subsp. *atlanticum* (Pomel) Maire, which is native to Algeria. The antioxidant activity was assessed using two different assays: 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and galvinoxyl (GOR) radical scavenging assays. Among the extracts tested, the ethyl acetate extract (EAE) exhibited the most potent antioxidant activity compared to the other samples. Specifically, the IC50 values for the DPPH and GOR assays were determined to be 69.45±5.49 µg/mL and 28.87±0.18 µg/mL, respectively. There appears to be a significant correlation between the type of extract and its potent antioxidant potential. This research highlights the strong antioxidant effect of Algerian Arctium minus subsp. Atlanticum, which has the potential to combat various cellular disorders. The use of this natural resource may be considered in the context of combating oxidative stress.

Keywords: Antioxidant, *Arctium minus* subsp. *atlanticum*, Oxidative stress

Autobiography and antifusaric activity of two acids from Juniperus oxycedrus Tar of Saoura region in Algeria

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ABSTRACT: This work is interested in the study of the antifusaric activity of the tarry and oily parts of (*Juniperus oxycedrus*). The activity is tested against five fungal strains (*Fusarium oxysporum* f. sp. *albedinis* (F1, F5, F13), *Fusarium graminearum* (FG4, FGa) at different concentrations. The autobiography revealed the existence of 2 anti-FOA molecules. The results of antifungal activity showed that the samples of tars and oils (T1, O1) completely inhibit the growth of fungal strains tested. For T1 inhibition was observed with an MIC of 0.182 mg / ml for F5, FGa and FG4. The MIC was 0.039 mg / ml for F13 and F1. While O1 inhibits growth with MIC of 0.232 mg / ml for F1, F5 and for strains FGa, FG4 and F13 the MIC is on the order of 0.312 mg / ml, 0.402and 0.214 mg / ml respectively. Analysis of tar samples from both shrubs by thin layer chromatography (TLC) showed 187 spots in the three mobile phases. The valorization of the TLC plates using an autobiography technique based on Iodo-nirtro tetrazolium as developer showed the presence of 2 spots of anti-FOA activity.

Keywords: Tar, Oil, *Juniperus oxycedrus*, Antifungal activity, TLC, Autobiography, *Fusarium oxysporum* f. sp. *albedinis*

Effect of Vacuum Drying on the Quality of Khalas Date Powder

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ABSTRACT: This study focuses on investigating the effect of vacuum drying on the quality aspects of Khalas date powder. The effect of drying temperature (50, 60, and 70 °C), drying pressure (10, 25, and 40 k Pa), three levels of maltodextrin ratio (0, 20, and 40% (w/w)), and grinding time (10, 20, 30, and 40 s) on the quality of Khalas date powder were studied. Quality analyses of the samples were carried out in terms of total phenolic content (TPC) and antioxidant activity (DPPH), total color difference (ΔE), and basic color parameters (L, a, and b). The total phenolic content (TPC) increased with increasing drying temperature and drying pressure, while increasing to its maximum value of 9.52 mg GAE/g dry weight with an increasing maltodextrin ratio to 20% (w/w) at 70 oC and 40 kPa. TPC decreased with an increasing maltodextrin ratio to reach 5.72 mg GAE/g dry weight at 40% (w/w), 70 oC, and 10 kPa. The antioxidant activity (DPPH) decreased with increasing drying pressure and maltodextrin ratio while increasing with increasing drying temperature. The total color difference (ΔE) decreased with increasing maltodextrin ratio and grinding time, while increasing with increasing drying temperature and drying pressure.

Keywords: Khalas Date, Powder, Dry, Vacuum, Mechanical

Microbiological Evaluation of Yoghurt with the Addition of Date Syrup and Study of Its Stability During Storage by Refrigeration*

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ABSTRACT: Our scientific research aims to evaluate a very useful and common food substance, which is dates and to determine the effect of date syrup on the overall quality of the yoghurt. The samples were kept at 4°C for 21 days. Where we prepared 3 types of yogurt with: 0%, 2.5% and 5% for each type and changes in selected microbiological and sensory parameters were monitored during storage. Each parameter studied is represented by three 30 ml pots, the following measurements and controls were carried out every 7 days on each processed product. From the beginning to the end of the post-acidification period, the acidity values of the yogurts measured are proportionally increased with the addition of date syrup. Overall average acidity levels between 78°D at the beginning of fermentation up to 82.34°D at the end of the post-acidification period. During the experiment, however, the acidity of the experiments did not exceed the commercially acceptable standards of 150°D. The microbiological quality of the date yoghurts was also acceptable and within the Algerian standard quality, due to the increased the acidity content, or may be due to the antimicrobial effect of dates fruit. Moreover, the number of specific yogurt germs proves to meet the standards required, and we remark the absence of pathogenic flora for every 21 days. The findings of this study may give an overall idea about manufacturing of date palm yoghurt supplementing with different level of date extraction or with low level of concentration of its syrup and appropriate technology of date extraction and its syrup preparation.

Keywords: Yoghurt, Date syrup, Microbiological profile, Storage

A Massage Oil Based on the Essential Oil of Citronella*

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ABSTRACT: Continuously solicited, the joints can be the source of joint pain such as arthritis, osteoarthritis, lumbartosis, rheumatism or polyarthritis. Linked to the passage of time or the repeated practice of a sport, these painful pathologies also cause difficulty in moving. Whether the pain is diffuse, recurrent or temporary, linked to an occasional or chronic pathology, it is possible to relieve it thanks to natural solutions. Used alone or in support of medical treatment, citronella essential oil is one of the essentials for joint pain. The objective of this work consists in the formulation of a recipe for the manufacture of a massage oil based on the essential oil of citronella. After research and consultation with the tutor at Aromabiol, a company specializing in vegetable and essential oils, and natural cosmetics, we chose the oils adapted to the body to prepare the product. For the materials used in the preparation of the product, citronella oil, sweet almond oil and argan oil. We harvested citronella with a company in the Wilaya of Biskra, where we collected the upper part of the plant. To extract the citronella oil from Java, the steam distillation method was used for 2 hours (from 10 am to 12 am), at 120°C. The use of essential oils requires strict and precautionary measures to limit the dangers. Therefore, it should be remembered that aromatherapy is by no means "alternative medicine", as its natural origin may indicate. Finally, further research is needed, including protocols for phytochemical analysis and evaluation of the bioactive potential of essential oils used in the manufacture of therapeutic products.

Keywords: Essential oil of citronella, Steam distillation, Aromabioil, Massage

Antimicrobial Activity of *Rubus ulmifolius* Leaves

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ABSTRACT: In the context of the discovery of natural extracts from plants used in traditional medicine, and with a view to using them as an alternative to synthetic antibiotics, we were interested in evaluating the antimicrobial activity of *Rubus ulmifolius* schott. (Wild Mulberry) leaves extracts, a medicinal plant belonging to the Rosaceae family. This plant species possesses biological properties due to its richness in secondary metabolites. We began by extracting the various families of predominant secondary metabolites (polyphenols, flavonoid fraction of ethyl acetate, flavonoid fraction n-butanol). We then tested their antimicrobial properties using the disk diffusion method. The results demonstrate the anti-hemolytic effect of the extracts compared with ascorbic acid at different concentrations, with the extract from our study plant having a strong activity where we found that the flavonoid extract (n-butanol) at a percentage equal to 95% for concentration 0.25 mg/ml very low concentration compared with 1.5 ascorbic acid. Leaves extracts were tested on five bacterial strains and one yeast according to the disk diffusion method, with inhibition diameters ranging from 8 to 17.25 mm. Then by micro-dilution on liquid medium to determine minimum inhibitory CMs concentrations. The results showed that the ethyl acetate phase was the most active against all the bacteria tested. The results show that *Rubus ulmifolius* extracts have a strong antimicrobial potential, and can be an alternative to drugs.

Keywords: *Rubus ulmifolius* Schott., Secondary metabolites, Antimicrobial activity

Effect of Potato by-Product on Growth Response and Digestive Function of Broilers during Different Feeding Periods*

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ABSTRACT: Potato and its by-products, due to having a high level of starch, can be partially replaced for corn in poultry rations. This experiment studied the effect of sliced potato by-product on broiler chickens' growth performance and nutrient digestibility. A total of 256 one-day-old Ross 308 broiler chicks were allocated into four dietary treatments with four replicates by employing a completely randomized design. The treatments included a control corn-based diet or 50% potato by-product replaced for corn and fed in throughout, grower and finisher or only finisher periods. The nutrients digestibility was determined at the end of the experiment by sample collection. Replacing 50% potato by-product reduced feed intake and weight gain in the starter period (1-10 d), and lowered weight gain and increased feed conversion ratio in the grower (11-24 d), finisher (25-42 d), and whole raising periods ($P<0.05$). Potato by-product also reduced the digestibility of organic matter and declined nitrogen retention in broilers ($P<0.05$). However, the treatments did not affect the digestibility of dry matter, crude ash, and crude fat. In conclusion, the consumption of 50% replaced sliced potato by-product instead of corn is not recommended in broilers' diets due to reduced growth performance and decreased nutrients digestibility.

Keywords: Potato by-product, Performance, Digestibility, Broiler chicken

*This study is a part of the Mir DaryoushShakouri's research project by University of Mohaghegh Ardsbili

Enhancing Tropane Alkaloid Production in *Hyoscyamus niger* L. Using LED Lighting and Glycine Treatment*

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ABSTRACT: LED lighting is promising in the field of agricultural lighting and secondary metabolites production. Features such as high-energy efficiency, long life, and flexibility in application make LEDs more suitable for the production of secondary metabolites in the future than traditional lighting systems. The present study aimed to investigate different LED light color and glycine treatments on tropane alkaloids production by *Hyoscyamus niger* L. The use of LED light treatments started from the transplant stage, in such a way that, the pots were transfered to light boxes and light treatments were applied at 4 levels (white (W), blue (B), red (R) LED, and normal conditions in the greenhouse without LED light as control (C). Glycine foliar spraying was applied in three stages with 10 days intervals (0, 40, and 80 mg.l^{-1}). 14 days after the last glycine foliar application, yield efficiency, tropane alkaloids, phenol, flavonoids were examined. Scopolamine and atropine assays with HPLC showed that LED color lights and glycine had a positive effect on the accumulation of these two important metabolites. The highest scopolamine (22.61 mg. g^{-1}) and atropine were observed in B LED light boxes under glycine 40 and 80 mg. L^{-1}.

Keywords: Amino acid, Atropine, Glycine, *Hyoscyamus niger* L., LED light, Scopolamine

*This study is a part of the Rasoul Heydarnajad Giglou's Ph.D. thesis accepted by University of Mohaghegh Ardabili

Quantification of Polyphenols and Flavonoids of *Matricaria chamomilla* L: Study of their Antioxidant Activity

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ABSTRACT: Phenolic compounds are common plant secondary metabolites which have not only physiological functions in plants but also positive effects for human health because they can act as antioxidants. Antioxidants play important roles in preventing pathogenic processes related to cancer, cardiovascular disease, macular degeneration, cataracts and asthma, and can enhance immune function. Antioxidant defenses protect the body from the detrimental effects of free radicals generated as by-products of normal metabolism. The objective of this study is the evaluation of the antioxidant activity of the aqueous and methanolic extract of *Matricaria chamomilla* L. Flower, root, and aerial part selected from Remeda Ain Hdjar, Boussaâda Djemila Setif regions of Algeria. The antioxidant activity of different extracts (aqueous and Methanol fractions) from *Matricaria chamomilla* L was evaluated by the DPPH scavenging test. The total phenolic and total flavonoid content of these extracts was determined according to the Folin-Ciocalteu procedure and Aluminum chloride colorimetric assay respectively. Total phenolic content was measured by Folin Ciocalteu reagent. All the extracts showed significant antioxidant activities and contained important levels of phenols. The methanol extract from *Matricaria chamomilla* L showed the greatest antioxidant capacity, which was probably due to its high content of polyphenols. Total flavonoid content was found equal for all extracts. Our results of antioxidant assays were justified and partially supported the popular usage of the tested plants. The high antioxidant activity found in the plant in this region suggested that *Matricaria chamomilla* L is a good source of natural compounds which might have benefits for health.

Keywords: *Matricaria chamomilla* L, Medicinal herb, Flavonoids contents, Polyphenols, Antioxidant activity

Food Quality, Micronutrition and Breast Cancer Risk in the N-West of Algeria -a Case Control Study

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ABSTRACT: Lifestyle and dietary factors play an important role in individual predisposition to the risk of cancer. The advantage is that they can be modified for primary, secondary or tertiary prevention of breast cancer (BC). The aim of this study was to describe the epidemiology of the BC and to investigate the relationship between food quality, lifestyle and BC risk in women in western Algeria. A case–control study of was directed with BC cases and healthy control subjects to determine the relationships between food frequency, micronutrients and serum fatty acids. The latter were analyzed by gas chromatography. Data were statistically treated by multivariate analysis. Our findings showed that the factors predicting BC in the cancer cases were food frequency, nutrients profile, late menopause, BMI >25, history of BC, low economic status, low level of education, sedentary lifestyle and physical activity. A significant increase in plasma concentrations of SFAs was noted in women with BC compared to controls, in contrast to PUFAs, which were found to be significantly reduced. This reduction was marked by a significant drop in the precursor of docosahexaenoïc acid: DHA (ALA, EPA) and eicosapentaenoïc acid: DPA. The multivariate analysis showed the significant interaction between the markers of the micronutrients, the age of the menopause, the lifestyle of BC cases. This study suggested that BC is associated with nutrients intake profile linked to low physical activity, the age of the menopause, the lifestyle, and the genetic predisposition are predictive factors of BC. A healthy, balanced diet plays a vital role in correcting this imbalance and reducing the associated risk.

Keywords: Lifestyle, Micronutrition, Plasma Fatty acids, DHA, EPA, Vitamins, Physical activity, Breast cancer

Insulin and Leptin Profile and Their Association with Nutritional Status in Breast Cancer Patients

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ABSTRACT: This paper aims to investigate the insulin and leptin profile and their association with nutritional status in cancer patients and to identify risk factors. The study sample included healthy subjects as controls and breast cancer (BC) patients who participated in a case –control study in the wilaya of Tlemcen. Dietary rations in control and cancer women are estimated using nutritional surveys based on the three-day food diary technique, including a weekend day, and supplemented by the 24-hour recall. The association of insulin and leptin levels with nutritional status was described and argued in cancer disease. Multivariate analysis with several predictors and Student test were used to compare the medians and find out risk factors. In terms of the breakdown of nutrient consumption by meal, total carbohydrates are expressed significantly in breakfast (38,30 ± 6,24 *vs* 51,47 ± 8,73g/day; p < 0,05) and snacks (18,95 ± 5,75 *vs* 30,08 ± 4,12g/day; p < 0,05). Daily consumption of simple carbohydrates was significantly higher in BC cases than in controls (141,04 ± 12,50 *vs* 94,10 ± 11,57g; p<0.05), in contrast, complex carbohydrate intake was significantly reduced (119,04 ± 13,05 *vs* 193,29 ± 18,15g; p<0.05). The proportions of the consumed fatty acids show a significant higher proportion of SFA consumed by BC patients compared with controls (51,98 ± 1,11 *vs* 47,23 ± 1,34 %; p < 0.05). In contrast, the percentages of MUFA were significantly lower (32,60 ± 1,34 *vs* 38,11 ± 1,55 %; p<0.05) in BC cases. The proportions of PUFA and the ratio of PUFA/SFA were relatively similar between the two groups (50,30 ± 0,04 *vs* 0,31 ± 0,03). Serum insulin (75.45 ± 4.39 *vs* 64.34 ± 5.72 pmol/L) and leptin levels (13.75 ± 1.79 *vs* 7.96 ± 1.33pmol/L) were significantly higher in cancer patients than in controls (p<0.05). The statistical analysis underlined that the predictor of BC was (a) low density lipoproteins – cholesterol (p=0,001), depending on late menopause (≥ 50years), overweight (p=0,001) and sedentary lifestyle (p=0,005), (b) triglycerides levels (p=0,001) depending on BMI (>25), (p=0,001) and sedentary lifestyle (p=0,001). This study confirms certain lifestyle and nutritional risk factors for BC, which are closely linked to the profile of digestive hormones, revealing hyperinsulinism and hyperleptinism. The latter act as initiating factors for tumour growth.

Keywords: Nutritional status, BMI, Breast Cancer, Insulin, Leptin, BC predictors

Evaluation of the Organoleptic and Physico-chemical parameters Quality of a Yoghurt with *Curcuma longa* L. Extract at The GIPLAIT TELL* Company in Mezloug South of Setif (Algeria)

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ABSTRACT: This study aims to the effect of enrichment with the extract of an aromatic plant of the genus *Curcuma longa* L. (*Zingiberaceae* family) at different concentrations respectively 0, 0.5, 1, 1.5 2, 2.5 and 3 ml after 10 days of maturation of a yogurt manufactured at GIPLAIT TELL Mezloug south of Setif (Algeria) and its effect on the physico chemical quality (pH, total dry extract and moisture) and organoleptic (color, taste, odour and adhesiveness) through a tasting panel of 25operators evaluated from a scale of 1 to 10 in order to elaborate a new type of yogurt brewed. Each parameter studied during the analysis was the subject of 5 repetitions of a capacity of 100ml. The results obtained show that for the selected physic-chemical parameters, the pH values measured decrease as extract concentration increases and the number of days after production is prolonged. While the values of the total dry extract are inversely proportional with the humidity according to the added concentration. At the concentration of 2ml of *Curcuma longa* L. extract which according to the tasting panel this new product had a fairly good organoleptic quality namely: acceptable acidic taste, fresh, had no aftertaste, a color and a fairly good adhesiveness.

Keywords: Extract, *Curcuma longa* L., Yoghurt, Physico-chemical and Organoleptic parameters, Functional product

Study of Plant Based Yoghurt Flavoring

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ABSTRACT: This work aims to know more about the effect of natural and synthetic and mixed aromas on yogurt, based on physico-chemical analysis (pH and titrable acidity) and organoleptic tests (taste, aftertaste, odor and texture). In this experiment, the used dose of the three types of aromas (lemon flavor (natural, artificial and mixed) was 0.1 ml. For physico-chemical analysis, there is an inversely proportional relationship between pH and yoghurt acidity. Fermentation of natural aroma yoghurt was slow, because this aroma had no effect on the activity of lactic acid bacteria. For organoleptic quality, the product is tested by a committee of 10 people. The results of this test showed that the mixed aroma yogurt was highly appreciated due to its good taste quality.

Keywords: Yoghurt, Aroma, Fermentation, Physico-chemical analysis, Organoleptic tests

Antibacterial activity of *Carthamus caeruleus* L. plant extracts

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ABSTRACT: This present work aims to evaluate the antibacterial activity of essentials oils, aqueous and methanolic extracts of *Carthamus caeruleus* L.. The extraction of essential oil was carried out by hydrodistilation, the extracts of this plant are obtained by decoction and maceration. The results of this method showed that the essential oil of the aerial part had a very strong antibacterial activity on *Staphylococcus aureus* ATCC 6538P, in contrast the aqueous extracts of the aerial part and roots have not showed any antibacterial activity, the bacterial strains tested were resistant. And the methanolic extracts of the aerial part and root have showed a weak antibacterial activity.

Keywords: *Carthamus caeruleus* L., Essential oil, Methanolic extracts, Aqueous extracts, Antibacterial activity

Changes in Bioactive Compounds and Antioxidant Activity Occurring during Olive Oil Heating

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ABSTRACT: This work was undertaken to evaluate the effect of heating on bioactive compounds contents and the antioxidant activity of olive oils derived from two local varieties. Methanol 80% was used to extracts preparation and spectrophotometric methods were performed to analysis of polyphenols, flavonoids, flavonols, orthodiphenols and pigments as well as their antioxidant potential. The quality of olive oil was also determined. The results indicate the accordance of the physicochemical parameters (acidity the peroxide index and the absorbance in the UV) with the standards established by COI (2015), for the category of extra virgin olive oils. The statistical analysis revealed that heating significantly affects the levels of phenolic compounds as well as the antioxidant potential. The variety 1 extracts which contain highest levels of total phenolic compounds, flavonoids and orthodiphenols exhibit the potent antioxidant power, this confirm the role of such substance in this activity. The results indicate that olive oil present a noted stability when heating at 180 ° C during the first forty minutes (45 min), then, the degradation of antioxidants progresses during the rest of the cooking period (from 45 to 120 min), until reaching its lowest concentration after two hours. Similar evolution is noted for antioxidant activity.

Keywords: Olive oil, Heating, Bioactive compounds, Antioxidant activity

Aloe Vera Gel Corrects the Lipid Profile and Modulates the Lipase Activities of Target Organs during Obesity

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ABSTRACT: Aloe barbadensis Miller is a species of aloe with multiple medicinal virtues due to its leaf, which contains a gel rich in active compounds that can be highly effective in preventing complications associated with obesity. The objective of our study is to determine the effects of consuming Aloe vera (Av) gel on the lipid profile, as well as the activity of lipases in the liver, muscle, adipose tissue, and intestines of obese rats. The experimental protocol consists of using different groups of control or obese male wistar rats following a cafeteria diet for a duration of 4 weeks. Subsequently, the gel is administered by gavage to the rats, at two concentrations, 100 and 200 mg/Kg/day during 4 weeks. The rats of the six groups are sacrificed, the organs are used for the measurement of lipid markers, namely cholesterol and triglycerides, as well as lipase activities. In obese rats, the increase in relative organ weight (adipose tissue, liver, muscle) and their lipid content coincides with the elevation of enzyme activities involved in lipid storage, such as LPL. Our results demonstrated a reduction in lipid accumulation in the target organs, which is consistent with the decrease in LPL activity and increase in HSL within adipose tissue in obese rats treated with the gel. AV gel corrects the lipid profile in target organs during obesity. Our study leads us to conclude that AV gel could be utilized as a natural anti-obesity treatment, with the potential to correct hyperlipidemia and modulate lipase activities.

Keywords: *Aloe vera* gel, Lipases, Lipid profile, Obesity, Rat

Anti-Parkinson Activity of *Thymus ciliatus* Essential Oil: Insights into Dopa Decarboxylase İnhibition

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ABSTRACT: Parkinson's disease, as the preeminent movement disorder, serves as the secondary most prevalent neurodegenerative ailment of the central nervous system, subsequent to Alzheimer's disease (AD). Its presentation takes the form of a triad of symptoms, which encompass bradykinesia, rigidity, and tremor while at rest. In the investigation concerning novel inhibitors of Dopa Decarboxylase, an established therapeutic target for the management of Parkinson's disease, we employed virtual screening method utilizing AutoDock Vina[3]. The results obtained from the molecular docking analysis demonstrated that, among the 19 compounds investigated, three of them exhibited a significant inhibitory effect on the activities of Dopa Decarboxylase when compared to the experimental inhibitor "Rasagiline". Based on the outcomes of the virtual screening procedure, it was observed that the top three compounds, namely p-cymene, carvacryl acetate, and γ-Terpinene, displayed the highest docking scores, which were calculated to be -6.3 kcal/mol, -6.1 kcal/mol, and -5.9 kcal/mol, respectively. Furthermore, the Analysis of the interactions between these compounds and Dopa Decarboxylase enables the identification of the specific functional groups implicated in the inhibitory activity of said interactions. Therefore, the identified natural compounds may be taken into further consideration for potential *in vitro* evaluation as DDC inhibitors, which would lead to the development of more potent and efficient anti-Parkinson drugs.

Keywords: Dopa decarboxylase, Parkinson's disease, Molecular docking, *Thymus ciliatus* essential oils

Antioxidant Activity of Ethyl Acetate Extract of *Arbutus Unedo* L.

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ABSTRACT: For the medicinal use, standardization of plant extracts is a necessity. This study aims to evaluate the *in-vitro* antioxidant effect of ethyl acetate extract prepared from leaves of an Algerian *Arbutus unedo*, and to estimate its phenolic and flavonoid content. Total phenolic and flavonoid content were estimated by Folin-Ciocalteau's reagent and Aliminium chloride colorimetric method, respectively. The antioxidant activity was evaluated by using ABTS, peroxidation of linoleic acid and phosphomolybdate (PPM) assays. The total polyphenol and flavonoid content of this extract was found to be 1054, 81 ± 95,02 µg/mg gallic acid equivalent and 53,99 ± 5,33 µg/mg quercetin equivalent, respectively. Results showed that the ethyl acetate extract exhibited a strong free radical scavenging activity against the free radical ABTS with IC_{50} = 1.59 µg/ml which is better than that obtained with BHT (IC_{50} = 6.10 µg/ml) used as standard antioxidant. Moreover, the extract inhibited strongly the oxidation of linoleic acid with percentage of 89%. In addition, the extract showed a good reducing activity in phosphomolybdate test with a total antioxidant capacity (TAC) of 986.66 ± 35.87 µg equivalent ascorbic acid/mg extract. These findings showed that the ethyl acetate extract of *Arbutus unedo* leaves possesses strong antioxidant potential, which may be attributed to the presence of high amount of polyphenolic phyto-constituents. So, this plant might be exploited for pharmaceutical and food applications as a potential source of effective antioxidant compounds to prevent the oxidative stress related diseases.

Keywords: Oxidative stress, Antioxidant effect, *Arbutus unedo* L., Phenolic content

Anti-Inflammatory Activity of Ethyl Acetate and Butanolic Extracts of *Arbutus unedo* L.

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ABSTRACT: The growing concern for the side effects and toxicity experienced with the use of currently available anti-inflammatory drugs makes it necessary to search for novel compounds. Medicinal plants provide a ready source of anti-inflammatory agents with minimal side effects. The present study is devoted to evaluate the anti-inflammatory activity of the ethyl acetate and butanolic extracts from the leaves of *Arbutus unedo* L. using croton oil-induced ear edema in mice and human red blood cell membrane stabilization method. Results showed that the topical application of 2 mg/ear of ethyl acetate or butanolic extract reduced the ear edema induced by croton oil with an inhibition of 74% and 66%, respectively. This value is better than those obtained with indomethacin, used as reference. On the other hand, the ethyl acetate and butanolic extracts inhibited the erythrocytes hemolysis by 96% and 97% respectively against 98% exerted by diclofenac. Our finding may indicate the possibility of using the extracts of *Arbutus unedo* L. to prevent the inflammatory processes.

Keywords: Inflammation, Anti-inflammatory activity, *Arbutus unedo* L., Edema, Medicinal plants

Anti-inflammatory Potential of *Malva sylvestris* Flowers Acetonic Extract

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ABSTRACT: *Malva sylvestris* is widely used in traditional medicine throughout the world. The objective of this study is to determine the amount of phenolic compounds and to evaluate the the anti-inflammatory properties of acetonic extract (AcE) of *Malva sylvestris* flowers. Phytochemical analysis revealed that the extract is rich in polyphenols (102.39 ± 7.06 mg E AG/g extract), flavonoids (0.99 ± 0.61 mg EQ/g extract) and in tannins (194.93 ± 4.37 mg EC/g of extract). *In vivo,* two models of inflammation (ear edema and air pouch) were conducted. Results showed that the topical treatment of mice with 2 mg/ear of AcE inhibited croton oil-induced inflammation (ear edema) by 85%. On the other hand, the treatment of mice with1mg/pouch of AcE significantly reduced the migration of the number of leukocytes towards the site of inflammation, with 678%. These effects are comparable to those of indomethacin, used as anti-inflammatory reference. *In vitro* AcE prevented the lysis of erythrocyte membranes induced by hypotonic solutions with an inhibition rate of 57%. In conclusion, the anti-inflammatory activity of the acetone extract of *Malva sylvestris* shows that this plant has good pharmacological power, which supports the traditional use to treat certain disorders related to inflammation.

Keywords: Air pouch, Anti-inflammatory, Edema, Inflammation, *Malva sylvestris,* Plant extract

Activity of *Mentha rotundifolia* Aqueous Extract on Carrageenan-Induced Rat Paw Edema

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ABSTRACT: *Mentha rotundifolia* is one of Lamiaceae species which is largely used in traditional medicine in Algeria and Mediterranean bassin. The aqueous extract obtained by ebullition from *Mentha rotundifolia* was assessed for anti-inflammatory activity by carrageenan-induced rat paw edema model. Albino rats were divided into five groups of six each; Group 1 was kept as control and received distilled water only. Groups 2, 3 and 4 received the extract at different concentrations (100, 250, 500 mg/kg respectively). Group 5 received diclofenac oraly (5 mg/kg). One hour after oral administration of the extract, 0.2 ml of λ-carrageenan (1%) was injected into the left hind paw of each rat. The results reveal that, the groups given the different doses of extracts as a preventive measure significantly reduced λ-carraginan-induced edema from the first hour. Inhibition was dose-dependent, with maximum percentages obtained six hours after λ-carraginan injection. In conclusion, *M. rotundifolia* could be exploited as a potential source for the treatment of certain inflammatory disorders.

Keywords: Inflammation, Albino rats, *Mentha rotundifolia,* λ-carraginan, Paw edema

In Vitro Cytotoxicity Test of *Mentha Rotundifolia* Acetonic Extract in Caco-2 Cells

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ABSTRACT: *Mentha rotundifolia* is a medicinal plant belonging to the Lamiaceae family. It is widely used in Algerian traditional medicine for the treatment of different illness such as: Gastrointestinal system disease, Hemorrhoids, Rheumatism, Gynecologic disease, Headache and inflammatory disease. The objectif of this study was to investigate its cytotoxicity on human cells. The acetonic extract was prepared by Soxhlet apparatus and the cytotoxic activity of extract in human epithelial Caco-2 cells was performed using the 3-(4, 5- dimethylthiazol- 2- yl) - 2, 5- diphenyl- tetrazolium bromide (MTT) assay. The results demonstrate that the incubation of human epithelial Caco-2 cell lines with different concentrations of the extract for 24 hours did not affect cell viability. In fact, the findings demonstrated that *Mentha rotundifolia* acetonic extract has non-toxic effect.

Keywords: *Mentha rotundifolia*, Cytotoxicity, Caco-2 cells, MTT assay

Antioxidant Activity and Phytochemical Screening of Hydromethanolic Extract of *Genista saharae* (Coss. & Dur.)

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ABSTRACT: Preventing oxidative stress through the use of effective antioxidant agents has become an urgent priority. The Sahara Desert is believed to harbor plants that contain valuable biomolecules with antioxidant properties. Genista saharae, a leafless spontaneous fabaceae endemic to Algeria and locally referred to as "Tellegit," is renowned for its chemical composition and antioxidant properties, making it a significant component of traditional pharmacopoeia. This study aimed to evaluate the antioxidant activity and phytochemical composition of the hydromethanolic extract of Genista Saharae (HMEG) under conditions that align with its traditional use. Precipitation and coloration reactions were employed to perform a phytochemical screening, assessing the qualitative chemical composition of HMEG. Additionally, spectrophotometric methods were used to determine the levels of total phenolics, flavonoids, tannins, and β-carotene in the extract. The antioxidant potential of HMEG was evaluated using three methods: the DPPH assay, reducing power determination, and β-carotene bleaching test. The findings confirmed the presence of various biomolecules, including phenolic compounds, flavonoids, alkaloids, tannins, terpenoids, glycosides, steroids, and saponins, in HMEG. Moreover, quantitative analysis revealed significant concentrations of total phenolics, tannins, and β-carotene. The results indicated that HMEG exhibited robust antioxidant activity, particularly in terms of β-carotene bleaching, thereby substantiating its traditional use by healers. In conclusion, Genista Saharae serves as a valuable source of antioxidants, with its potent antioxidant activity likely attributed to the presence of tannins or other phenolic compounds, such as terpenoids, β-carotene, and saponins.

Keywords: Antioxidant activity, Phytochemical screening, Hydromethanolic extract, *Genista saharae*, DPPH test, Reducing power, β-carotene, Tannins

Calretinin to Diferentiate Malignant Pleural Mesothelioma from Lung Adenocarcinoma

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ABSTRACT: Calretinin is a calcium-binding protein originally found in neurons. It is also overexpressed in most types of malignant mesothelioma. Pathologists use calretinin as a selective marker to diagnose mesothelioma, and researchers are testing the protein as a target for cancer therapy. The aim of this study was to confirm the use of Calretinin in the differential diagnosis of epithelioid MPM *versus* lung ADC on human biopsies. 41 human biopsies (13 epithelioid MPM and 28 pulmonary ADC were used in this study by performing histology and immunohistochemistry. Our findings reveal a total lack of expression of Calretinin in 28 cases of lung ADC (100%) and a strong expression of this protein in 10 cases (77%) of MPM. The remaining three cases (23%) of MPM were found to be negative for calretinin after immunostaining with anti-calretinin Antibody. Confirming the results of previous studies. Calretinin appears to be very useful in discriminating between the lung ADC and epithelioid MPM because its expression formally excluded a diagnosis of lung ADC.

Keywords: Calretinin, Adenocarcinoma, Malignant pleural mesothelioma (MPM), Differential diagnosis

Changes in Bioactive Compounds and Antioxidant Activity Occurring during Olive Oil Heating

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ABSTRACT: This work was undertaken to evaluate the effect of heating on bioactive compounds contents and the antioxidant activity of olive oils derived from two local varieties. Methanol 80% was used to extracts preparation and spectrophotometric methods were performed to analysis of polyphenols, flavonoids, flavonols, orthodiphenols and pigments as well as their antioxidant potential. The quality of olive oil was also determined. The results indicate the accordance of the physicochemical parameters (acidity the peroxide index and the absorbance in the UV) with the standards established by COI (2015), for the category of extra virgin olive oils. The statistical analysis revealed that heating significantly affects the levels of phenolic compounds as well as the antioxidant potential. The variety 1 extracts which contain highest levels of total phenolic compounds, flavonoids and orthodiphenols exhibit the potent antioxidant power, this confirm the role of such substance in this activity. The results indicate that olive oil present a noted stability when heating at 180 ° C during the first forty minutes (45 min), then, the degradation of antioxidants progresses during the rest of the cooking period (from 45 to 120 min), until reaching its lowest concentration after two hours. Similar evolution is noted for antioxidant activity.

Keywords: Olive oil, Heating, Bioactive compounds, Antioxidant activity

Incorporation of Xanthan and LBG into a Gluten-Free İnfant Biscuit Formula Made from Rice-Dry Pea Flour

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ABSTRACT: The replacement of gluten presents a major technological challenge, as it is an essential structure-building protein in flour, which is responsible for the viscoelastic characteristics of dough, and contributes to the appearance and structure of many cereal-based baked products. In this study, a composite flour of rice-dry pea was used to develop a nutritionally enriched infant gluten-free biscuit. The effect of incorporated xanthan and locust bean gum (LBG) (1 g each per 100 g of composite flour) was studied by evaluating the rheological characteristics of the dough and final quality of the baked biscuit compared with control wheat flour (WF). Results showed a visible improvement in the rheological behavior of the gluten-free dough, particularly during kneading and shaping. The viscosity curves revealed a significant increase in the viscosity of the gluten-free dough after addition of gums, of which that with LBG had the highest viscosity. A significant improvement in the specific volume (Vsp) of the biscuit is found for the formula incorporated with xanthan, but which remains lower than that of the WF. The biscuit added with LBG gave the lowest Vsp. The xanthan-based biscuit showed the highest final moisture, while the one with added LBG showed the lowest.

Keywords: Gluten-free, Infant biscuit, Rice, Dry pea, Xanthan, LBG

Allicin, Phytochemicals and Pyruvic Acid Contents in Processed Garlic

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ABSTRACT: This study aimed to evaluate the stability of allicin in fresh whole peeled garlic, fresh sliced garlic, fresh crushed garlic, and dried garlic slices under different storage conditions of time and temperature (from zero time to two days at 4 ºC and 20 ºC). In addition, to investigate the chemical composition, phytochemical content, and sensory properties, Allicin content in fresh garlic (sliced and crushed) ranged from 0.6 to 32.14 mg/g, and in dried treatments, it ranged from 3.77 to 6.68 mg/g. Stability exhibited some varieties between the different forms of garlic. The results revealed that allicin in fresh garlic (sliced and crushed) reaches its maximum rate after a 10-minute cutting process at 20 ºC. Pyruvic acid in fresh garlic (sliced and crushed) reaches its maximum rate after a 10-minute cutting process at 4 ºC. The results show that the highest values of total phenol content and antioxidant activity were achieved when cutting garlic tissue at zero time. The dried garlic slices have less content of allicin, pyruvic acid, total phenol, and antioxidant activity than fresh garlic due to the thermal process. The results of consumer testing on a hedonic scale showed a higher value of overall acceptability found in hummus with fresh crushed garlic (10 min, 20 ºC). While the results of consumer testing are just about right scale, the higher values of flavour, aroma, and pungency are found in hummus with dried garlic slices at 20 minutes 4 C, and the lowest values are found in fresh crushed garlic at 20 minutes 4 ºC. In conclusion, pyruvic acid and allicin have more content in fresh crushed garlic than fresh sliced garlic, and total phenol and antioxidants have more content in fresh sliced garlic than fresh crushed garlic.

Keywords: Allicin, Stability, Garlic, Temperature

Characterization, Quantification, and Activities of Phenolic Compounds in *Phagnalon saxatile*

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ABSTRACT: *Phagnalon saxatile* subsp. *saxatile* is a wild species widespread in Algeria which is utilized for medicinal purposes as analgesic and anticholesterolemic. However, information is still scarce regarding its phytochemical content. The objective of this study was to identify and quantify the phenolic compounds from different extracts of its leafy stems. For this purpose, the effects of four extracting solvents were investigated on the content of phenolic compounds and the antioxidant activity of this plant. The extracts prepared with polar solvents (methanol and water) contained higher amounts of phenolic compounds and showed better antioxidant activity than the extracts with apolar solvents (hexane, dichloromethane). The methanolic extract, richest in total phenolic and total flavonoid, had significant antioxidant activity as regarded by DPPH scavenging capacity (IC50 of 5.5 g/mL), ABTS+ scavenging capacity (IC50 of 63.8 g/mL) and inhibition of oxidation of linoleic acid (IC50 of 22.7 g/mL), when compared to synthetic antioxidants. Chlorogenic acids and several flavonoids were identified and quantified by UPLC-DAD-MSn. The di-O-caffeoylquinic acids isomers were the most concentrated phenolics (25.4 mg/g DW) in the methanolic extract.

Keywords: *Phagnalon saxatile* subsp. *saxatile*, Phenolic compounds, Polarity, Antioxidant activities, UPLC-DAD-ESI-MSn, Chlorogenic acids

Evaluation of Biochemical Composition and Antioxidant Properties of Date Palm (*Phoenix dactylifera* L.) Kernel Oil from Algeria

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ABSTRACT: Date production is increasing every year. In addition, pitted date exportation is rising and great amounts of date seeds are produced. This biomass represents a problem for manufacturing companies. Now, date seeds are normally discarded or used as animal feed ingredients. However, this co-product can be used for many other applications due to its valuable chemical composition. Oil is one of the most interesting components of the date seed. This study focuses on the analysis of oil extracted from date pits of the *Takerboucht* variety, from the Adrar region. The main objective of this research is to determine the content of phenolic compounds in this oil, including total polyphenols, flavonoids and condensed tannins. Additionally, we aim to assess the antioxidant capacity of this oil using (DPPH) test. The oil was extracted using the Soxhlet method by hexane as a solvent. The yield obtained was 9%. The phenolic compounds were extracted by a liquid-liquid extraction method, and their content was determined by specific colorimetric assays. Total polyphenols were quantified using the Folin-Ciocalteu method, condensed tannins with the vanillin reaction, and flavonoids were evaluated using aluminum trichloride. The total polyphenol content of date stone oil is 0.73 mg/ml, condensed tannins amount to 0.47 mg/ml, and flavonoids are present at a concentration of 0.53 mg/ml. The IC50 results of the DPPH test indicate an antioxidant activity of 0.09 mg/ml. This study demonstrated that the oil from date pits of the Takerboucht variety is rich in phenolic compounds. These compounds give the oil a strong antioxidant capacity. Our study demonstrates that since date seeds produce an oil rich in bioactive compounds, which are excellent ingredients for the nutraceutical, pharmaceutical, and cosmetic industries, it should not be treated as "simple waste" but as a raw material. The production of seed oil should be considered a new economic resource that could help in the disposal of by-products derived from date processing.

Keywords: Date, Kernels, Phenolic compounds, DPPH, Antioxydant

Proposals for Food Preparation Based on date for Children in Algeria: Description and Fabrication Diagrams

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ABSTRACT: Algeria is the 3rd best producer of dates in the Arab world and 4th in the world with a production of around 1.2 million tonnes in 2021, according to the latest ranking established by the United Nations Food Organization and agriculture (FAO).The objective of this work consists, through a descriptive survey, carried out in Algeria where the know-how in the field of date processing remains anchored among the populations, particularly urban populations, to inventory and describe the food preparations (artisanal and domestic: Rob, flour date powder, R'ouina, date vinegar, date paste, "Honey" date exudate, "Coffee" roasted date stone flour, date flour and cereals, Makroudh, B'radj, R'fiss, . ..) based on dates, as well as to describe their methods of preparation, in order to analyze their production processes and to establish their manufacturing diagrams. The products derived from dates on the national market remain weak quantitatively in view of the importance of production. Date paste and more recently rob, remain the most present products on the national market, as processed date-based products; On the other hand, there is traditional knowledge and know-how in the transformation of dates into various food products (vinegar, jam, juice, rob, flour, etc.), in all this has led us to propose improvements to the production process. of date-based products for children, our work consists in inventorying and describing the different knowledge and know-how in Algeria, with a view to proposing valorization actions. To achieve this, the following points will be studied:

1. Inventory of food preparations (artisanal and domestic) based on dates;
2. Description of food preparations (artisanal and domestic) based on dates;
3. Description of the method of manufacture of these date-based food preparations;
4. Proposals for technical diagrams of semi-industrial production and possible improvements.

Keywords: Survey, Dates, Food, Children, Inventory, Description, Process, Improvement

Evaluation of Antibacterial Potential of the Mucilage of *Arbutus unedo* Fruits

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ABSTRACT: Mucilages are carbohydrate polymers that are found in most plants, such as *Arbutus unedo*, a medicinal plant of the traditional pharmacopoeia of Algeria, belongs to the Ericaceae family. Also, it is used in traditional medicine to treat several diseases such as urinary infections. The aim of this study was to evaluate the antibacterial activity of the mucilage obtained from the fruits of *Arbutus unedo*. After harvesting, the fruits were decocted to obtain mucilage extracts. The antibacterial activity was tested by the diffusion method (disc method) against seven reference bacterial strains: *Esherichia coli* ATCC 8739, *Pseudomonas aeroginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 10876, *Salmonella typhimurium* ATCC 13311, *Enterococcus faecalis* ATCC 49452 and *Klabsiella pneumoniae* ATCC 700603. Phytochemical analysis shows that the mucilage of *Arbutus unedo* contains proteins, sugars and amino acids. The results of the antibacterial tests indicate that mucilage showed antibacterial activity against *Staphylococcus aureus, Enterococcus faecalis* and *Salmonella typhimurium*. The best activity was recorded against *Salmonella typhimurium*, with a zone of inhibition of 14 mm. On the basis of the results obtained, we conclude that the mucilages of *Arbutus unedo* fruits have very significant antibacterial activity.

Keywords: *Arbutus unedo*, Antibacterial activity, Mucilage

Aloe vera Gel Corrects the Lipid Profile and Modulates the Lipase Activities of Target Organs During Obesity

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ABSTRACT: *Aloe barbadensis* Miller is a species of aloe with multiple medicinal virtues due to its leaf, which contains a gel rich in active compounds that can be highly effective in preventing complications associated with obesity. The objective of our study is to determine the effects of consuming *Aloe vera* (Av) gel on the lipid profile, as well as the activity of lipases in the liver, muscle, adipose tissue, and intestines of obese rats. The experimental protocol consists of using different groups of control or obese male wistar rats following a cafeteria diet for a duration of 4 weeks. Subsequently, the gel is administered by gavage to the rats, at two concentrations, 100 and 200 mg/Kg/day during 4 weeks. The rats of the six groups are sacrificed, the organs are used for the measurement of lipid markers, namely cholesterol and triglycerides, as well as lipase activities. In obese rats, the increase in relative organ weight (adipose tissue, liver, muscle) and their lipid content coincides with the elevation of enzyme activities involved in lipid storage, such as LPL. Our results demonstrated a reduction in lipid accumulation in the target organs, which is consistent with the decrease in LPL activity and increase in HSL within adipose tissue in obese rats treated with the gel. AV gel corrects the lipid profile in target organs during obesity. Our study leads us to conclude that AV gel could be utilized as a natural anti-obesity treatment, with the potential to correct hyperlipidemia and modulate lipase activities.

Keywords: *Aloe vera* gel, Lipases, Lipid profile, Obesity, Rat

The Chemical Composition and Antifungal Activity of The Essential Oil of *Crithmum maritimum* from Eastern Algeria

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ABSTRACT: Essential oils have found their place in aromatherapy, pharmacy, perfumery, cosmetics, and in food preservation, owing to their widely recognized spectrum of biological activities. In this study, the essential oil was extracted via steam distillation from the aerial parts of *Crithmum maritimum* from the Eastern Algeria (W. Annaba-Algeria) with a yield of 2.11%. Gas Chromatography/Mass Spectrometry (GC/MS) analysis revealed sabinene (26.90%), limonene (24.2%), γ-terpinene (19.3%), and terpinen-4-ol (9.0%) as the major constituents. The antifungal activity was tested against Candida albicans, and the determination of the Minimum Inhibitory Concentration (MIC) was conducted using the Sabouraud broth microdilution method followed by spectrophotometric reading. The essential oil of *Crithmum maritimum* demonstrated antifungal efficacy against Candida albicans, with an MIC 80% value of 18.19 µl/ml.

Keywords: Essential oil, *Crithmum maritimum*, GC/MS, Antifungal activity, MIC

Germination Characteristics of Argan Seeds

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ABSTRACT: The argan tree is an Algerian-Moroccan endemic species, which belongs to the *Sapotaceae* family. In Algeria, the tree covers the southeast margins of the distribution. This taxon is a source of socio-economic and ecological importance. However, the argan forest is facing degradation and natural regeneration is mainly absent. The study's aim is to improve knowledge of regeneration ecology by researching optimal germination conditions in order to ensure reforestation establishment by planting. To evaluate the morpho-physiological variability in response to improving different factors (harvest period, longevity, envelope, mother tree and biometrics of the seeds), we collected Argan fruits from Oued Elma (Tindouf) in June, July and August for four consecutive years. A quantitative evaluation of the amylase activity evolution of germinating seeds has been done. On average, walnuts showed the following dimensions: 1.96 cm long, 1.37 cm wide, 0.71 circularity index, 2.58 g weight, and two loculi. There was a significant correlation between weight and width and circularity index (p=0.01). The harvest period has a significant effect on germination (P <0.01). The late-season seeds have a low germination potential, from which 79% was recorded for the June harvest, and 67% for the month of August. there was a decline in germination as storage durations increase, removal of shell improved germination parameters, with a shorter latency period (2-6 days) and an 80% germination rate for seeds harvested in June. These showed a rapid increase in amylase activity with peaked at 3rd week. In addition, the effect of the mother tree is not significant, which suggests that the Tindouf argan tree has very similar characteristics in terms of germination. The results obtained in this study provide a seed selection basis for reforestation programs.

Keywords: Argania spinosa, Biometry, Amylase activity, Germination, Harvest date, Longevity

Spatio-temporal Evolution of Total Coliforms in the Waters of Boussellam Valley, Sétif, North-east Algeria

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ABSTRACT: Water pollution is a global phenomenon that threatens life on earth and the daily life of individuals, the sources of pollution can be divided into three main axes: Domestic, Industrial and Agricultural, the Boussellam valley is considered as the main tributary of the Boussellam valley upstream, which crosses the town of Sétif, the largest population center in the region, and which empties into the Ain Zada am. This dam supplies the towns of Sétif, El Eulma, Ain Arnat and Bougaa, disposes of all types of pollution that threaten the lives of the inhabitants of these agglomerations. For this reason, we have monitored the evolution of the water contamination of the valley by bacteria Total Coliforms over a period of 3 years 2021,2022, and 2023 to know the rate of water pollution by this bacterium and its annual evolution. Where we carried out water sampling from 5 stations along the valley from the source area to the mouth of Boussellam valley in the Ain Zada dam. The results obtained show the high rate of this bacterium in the water of the valley, this increase is probably due to wastewater discharges and the intensive use of fertilizers, septic tanks, leaching of animal manure, runoff water and raising domestic animals or wild animals. This increases the risk of the vulnerability of the waters of the plain to pollution and the degradation of surface waters and the environment in general.

Keywords: Water, Pollution, Total coliforms, Boussellam valley, Sétif

FULL TEXT

ORAL PRESENTATIONS

Determination of the Total Phenolic and Flavonoid Contents of Lyophilized Aqueous Extracts of Ribwort Plantain (*Plantago lanceolata* L.)*

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ABSTRACT: Ribwort plantain (*Plantago lanceolata* L.) belongs to *Plantago* genus and Plantaginaceae family. Most of the *Plantago* species are used in traditional medicine. Infusion is one of the consumption methods of *Plantago lanceolata*. The aim of this study was to investigate the total phenolic and flavonoid contents of *Plantago lanceolata* leaves in different concentrations. For this purpose, infusions of *Plantago lanceolata* (PL) leaves, purchased from a local market, for different concentrations (2.5% (PL-2.5), 5% (PL-5), 10% (PL10) and 20% (PL-20), w/v) were prepared. Leaves were ground into small pieces and 100mL of boiling water was added onto leaves. After 10 minutes incubation, mixture was filtrated and water was removed using a rotavapor. Lyophilized extracts were prepared using a freeze dryer. Extract yield, total phenolic and flavonoid contents were determined. The highest extract yield was in PL-2.5 (23.6%). On the other hand, the lowest extract yield was in PL-20 (9.85%). The PL-2.5 (63.3 mg GAE/g extract) had the significantly more phenolic content than PL-5 (59.7 mg), PL-10 (50.2 mg) and PL-20 (45 mg). Similarly, PL-2.5 (10.36 mg QE/g extract) had the highest total flavonoid content. In addition, PL-2.5 had the significantly higher flavonoid content than PL-5 and PL-20. In conclusion, lyophilized extract obtained from the lowest concentration of PL showed the best results.

Keywords: *Plantago lanceolata*, Phenolic content, Flavonoid content, Extraction yield

INTRODUCTION

Medicinal and aromatic plants have been used in many areas such as food, cosmetics, paint, textile, medicine and agriculture (Hakverdi and Yiğit, 2017). There is a change in therapeutic use medicinal plants depending on the development level of the countries. 80% of people live in developing countries are treated with herbal products. While this rate is up to 95% in some countries in the Asia, Middle East, and Africa. This rate is less in developed countries.

Turkey having a rich flora contains lots of plant species. There are approximately 11000 plant taxa, and about five hundred of them are used for alternative treatment of diseases (Türkan et al., 2006). These plants, which can be used fresh, can also be used dried. All organs of the plant, such as its trunks (stems), leaves, flowers, seeds, tubers and barks, are used for different purposes and with different methods. Medicinal teas are aqueous preparations having therapeutic and protective properties (Aytaç and Yiğen, 2016). These types of herbal teas with functional properties are used to alleviate problems such as the common cold, psychosomatic illnesses, gastrointestinal diseases, congestion, urinary system diseases, constipation, diarrhea, local use as mouthwash or gargle, physical and menstrual complaints, and mental fatigue (Toker et al., 2015). Infusion, decoction and maceration are the most widely preparation methods (Ghenabzia et al., 2023). *Infusion:* The finely ground plant parts are placed in a closed container after hot water is poured over them. The mixture is left to steep over low heat for 5 minutes with frequent stirring. The cooled infusion is then filtered (Fotsing et al., 2021). *Decoction (Boiling Method):* Finely ground plant parts (bark, root, fruit) are covered with a sufficient amount of cold water. The mixture is then stirred frequently, kept on low heat for 30 minutes, and filtered while still hot (Fotsing et al., 2021). *Maceration (Room Temperature Preparation Method):* The plant is prepared by soaking in an organic solvent or cold water. After a few days of soaking, the plant is then prepared by filtering (Bitwell et al., 2023).

Numerous studies have found that these teas generally have high antioxidant activities (Kılıç et al., 2017). It is indicated that the preparation method, plant composition, storage conditions, and process are connected to the antioxidant content of these teas (Piljac-Žegarac et al., 2013). However, the characteristics and quantities of the used medicinal teas and process time may vary depending on the type of plants used. Additionally, consulting with an expert in herbal use or a healthcare professional is important, especially if you have any health issues or if you are pregnant. Ribwort plantain (*Plantago lanceolata* L.) belongs to *Plantago* genus and Plantaginaceae family (Baytop, 2018). Most of the *Plantago* species are used in traditional medicine (Gonçalves and Romano, 2016). Infusion is one of the consumption methods of *Plantago lanceolata* (Abate et al., 2022). The beneficial effects of *Plantago lanceolata* have been reported for cancer, wound healing, respiratory system, inflammation, reproductive system and blood circulation (Gonçalves and Romano, 2016; Abate et al., 2022). In addition, *Plantago lanceolata* has antioxidant, antibacterial and anti-inflammatory effects (Gonçalves and Romano, 2016; Abate et al., 2022). *Plantago lanceolata* is a rich source for verbascoside, chlorogenic acid, rosmarinic acid, hesperidin and hyperoside (Bahadori et al., 2020).

The aim of this study was to investigate extract yield, the total phenolic content (TPC) and flavonoid content (TFC) of *Plantago lanceolata* leaves in different concentrations.

MATERIAL AND METHOD

Commercially available ribwort plantain (*Plantago lanceolata* L.) leaves were used in this study (Figure 1).

Figure 1. Ribwort plantain. **A.** Leaves **B.** Blended leaves.

Infusion Preparation: Different concentrations of for different concentrations (weight/volume) 2.5% (PL-2.5), 5% (PL-5), 10% (PL-10) and 20% (PL-20) were prepared. Leaves were ground into small pieces and 100 mL of boiling water was added onto leaves. After 10 minutes incubation, mixture was filtrated and water was removed using a rotavapor. Lyophilized extracts were prepared using a freeze dryer.

Determination of Extract Yield: Yield (%) = (Weight of lyophilized extract/Weight of dried plant sample) X 100 (Orak et al., 2021)

Determination of Total Phenolic Content: Total phenolic content (TPC) was determined according to Singleton and Rossi (1965). Gallic acid was used to calculate a calibration curve. Results were expressed as mg gallic acid equivalent (GAE)/g extract.

Determination of Total Flavonoid Content: Total flavonoid content (TFC) was determined according to Woisky and Salatino (1998). Quercetin was used to calculate a calibration curve. Results were expressed as mg quercetin equivalent (QE)/g extract.

Statistical Analysis: Data was presented as average±standard error of mean (SEM). GraphPad Prism 8 package program one-way anova was used to compare groups. All experiments were six replicates. *P* value was accepted as lower than 0.05.

RESULTS AND DISCUSSION

Extract yield

The highest extract yield was in PL-2.5 (23.6%). On the other hand, the lowest extract yield was in PL-20 (9.85%) (Table 1). The extract yield decreased according to increasing PL concentrations.

Table 1. Yield extract

Groups	Obtained Lyophilized Extract (g)	Yield (%)
PL-2.5	0.59	23,6
PL-5	1.03	20,6
PL-10	1.62	16.2
PL-20	1,97	9,85

Slope of the calibration curve was found as y=0.6112x+0.0073. The PL-2.5 (63.3 mg GAE/g extract) had the significantly more phenolic content than PL-5 (59.7 mg) ($p<0.05$), PL-10 (50.2 mg) ($p<0.0001$) and PL-20 (45 mg) ($p<0.0001$). The lowest TPC was determined in PL-20. PL-5 vs. PL-10, $p<0.0001$. PL-5 vs. PL-20, $p<0.0001$. PL-10 vs PL-20, $p<0.0001$. (Figure 2)

Slope of the calibration curve was found as y=3.4789x-0.0061. Similarly, PL-2.5 (10.36 mg QE/g extract) had the highest total flavonoid content. In addition, PL-2.5 had the significantly higher flavonoid content than PL-5 ($p<0.05$) and PL-20 ($p<0.01$). The lowest TPC was determined in PL-20. (Figure 2)

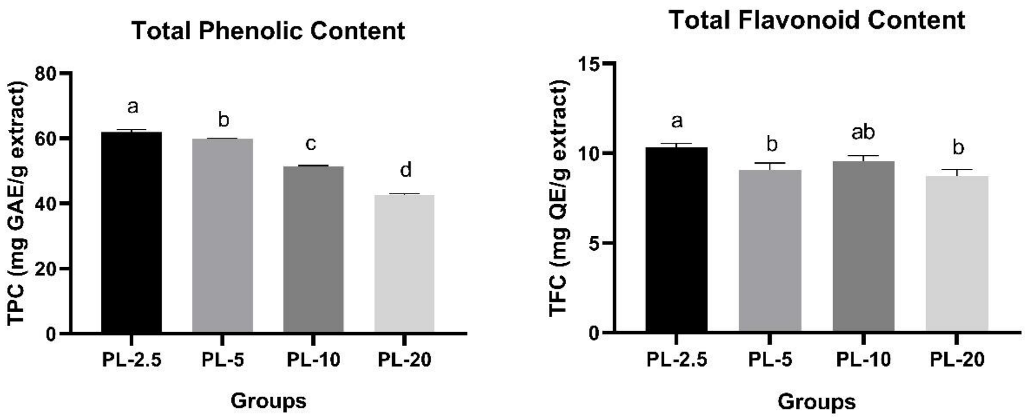

Figure 2. Total phenolic and flavonoid contents

CONCLUSION

Lyophilized fine powder infusion extracts of PL were obtained. Yield extract, TPC and TFC were determined. In conclusion, lyophilized extract obtained from the lowest concentration (PL-2.5) of PL showed the best results.

Statement of Conflict of Interest

The author(s) should declare that they are no conflict of interest.

Authors' Contributions

AB and RA designed and analyzed the research, AB, RA and AD studies arranged. RA worked on the preparation of figures and tables. All authors contributed to the writing of the article and took part in the process of publication of the article and read and approved it.

REFERENCES

Abate, L., Bachheti, R. K., Tadesse, M. G., Bachheti, A., 2022. Ethnobotanical Uses, Chemical Constituents, and Application of *Plantago lanceolata* L. Journal of Chemistry, 2022: 1532031.

Acıbuca, V., Budak, D.B., 2018. Dünya'da ve Türkiye'de Tıbbi ve Aromatik Bitkilerin Yeri ve Önemi, Çukurova Tarım ve Gıda Bilimleri Dergisi., vol. 33, no. 1, p. 37-44, 2018.

Aytaç, S., Yiğen, Ç., 2016. Tıbbi ve Aromatik Bitkilerin Önemli Kullanım Alanları, III. Tıbbi ve Aromatik Bitkiler Sempozyumu; 4-6 Ekim 2016; Antalya, pp.183.

Bahadori, M. B., Sarikurkcu, C., Kocak, M. S., Calapoglu, M., Uren, M. C., Ceylan, O., 2020. *Plantago lanceolata* as a source of health-beneficial phytochemicals: Phenolics profile and antioxidant capacity. Food Bioscience, 34, 100536.

Baytop, T., 1999. Türkiyede bitkiler ile tedavi. İstanbul Eczacılık Fakültesi Yayınları, İstanbul, 444.

Bitwell, C., Sen, I. S., Luke, C., Kakoma, M. K., 2023. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. Scientific African, e01585.

Fotsing, F., Stéphane, Y., Kezetas, B., Jules, J., El-Saber Batiha, G., Ali, I., Bruno, L. N., 2021. Extraction of Bioactive Compounds from Medicinal Plants and Herbs.

Ghenabzia, I., Hemmami, H., Ilham, B. E. N., Zeghoud, S., Seghir, B. B., Hammoudi, R., 2023. Different methods of extraction of bioactive compounds and their effect on biological activity: A review. International Journal of Secondary Metabolite, 10(4), 469-494.

Gonçalves, S., Romano, A., 2016. The medicinal potential of plants from the genus *Plantago* (Plantaginaceae). Industrial Crops and Products, 83, 213-226.

Hakverdi, A. E., Yiğit, N., 2017. "Yozgat-Akdağmadeni Yöresinde Bulunan Bazı Tıbbi ve Aromatik Bitkiler," Bartın Orman Fakültesi Dergisi., vol. 19, no. 2, p. 82-87.

Kılıç, C., Can, Z., Yılmaz, A., Yıldız, S., Turna, H., 2017. Antioxidant properties of some herbal teas (green tea, senna, corn silk, rosemary) brewed at different temperatures. International Journal of Secondary Metabolite, 4(3, Special Issue 1), 142-148.

Orak, H. H., KARAMAĆ, M., Adnan, O., Tenikecier, H. S., Amarowicz, R., 2021. Antioxidant Activity and Total Phenolic Contents of Different Alfalfa (*Medicago sativa* L.) Varieties Grown in Turkey. Yuzuncu Yıl University Journal of Agricultural Sciences, 31(4), 995-1000.

Piljac-Žegarac, J., Šamec, D., Piljac, A., 2013. Herbal teas: A focus on antioxidant properties. In Tea in health and disease prevention (pp. 129-140). Academic press.

Singleton, V. L., Rossi, J. A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture, 16(3), 144-158.

Toker, R., Gölükcü, M., Tokgöz, H., 2015. Tıbbi Ve Aromatik Bitkilerin Gıda Sanayisinde Kullanım Alanları. Türkiye Tohumcular Birliği Dergisi. 4(1): 54-59.

Türkan, Ş., Malyer, H., Aydın. S.Ö., Tümen, G., 2006. Ordu İli ve Çevresinde Yetişen Bazı Bitkilerin Etnobotanik Özellikleri. Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü Dergisi. 10(2): 162-166.

Woisky, R. G., Salatino, A., 1998. Analysis of propolis: some parameters and procedures for chemical quality control. Journal of apicultural research, 37(2), 99-105.

Isolation and Characterization of Potential Plant Growth-Promoting Bacteria (PGPB) from Erzurum Kuruçalı Microcatchment

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ABSTRACT: In this study; Soil samples were taken from 15 different areas in the Kuruçalı microcatchment and brought to the laboratory in a vehicle refrigerator at +4 °C. A total of 85 different bacterial strains were obtained in different numbers from each of these soil samples. Phosphate solubility, nitrogen fixation and siderephore production tests were carried out to determine the plant growth-promoting properties of all the bacteria obtained. According to the results obtained; In the nitrogen fixation test, 25 bacteria gave strong positive results, 8 bacteria gave weak positive results, and 52 bacteria gave negative results. Among all bacteria, 5 bacterial strains gave positive results in phosphate solvent tests and the others gave negative results. As a result of the siderophore tests, 17 bacterial strains gave positive results. According to all the results obtained, it is thought that strains that are positive in terms of all three features may be potential PGPB.

Keywords: Biofertilizer, PGPB, Phosphate solubility, Kuruçalı Microcatchment

INTRODUCTION

Bacteria that are free-living, promoting plant growth, used in biological control or as biological fertilizers (BG) are called plant growth promoting bacteria (PGPB). PGPB are able to exert a beneficial effect upon plant growth, N_2 fixing and P solubilizing and playing a significant role as PGPB in the biofertilization of crops. These microorganisms are found in several genera including *Acinetobacter, Alcaligenes, Arthrobacter, Azospirillium, Azotobacter, Bacillus, Burkholderia, Enterobacter, Flavobacterium, Rhizobium and Serratia*. Although the mechanisms of PGPB are not fully understood, are thought to include: the ability to produce plant hormones; such as auxins, cytokinins and gibberellins, asymbiotic N_2 fixation, solubilization of inorganic phosphate and mineralization of organic phosphate and mineralization of organic phosphate and/or other nutrients and antagonism against phytopathogenic microorganisms by production of siderophores the synthesis of antibiotics enzymes and/or fungicidal compounds, and competition with detrimental microorganisms (Antoun and Prevost 2006; Fuentes-Ramirez and Caballero-Mellado 2006; Bloemberg and Lugtenberg 2001; Burdman et al, 2002; Çakmakçı, 2005a, b; Vessey 2003; Niranjiyan et al. 2006).

MATERIAL AND METHODS

Isolation and Stocking of Bacterial Isolates

Soil samples were weighed 1 gr and transferred to tubes containing 2 ml of sterile water and left to mix in a hematological shaker for about 2 hours. Serial dilutions were then prepared from the solution in the tube with a sterile pipette. Nutrient Agar (NA) medium was used as isolation medium. Cultures were incubated at 25-30°C for 24-72 hours and transferred to new media from each colony with different characters, especially those with dense growth, as much as possible from the formed colonies (Klement et al., 1990).

By giving a separate code number to each isolate, information about the isolation (location of isolation, date, etc.) was recorded; were stored at -80°C in stock media containing 30% glycerol and Lauryl Broth (LB) to be used in diagnosis and characterization processes and other studies.

Determination of Phosphate Solubizing Potential of Bacteria

24-hour bacterial cultures grown on nutrient agar were suspended in sdH_2O and their density was adjusted to 10^8 CFU/ml. Tubes containing 5 ml of NBRIP-BPB in each suspension were inoculated. After a 15-day incubation period, the phosphate solubilization ability of bacteria that showed discoloration in the medium was evaluated as positive (Metha and Nautiyal 2001). In addition, the potential of the isolates to dissolve Mazıdağı Rock Phosphate was tested by adding Mazıdağı Rock Phosphate to the $Ca_3(PO_4)_2$ medium contained in NBRIP (Nautiyal, 1997).

Detection of Nitrogen Fixation of Bacteria

Bacteria were contaminated by streaking from stock cultures into NA medium. After 2-7 days of incubation, bacterial colonies were drawn onto nitrogen-free medium Burk's and Ashby's solid medium using the scatter plate method (N-Free Solid Malate Sucrose Medium) and allowed to grow in an incubator set at 27 °C for 7-10 days. Bacterial growth in the medium was evaluated as nitrogen fixation positive.

Production of siderophores tests

CAS assay was used to detect siderophores produced by bacteria. Siderophore production was tested on Petri dishes contained CAS-agar. The composition of CAS blue solution for this assay was prepared according to Schwyn and Neilands (1987). Pure isolates were stabbed on CASagar plates using sterile toothpicks and incubated at 28C for 2 weeks in the dark. The colonies with orange zones were considered as siderophore–producing strains (Schwyn ve Neilands 1987).

Coordinates of soil samples

The map image of Kuruçalı microcatchment in Erzurum province is given in Figure 1 and the coordinates of the soil samples taken are given in Table 1.

Figure 1. Kuruçalı microcatchment

Table 1. Coordinates of soil samples

Sample Number	Coordinates
1	743435.08 d D 4477930.60 m K
2	743684.16 d D

	4477911.75 m K
3	743578.23 d D 4477910.62 m K
4	743667.84 d D 4477741.89 m K
5	743473.58 d D 4478127.65 m K
6	743759.31 d D 4478036.28 m K
7	743553.25 d D 4477606.80 m K
8	743746.22 d D 4477835.29 m K
9	743798.60 d D 4477738.98 m K
10	743292.65 d D 4477218.46 m K
11	743488.24 d D 4477203.21 m K
12	743900.70 d D 4477693.06 m K
13	743660.98 d D 4477297.67 m K
14	743803.46 d D 4477342.99 m K
15	743978.68 d D 4477414.78 m K

RESULTS AND DISCUSSION

Bacteria used as biological control agents or biofertilizers in agriculture are called plant growth promoting bacteria= PGPB. A total of 85 bacterial isolates were obtained as a result of the isolations made from soil samples taken from the in different areas in Erzurum Kuruçalı Microcatchment. In order to determine whether the isolates obtained have PGPB potential, their phosphate dissolving, production of siderophores and nitrogen fixation properties were determined and the results were shown in Table. 2. In the nitrogen fixation test, 25 bacteria gave positive results, 8 bacteria gave weak positive results and 52 bacteria gave negative results. Among all bacteria, 5 bacterial strains gave positive results in phosphate solvent tests and the others gave negative results. As a result of the siderephor tests, 17 bacterial strains gave positive results.

Considering all these results; As a result of the study, it was determined that there are many bacteria with high potential to become PGPB among the bacterial isolates obtained. It is planned to carry out studies on the use of these bacteria individually or in combination in the cultivation of different plants.

Table.2 Some plant growth-promoting properties of potential biofertilizer bacteria, host and location data

Strain no	Coordinates	N-Free Medium	Phosphate solubility (P)	Production of siderophores
BORM1		Z+	-	-
BORM2		Z+	-	+
BORM3		K+	-	-
BORM4	743435.08 d D	-	-	-
BORM5	4477930.60 m K	-	-	-
BORM6		-	-	-
BORM7		K+	-	-
BORM8		K+	-	-
BORM9	743684.16 d D	-	-	+
BORM10	4477911.75 m K	-	-	+
BORM11		-	-	-
BORM12	743578.23 d D	K+	-	-
BORM13	4477910.62 m K	K+	-	-
BORM14		-	-	-

BORM15		-	-	-
BORM16	743667.84 d D	K+	-	-
BORM17	4477741.89 m K	-	-	-
BORM18		-	-	-
BORM19		-	+	+
BORM20		Z+	-	-
BORM21	743473.58 d D	-	-	-
BORM22	4478127.65 m K	-	-	-
BORM23		-	-	-
BORM24		Z+	-	-
BORM25	743759.31 d D	-	-	+
BORM26	4478036.28 m K	-	-	-
BORM27		-	-	+
BORM28		K+	-	-
BORM29		-	-	-
BORM30		K+	-	-
BORM31	743553.25 d D	-	-	-
BORM32	4477606.80 m K	-	-	-
BORM33		-	+	+
BORM34		-	-	-
BORM35		K+	+	+
BORM36	743746.22 d D	-	-	-
BORM37	4477835.29 m K	K+	-	-
BORM38		-	-	-
BORM39		-	-	+
BORM40		-	-	+
BORM41	743798.60 d D	K+	-	+
BORM42	4477738.98 m K	-	-	-
BORM43		-	-	-
BORM44		-	-	-

BORM45		-	-	+
BORM46		-	-	-
BORM47		K+	-	-
BORM48		K+	-	-
BORM49		-	-	-
BORM50		-	-	-
BORM51	743292.65 d D	-	-	-
BORM52	4477218.46 m K	-	-	-
BORM53		-	-	-
BORM54		K+	-	-
BORM55		-	+	+
BORM56		K+	-	-
BORM57		-	-	-
BORM58		-	-	-
BORM59	743488.24 d D	K+	-	-
BORM60	4477203.21 m K	K+	-	-
BORM61		K+	-	+
BORM62		-	-	-
BORM63		-	-	-
BORM64		K+	-	-
BORM65	743900.70 d D	K+	-	-
BORM66	4477693.06 m K	K+	-	-
BORM67		-	-	+
BORM68		Z+	-	-
BORM69		-	-	-
BORM70	743660.98 d D	Z+	-	-
BORM71	4477297.67 m K	-	-	-
BORM72		-	-	-
BORM73		K+	-	-
BORM74		-	-	-

BORM75		-	-	-
BORM76		K+	-	-
BORM77	743803.46 d D	K+	-	-
BORM78	4477342.99 m K	-	-	-
BORM79		-	+	+
BORM80		-	-	-
BORM81		-	-	-
BORM82		Z+	-	-
BORM83	743978.68 d D	Z+	-	-
BORM84	4477414.78 m K	K+	-	-
BORM85		-	-	+

Z+: Weak positive, K+: Strong positive, -: Negative

REFERENCES

Antoun, H., Prevost, D., 2006. Ecology of Plant Growth Promoting Rhizobacteria. PGPR: Biocontrol and Biofertlization. Edited by Zaki A. Sıddıqui. S 1-38, Springer, The Netherlands.

Bloemberg, G.V., Lugtenberg, B.J.J., 2001. Molecular Basis of Plant Growth Promotion and Biocontrol by Rhizobacteria. Current Opinion in Plant Biotechnology 4, 343-350. Anonim, 2006. Effects of Inoculation with PGPR on Seedlings Growth of Different Tomato and Pepper Varieties in Axenic Conditions, Universidad S. Pablo CEU. Department De Biologia, Madrid.

Burdman, S., Jurkevitch, E. and Okon, Y., 2000. Recent advances i the use of plant Growth Promoting Rhizobacteria (PGPR) in agriculture. In Microbiol Interactions in Agriculture and Forestry, Vol II Eds. Subba Rao N and Dommergues Y.R. Chapter 10 pp 29-250. ci. Pub. Inc. UK.

Çakmakçı, R., 2005a. Bitki gelişiminde fosfat çözücü bakterilerin önemi. S. Ü. Ziraat Fak. Derg. 35, 93-108.

Çakmakçı, R., 2005b. Bitki gelişimini teşvik eden rizobakterilerin tarımda kullanımı. Atatürk Üniv. Ziraat Fak. Derg.36, 97-107.

Fuentes-Ramirez, E.L., Caballero-Mellado, J. 2006. Bacterial Biofertilizers. PGPR: Biocontrol and Biofertlization. Edited by Zaki A. Sıddıqui. P 143-172, Springer, The Netherlands.

Klement, Z., Rudolph, K. and Sands, D.C., 1990. Methods in phytopathology. Akademiai Kiado, Budapest, 153-180.

Nautiyal, C.S. (1997) Rhizosphere competence of *Pseudomonas* sp. NBRI9926 and *Rhizobium* sp. NBRI9513 involved in the suppression of chickpea (*Cicer arietinum* L.) pathogenic fungi. FEMS Microbiol. Ecol. 23, 145^158.

Schwyn B, Neilands JB 1987. Universal Chemical Assay for Detection and Determination of Siderophores. Analytical Biochemistry, 160: 47-56.

Niranjiyan RAJ, S., Shetty, H.S., Reddy, M.S., 2006. Plant Growth Promoting Rhizobacteria: Potential Gren Alternative for Plant Productivity. PGPR: Biocontrol and Biofertlization. Edited by Zaki A. Sıddıqui. P 197-216, Springer, The Netherlands.

Wood Vinegar from The Tea Residues Improves Growth of Pepper Seedling

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ABSTRACT: In recent years, there has been an intense effort to re-evaluate agricultural residues. It is an important issue to evaluate the waste generated by tea production, which has a high production and consumption in Türkiye and the world. In this study, the effects of wood vinegar obtained by the processing wastes from tea production on plant development in pepper seedlings were investigated. Different doses (0, 1/25, 1/50, 1/75, 1/100, 1/250 and 1/500; wood vinegar/water) of wood vinegar were applied to the plant in two ways (spraying on the leaves and drenching into the soil). It has been determined that the wood vinegar applications (especially lower doses) have a positive effect on pepper seedling development. With the applications, plant fresh and dry weight, root fresh and dry weight, stem diameter and plant height increased significantly. In addition, there were significant increases in the contents of macro and micro elements in the plant with the applications compared to the control. As a result, it was determined that wood vinegar applied to the soil, especially in low doses, would be more beneficial for plant growth in pepper.

Keywords: *Pepper*; Seedling; Wood vinegar; Plant growth

INTRODUCTION

In recent years, industrial waste has become an important problem. Disposal of some wastes is difficult and takes a long time. Particular attention is paid to the evaluation and reuse of wastes due to their environmental effects. There are many by-products that arise as a result of processes such as agriculture and forestry, and are destroyed by burning or abandoned in nature. These damage the environment and the living things in it over time (Hamid et al., 2010).

Tea is one of the most preferred beverages worldwide and therefore its production is intensive. It is predicted that global tea consumption will be approximately 6.3 million tons in 2020 and may increase to 7.4 million tons by 2025 (Debnath et al., 2021). Tea is one of the products with the most waste problems as a result of agricultural production and processing. Many tea wastes, such as tea residues generated during the production of tea extracts, uncollected tea leaves and pruned branches, cause biomass loss and environmental problems (Wang et al., 2011; Guo et al., 2021; Debnath et al., 2021). Tea waste is a lignocellulosic biomass consisting of cellulose, hemicellulose, lignin, polyphenols, proteins and tannins, and contains biologically active substances such as polyphenols, methylxanthines, alkaloids, vitamins, minerals, terpenoids, pigments, amino acids and polysaccharides (Barathi et al., 2017;

Debnath et al., 2021; Shang et al., 2021). It is known that tea waste generally has similar components to regular tea (Sui et al., 2019; Debnath et al., 2021). In recent years, products such as compost and biochar have been obtained from tea waste and used in the production of agricultural products. One of these is the use of vinegar obtained from tea waste, which is not yet known.

Studies focus on the processes of converting biomass into gas, liquid and solid forms by various processes such as pyrolysis, gasification, carbonization and liquid extraction in order to reduce this environmental pollution. Wood vinegar, is a product obtained by the pyrolysis of biomass, and is the product formed after resting and waiting for the liquid formed by distilling the steam, gas and smoke released as a result of the pyrolysis process. Wood vinegar, also known as pyroligneous acid, is a byproduct of burning coal (Namlı et al. 2014). Wood vinegar is environmentally friendly, has a compound effect that promotes plant growth and development, and can even increase the resistance of plants to biological and abiotic stresses (Zhu et al., 2021).

In this study, the effects of wood vinegar obtained from tea residues on plant growth and mineral content in pepper seedlings were examined.

MATERIAL AND METHOD

In the study, pepper (Capsicum annum L. cv Yalova) seedlings were used as plant material. Pepper seeds were planted in multi celled seedling trays containing peat:perlite (2:1), and then 2-3 ture leaves seedlings were transferred to 1.5 liter pots. There is a mixture of soil:peat:sand (2:1:1) in the pots. Different doses (0, 1/25, 1/50, 1/75, 1/100, 1/250 and 1/500; wood vinegar/water) of wood vinegar were applied to the plant in two ways (spraying on the leaves and drenching into the soil). The applications were made 3 times at one-week intervals. The study was designed according to the random plot design with 3 replications and 6 plants in each replication.

The study was completed 40 days after planting the seedlings in pots and various measurements were made. The effects of the treatments on plant and root fresh weight and plant and root dry weight, stem diameter, plant height and mineral content of plant were examined. Determination of the total N was achieved by the Kjeldahl method using a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) (Brenmer, 1996). An inductively coupled plasma spectrophotometer (Optima 2100 DV, ICP/OES; Perkin-Elmer, Shelton, CT) was used to determine mineral content (Mertens, 2005 a; Mertens 2005 b). By averaging the data obtained, the differences between the applications were analyzed statistically according to the Duncan Multiple comparison test using the SPSS program.

RESULTS AND DISCUSSION

In the study, it was determined that the applications had a positive effect on pepper seedling development. Plant fresh and dry weight, root fresh and dry weight, stem diameter and plant height increased significantly with the applications. In the study, the highest plant fresh and dry weight was obtained from the 1/250 dose of foliar application, while the root fresh weight and root dry weight were measured in the highest soil 1/500 application, and stem diameter and plant height were measured in the highest soil 1/250 application (Figure 1).

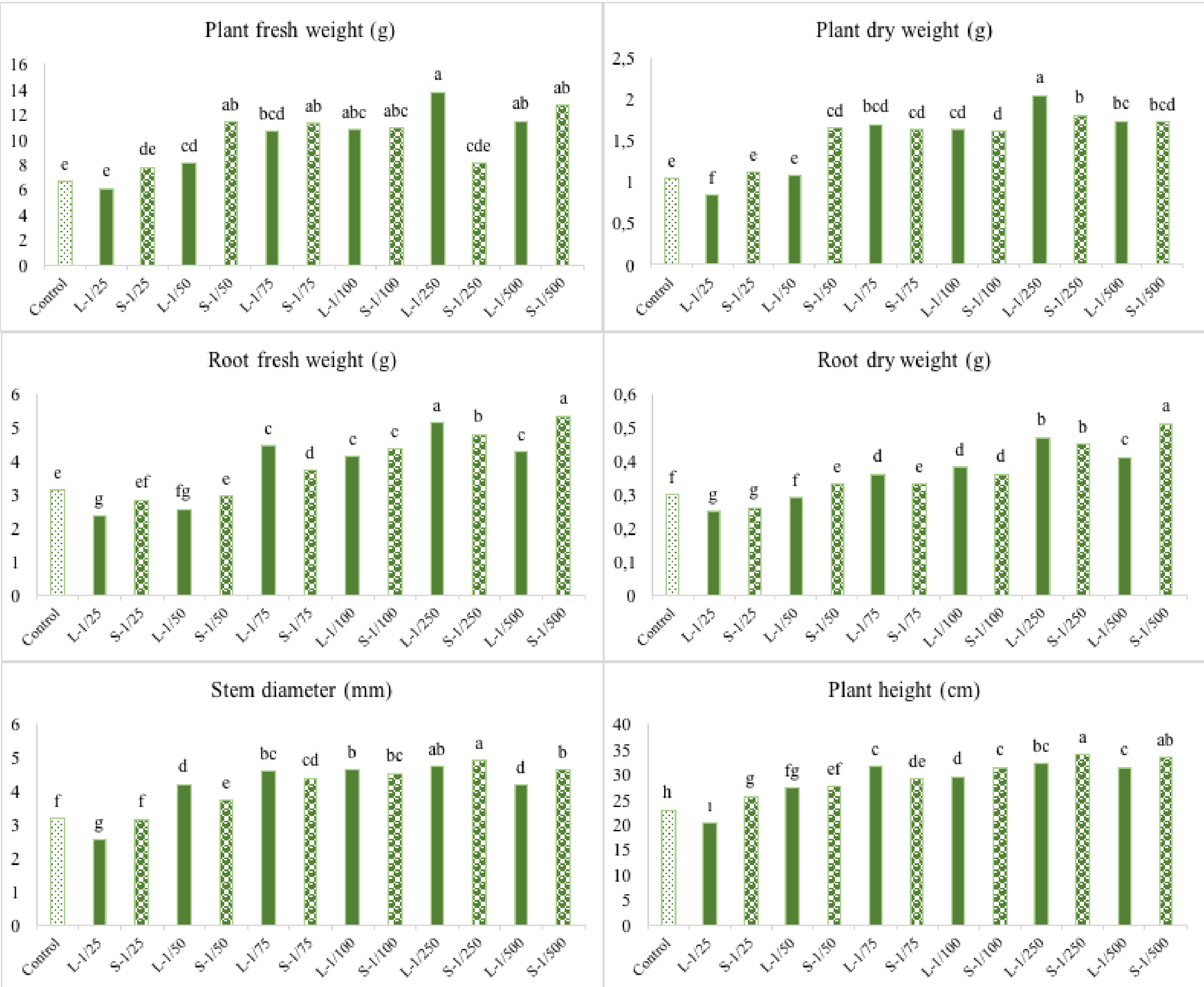

Figure 1. Effect of wood vinegar from the tea residues on growth of pepper seedling. There is no statistically (p<0.001) difference between the means shown with the same letter in the bar.

In addition, significant effects were observed on the plant nutrient content, and with the treatments, there were increases in the macro and micronutrient elements in pepper seedlings compared to the control. In the study, 1/500 wood vinegar applied foliar to pepper seedlings gave the highest values in N, Mn and Fe content in the plant. With the application of 1/250 wood vinegar to the soil the P, Ca and Cu contents in the plant increased, while 1/75 soil application increased the K and Zn content, 1/100 soil application increased the B content, 1/500 soil and 1/500 foliar application increased the Mg and S content (Figure 2 and Figure 3).

Generally, while especially low doses of wood vinegar (1/100, 1/250 and 1/500) gave higher results, the effectiveness of leaf and soil application varied.

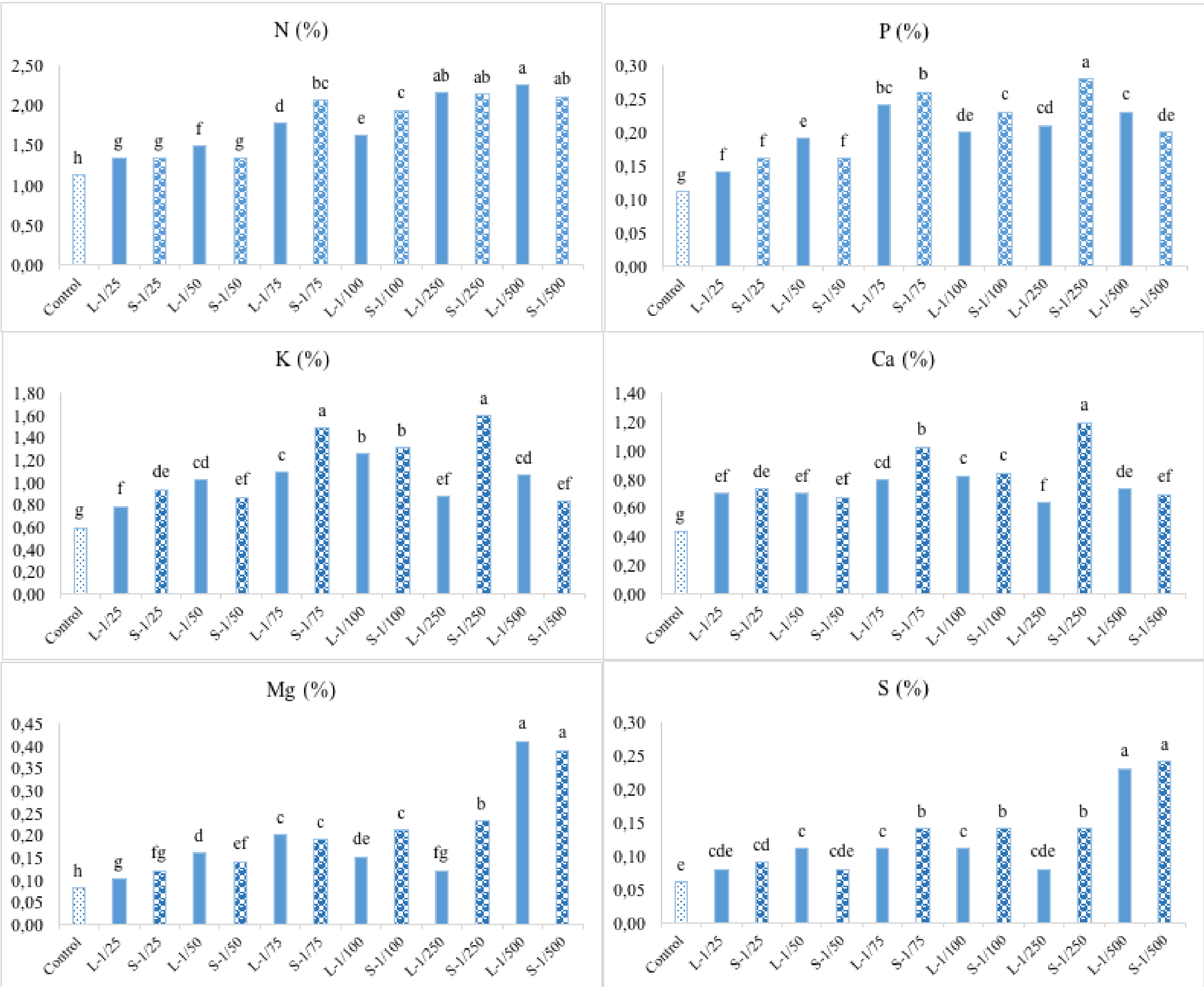

Figure 2. Effect of wood vinegar from the tea residues on N, P, K, Ca, Mg and S content of pepper seedling. There is no statistically (p<0.001) difference between the means shown with the same letter in the bar.

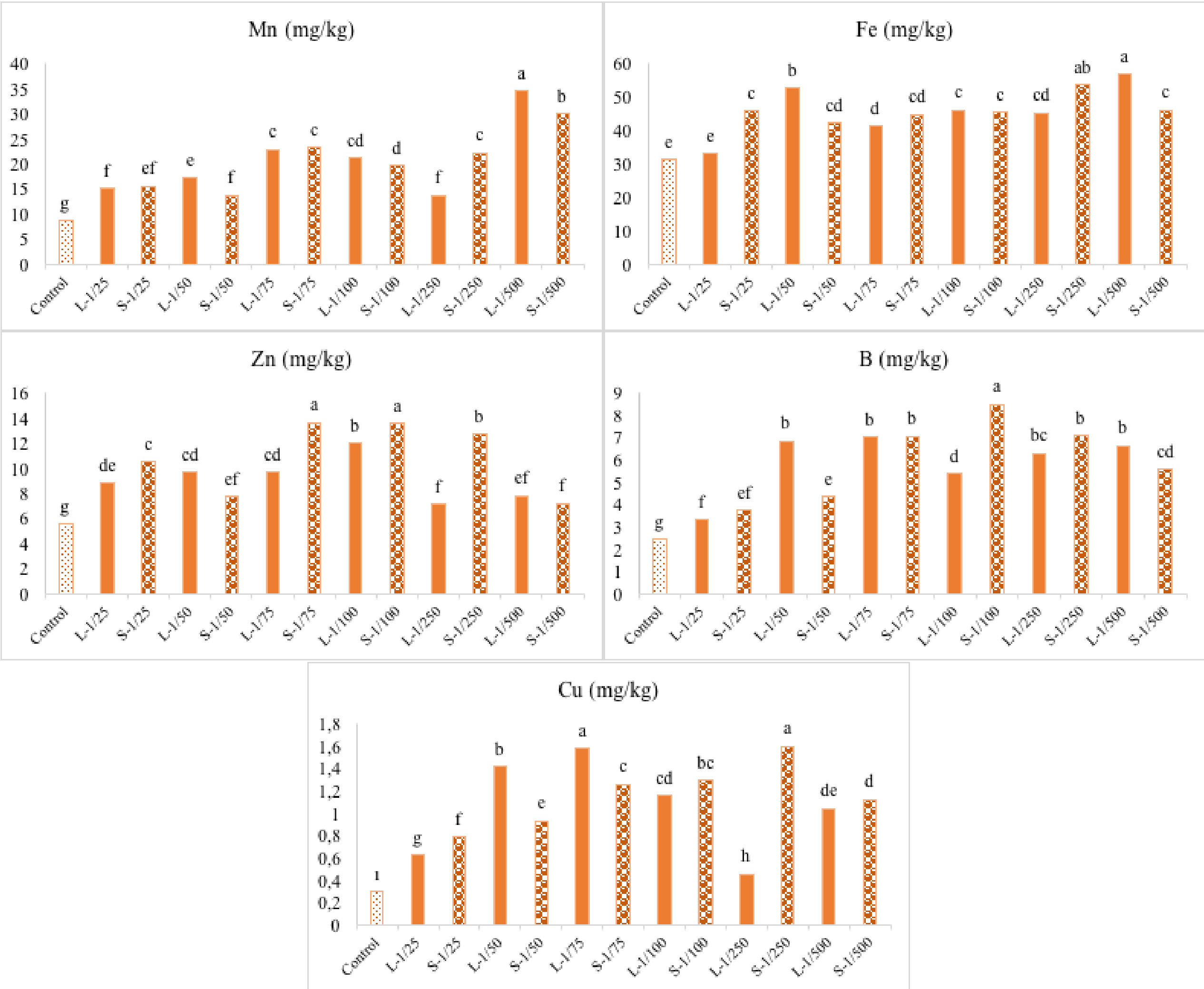

Figure 3. Effect of wood vinegar from the tea residues on Mn, Fe, Zn, B and Cu content of pepper seedling. There is no statistically ($p<0.001$) difference between the means shown with the same letter in the bar.

In this study, the effect of different doses of wood vinegar on plant growth and mineral content in pepper seedlings was examined, and the positive effects of the applications were observed depending on the dose. Wood vinegar is a condensed liquid produced in biochar production under high temperature and hypoxia from agricultural and forestry wastes (Maleki Lajayer, 2023).

The content of wood vinegar varies depending on the type of product used in biochar production and the pyrolysis conditions. Generally, in addition to water, it contains acetic acid, other acids, alcohols, phenols, esters, carbonyl, furans and other organic components. It improves soil quality with the organic matter and nutrients it provides to the soil, and is effective in combating various diseases and pests. Additionally, it improves seed germination, plant growth, yield and fruit quality (Birol and Günal, 2022).

It has been determined that wood vinegar is formed by the condensation of smoke produced by biochar production, contains compounds such as acetic acid, butyric acid, catechol and phenol, and promotes plant growth by providing effects similar to plant growth regulators. It also provides a resistance-increasing effect in plants against various biological and abiotic stresses (Zhu et al., 2021). All these effects may vary depending on the plant from which wood vinegar is obtained and the dose. In the researches, it has been determined that it contains different types of organic compounds to a large extent (Wu et al., 2015). Studies have also shown that wood vinegar can be considered as a plant growth regulator and its appropriate concentrations can have a positive effect on crops. Similarly, in pepper, poplar derived wood vinegar has been shown to increase root length and root development, and significantly increase shoot and root biomass (Luo et al., 2019). In the study, the components (such as phenols, alkaloids, minerals, amino acids etc.) contained in tea waste, which is the raw material of wood vinegar, provide this positive effect on pepper seedlings. In particular, the optimal concentration of wood vinegar contains various functional substances that can increase the resistance to abiotic and biotic stresses, as well as the effects on the growth of plants, and can also increase yield and quality. In a study, it was determined that poplar-derived wood vinegar did not have a significant effect on seed germination in tomatoes and peppers, but applications at low concentrations increased root and shoot length (Luo et al., 2019). In a study, it was determined that wood vinegar added with a 10000 times dilution significantly increased cucumber root length and dry biomass (Lei et al., 2018). Similarly, in this study, especially the effect of low doses of wood vinegar on pepper seedlings is better, and as the concentration increases, plant development may remain at lower levels.

CONCLUSION

In recent years, interest in the production of wood vinegar and its use in agriculture has increased. As a result of the study, it was determined that the effect of the wood vinegar used in the study on pepper seedlings varied depending on the dose. However, it would be useful to conduct further studies to detail the effects of wood vinegar, obtained as a by-product of the pyrolysis process, in agricultural activities, especially as biopesticide, biofertilizer and soil conditioner, etc. Also, in our literature research, we could not find any studies examined the effect of wood vinegar obtained from tea waste on plant growth. Based on the results obtained, it would be useful to conduct different studies to evaluate the wastes resulting from tea production and processing, which has an important waste potential in agricultural production.

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REFERENCES

Barathi, M., Kumar, A. S. K., Kodali, J., Mittal, S., Samhith, G. D., Rajesh, N., 2017. Probing the ınteraction between fluoride and the polysaccharides in Al (III)-and Zr (IV)-modified tea waste by using diverse analytical characterization techniques. ChemistrySelect, 2(31), 10123-10135.

Birol, M., Günal, E., 2022. Agricultural use of wood vinegar. Bilecik Seyh Edebali University Journal of Science, 9(1), 596-608.

Bremner, J.M., 1996. Nitrogen-total. In: Sparks DL, editor. Methods of Soil Analysis. Part 3—Chemical Methods. 2nd ed. Madison, WI, USA: American Society of Agronomy, pp. 1085–1122.

Debnath, B., Haldar, D., Purkait, M. K., 2021. Potential and sustainable utilization of tea waste: A review on present status and future trends. Journal of Environmental Chemical Engineering, 9(5), 106179.

Guo, S., Awasthi, M. K., Wang, Y., Xu, P., 2021. Current understanding in conversion and application of tea waste biomass: A review. Bioresource Technology, 338, 125530.

Hamid, A. A., Usman, L. A., Elaigwu, S. E., Zubair, M. F., 2010. Environmental and health risk of bush burning. Advances in Environmental Biology, 4(2), 241-249.

Lei, M., Liu, B., Wang, X., 2018. Effect of adding wood vinegar on cucumber (Cucumis sativus L) seed germination. In IOP Conference Series: Earth and Environmental Science (Vol. 128, No. 1, p. 012186). IOP Publishing.

Luo, X., Wang, Z., Meki, K., Wang, X., Liu, B., Zheng, H., Yuo, X., Li, F., 2019. Effect of co-application of wood vinegar and biochar on seed germination and seedling growth. Journal of Soils and Sediments, 19, 3934-3944.

Maleki Lajayer, H., 2023. Evaluation of ınfluence of wood vinegar and light quality on growth and biochemical traits of chervil plant (Antriscus cerifolium). Journal of Vegetables Sciences, 7(1), 198-209.

Mertens, D., 2005a. AOAC Official Method 922.02. Plants preparation of laboratuary sample. Official Methods of Analysis, 18th edn. Horwitz, W., and G.W. Latimer, (Eds). Chapter

3, AOAC-International Suite 500, 481. North Frederick Avenue, Gaitherburg, Maryland, USA. pp. 1–2 .

Mertens, D., 2005b.AOAC Official Method 975.03. Metal in plants and pet foods. Official Methods of Analysis, 18th edn. Horwitz, W., and G.W. Latimer, (Eds). Chapter 3, AOACInternational Suite 500, 481. North Frederick Avenue, Gaitherburg, Maryland, USA. pp. 3–4.

Namlı, A., Akca, M.O., Turgay, E.B., Soba, M.R., 2014. Investigation of potential agricultural use of wood vinegar. Soil Studies, 3(1): 44-52.

Shang, A., Li, J., Zhou, D. D., Gan, R. Y., Li, H. B., 2021. Molecular mechanisms underlying health benefits of tea compounds. Free Radical Biology and Medicine, 172, 181-200.

Sui, W., Xiao, Y., Liu, R., Wu, T., Zhang, M., 2019. Steam explosion modification on tea waste to enhance bioactive compounds' extractability and antioxidant capacity of extracts. Journal of Food Engineering, 261, 51-59.

Wang, R. R., Wang, H. L., Liu, X., Xu, C. C., 2011. Effects of different additives on fermentation characteristics and protein degradation of green tea grounds silage. Asian-Australasian Journal of Animal Sciences, 24(5), 616-622.

Wu, Q., Zhang, S., Hou, B., Zheng, H., Deng, W., Liu, D., Tang, W., 2015. Study on the preparation of wood vinegar from biomass residues by carbonization process. Bioresource technology, 179, 98-103.

Zhu, K., Gu, S., Liu, J., Luo, T., Khan, Z., Zhang, K., Hu, L., 2021. Wood vinegar as a complex growth regulator promotes the growth, yield, and quality of rapeseed. Agronomy, 11(3), 510.

Characterisation of Bacterial Blight Disease in Walnut Caused by (*Xanthomonas arboricola* pv. *juglandis*)*

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ABSTRACT: Bacterial walnut blight is a plant disease caused by the bacterium *Xanthomonas arboricola* pv. *juglandis (Xaj)* which significantly impacts the health and productivity of walnut trees, leading to reduced yields and economic losses for walnut growers. In this study, characterisation of *Xanthomonas arboricola* pv. *juglandis (Xaj)*, the causative agent of walnut blight disease, was investigated. Bacterial isolates were collected from diseased walnut leaves and fruits, and tests were conducted to determine their pathogenicity. Five bacterial isolates tested positive in HR tests and displayed bright mucoid yellow-colored colonies. In these results, all isolates tested positive in tomato plants, whereas only the *Xaj*-2 isolate tested positive in tobacco plants, and the other isolates gave negative results in the HR test. Additionally, all isolates yielded positive results in the KOH test and showed growth in YDCA medium as bright mucoid yellow colonies. In the starch hydrolysis test, *Xaj*-2 and *Xaj*-3 isolates tested positive, while the remaining isolates yielded negative results. All isolates grew in 2% and 5% NaCl medium, but *Xaj*-1, *Xaj*-2, and *Xaj*-3 exhibited weak growth in 5%. None of the isolates grew in 8% NaCl medium. Lastly, all isolates tested negative on SNA medium. Among the isolates, three showed positive results in the pathogenicity test, but the *Xaj*-2 isolate was more effective than the others. After 7 days, the isolates caused brown-black spots on the leaves, and these spots grew and merged after 14 days, leading to the formation of necrotic tissue. After 28 days, these necrotic formations expanded further, causing drying at the leaf edges and wilting throughout the leaf.

Key words: *Xanthomonas arboricola* pv. *juglandis*, Bacterial walnut blight, Plant disease

INTRODUCTION

Walnut, being one of the oldest edible fruits globally, has significant production in China, with 158,6367 tons in 2018. Following China, the largest walnut producers are the United States, Iran, and Turkey. According to production data, Turkey ranks fourth in the world with 215,000 tons of walnut production, accounting for approximately 6% of the global walnut production (FAO, 2019).

Bacteria of the *Xanthomonas* genus are Gram-negative, rod-shaped, and have a yellow color, smooth margins, and a mucoid consistency. The yellow color is due to the secretion of pigments called xanthomonadines, while the presence of mucus surrounding the cells is determined by the presence of xanthan exopolysaccharide (Swings, et al., 1993; Vauterin, et al., 1995). According to Vauterin et al. (1995), bacteria of this genus have catalase activity, but these lack urease or oxidase activity. These cannot produce indole or acetoin, and these cannot reduce nitrates to nitrites. Their growth is inhibited at concentrations of over 6% NaCl and in 30% glucose.

In Turkey, walnut cultivation holds a prominent position due to its importance in terms of production, health, and nutrition. However, it faces significant threats from various diseases, including fungal and bacterial infections. Among the most critical and widespread bacterial diseases affecting walnut (*Juglans regia* L.) plant production is walnut bacterial blight caused by *Xanthomonas arboricola* pv. *juglandis* (*Xaj*). Typical disease symptoms become evident within 10-15 days after the pathogen enters the plant tissues. The disease primarily affects parenchymal tissues and can even invade vascular bundles. This indicates that bacteria target rapidly growing green and tender tissues, including buds, leaf petioles, male and female flowers, small fruits, and buds (Sinavimo, 2018; Yörük and Mirik, 2018).

The initial symptoms of the disease appear on the leaves. The disease causes the entire leaf to turn yellow, forming brown-black spots. These spots can vary in size from small dot-like lesions to angular spots of 2-3 mm in length, limited by the veins. Countless spots and line-shaped formations completely cover the leaf surface, leading to leaf distortion and deformations (Fırat, 2006; Yılmaz, et al., 2017).

As for the fruits, these are highly susceptible during their early development. Small, initially olive-green spots appear on the fruit surface, later turning black and slightly sunken. Over time, these spots spread and lead to decay. The spots on the fruit often extend beyond the peel and can reach the inner parts of the walnut. The color of the walnut changes, and its taste becomes bitter. Early infection of the fruits can cause significant fruit drop. In walnut orchards affected by the disease, yield losses of over 50% can had observed (Fırat, 2006; Yılmaz, et al., 2017). Chandler, Çebin, Payne, and other foreign varieties are highly susceptible to this disease, and more attention should be paid to their cultivation (Erdal and Özaktan, 2011; Aysan et al., 2013).

In this study, the characterization of the *Xaj* was investigated, a significant pathogen in walnut trees in Turkey.

MATERIAL AND METHOD

Material

Pathogen bacteria and biological control agent bacteria

Pathogen isolates used in this study were identified using the microbial identification system MIS and BIOLOG system. Five pathogenic bacterial isolates were obtained from plant parts showing symptoms in walnut orchards, and these isolates were included in the collection of the Department of Plant Protection, Faculty of Agriculture at Atatürk University, Erzurum, Turkey

Method

In this study, a total of 81 potential biocontrol bacterial isolates obtained from the rhizosphere of different plants were subjected to the Hypersensitive Response (HR) test. Six isolates with a positive HR response were selected, while the remaining 75 isolates were tested against pathogens under in vitro conditions. Among five potential *Xanthomonas* bacterial isolates obtained from walnut plants and preserved, the most virulent isolate, *Xaj*-2, was chosen based on colony characteristics, biochemical tests, HR, and pathogenicity tests, and it was used for *in vitro* and *in vivo* experiments.

Hypersensitive Response (HR) test.

Hypersensitive Response (HR) test involved transferring bacterial isolates, previously developed in nutrient agar (NA) for one day, into Eppendorf tubes containing 1.5 mL of sterile distilled water. After vortexing, the mixture concentration was adjusted to 108CFU/ml using a biolog device. Then, tomato (*Solanum lycopersicum*) and tobacco (*Nicotiana tabacum*) leaves' lower surfaces were injected in the vein regions. After 24-48 hours, observed necrotic lesions were assessed as confirming a positive HR response (Klement and Goodman, 1967).

Pathogenicity and virulence test

Prior to bacterial application, walnut saplings were watered, and their exposure was sealed using airtight plastic bags. Then, a bacterial suspension of 0.1 mL containing 108 CFU/mL, developed and suspended in nutrient broth (NB), was sprayed onto the undersides of the leaves. Negative control experiments utilized sterile distilled water. Subsequently, the leaf surfaces were observed for lesion formation at 7, 14, and 28 days to evaluate pathogenicity, lesion development rate, and size, thereby indicating the severity of virulence (Tsiantos, et al., 2007). As for pathogenicity tests, healthy walnut fruits with a diameter of 2-3 cm were utilized. These fruits were disinfected through a 10-minute immersion in 5% liquid bleach, followed by five rinses with sterile water and drying using sterile paper towels. Each bacterial isolate was inoculated in four marked areas under the fruit pericarp using a sterile syringe (30 µl/inoculation point), with three replicates per fruit. To achieve a bacterial concentration of 108 CFU/mL, 48-hour fresh bacterial cultures developed in Tryptic Soy Broth (TSB) were suspended. A negative control with sterile water was also included. Subsequent to application, the fruits were placed in sealed plastic bags, alongside moistened sterile paper towels to maintain high humidity, and then incubated at 24°C for 6-7 days.

Figure 1. Pathogenicity test application on walnut saplings and walnut fruits. (A) walnut saplings, (B) walnut fruits.

Biochemical tests of pathogenic bacteria isolates

Biochemical tests were conducted on five potential bacterial isolates obtained from diseased walnut plants. These tests included NaCl tolerance, Potassium hydroxide (KOH), Starch hydrolysis, and *Xanthomonas* detection on Yeast Dextrose Calcium Carbonate Agar (YDCA) and Sucrose Nutrient Agar (SNA) medium.

For the NaCl tolerance test, bacterial cultures were streaked on a NA medium containing 2% and 5% NaCl and incubated at 25°C for 48-60 hours. Bacterial growth was evaluated to assess their tolerance to NaCl (Schaad, et al., 2001).

The Potassium hydroxide (KOH) test involved applying 3% KOH solution to a slide, mixing it with a 48-hour culture of *Xaj* isolates, and observing the reaction. If the mixture formed a viscous, thread-like extension, the isolates were considered gram-negative; if it remained in a liquid state, they were considered gram-positive (Fahy and Hayward, 1983).

For Starch hydrolysis, bacterial isolates and original cultures were streaked on a medium containing starch and incubated at 25°C for 7-14 days. After incubation, melted Lugol's solution was added to the cultures, and hydrolysis of starch was indicated by the appearance of a clear zone around the stained bacterial colony (Lelliot and Stead, 1987).

Xanthomonas detection on YDCA medium involved streak inoculation on freshly prepared YDCA medium, followed by incubation at 25°C for 48-60 hours. Evaluation was based on the development of bright yellow mucoid colonies (Lelliot and Stead, 1987).

Lastly, Sucrose Nutrient Agar (SNA) medium was used to differentiate bacteria based on their carbohydrate utilization. Bacteria showing white, dome-shaped, and mucoid growth after incubation at 25°C for 2-3 days were considered positive, while those lacking this growth were considered negative (Lelliot and Stead, 1987)

RESULTS AND DISCUSSION

Biochemical and Hypersensitive Response (HR) test results of pathogen isolates

Five bacterial isolates tested positive in HR tests and displayed bright mucoid yellow-colored colonies. In these results, all isolates tested positive in tomato plants, whereas only the *Xaj*-2 isolate tested positive in tobacco plants, and the other isolates gave negative results in the HR test. Additionally, all isolates yielded positive results in the KOH test and showed growth in YDCA medium as bright mucoid yellow colonies. In the starch hydrolysis test, *Xaj*-2 and *Xaj*-3 isolates tested positive, while the remaining isolates yielded negative results. All isolates grew in 2% and 5% NaCl medium, but *Xaj*-1, *Xaj*-2, and *Xaj*-3 exhibited weak growth in 5%. None of the isolates grew in 8% NaCl medium. Lastly, all isolates tested negative on SNA medium.

Table 1. Biochemical and Hypersensitive Response (HR) test results of pathogen isolates

Isolates	HR test		YDCA medium	KOH test	SNA medium	Starch hydrolysis	NaCl tolerance test		
	Tütün	Domates					%2	%5	%8
Xaj-1	-	+	+	+	-	-	+	W+	-
Xaj-2	+	+	+	+	-	+	+	W+	-
Xaj-3	-	+	+	+	-	+	+	W+	-
Xaj-4	-	+	+	+	-	-	+	+	-
Xaj-5	-	+	+	+	-	-	+	+	-

(+: Positive reaction, -: Negative reaction, W+: Weak positive)

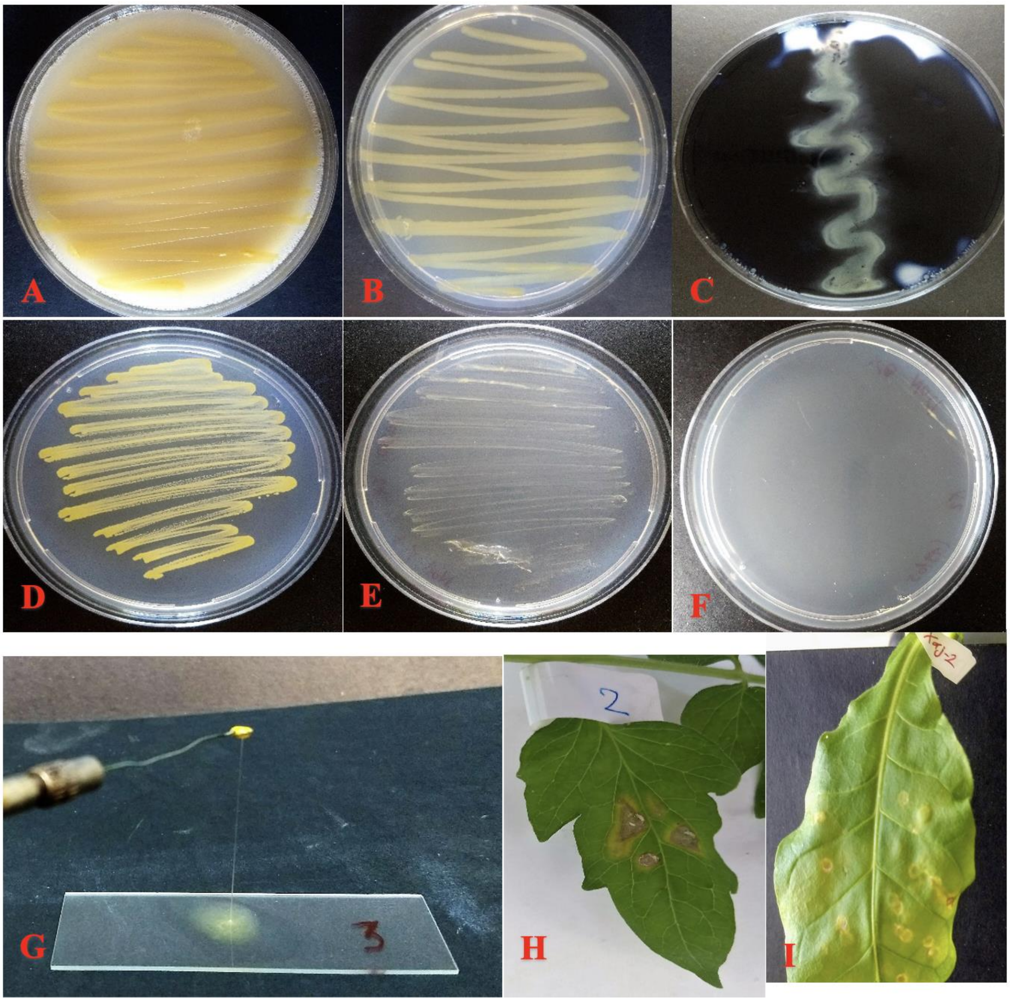

Figure 3. Biochemical and Hypersensitive Response (HR) test results of pathogen isolates. A: YDCA medium, B: SNA medium, C: Starch hydrolysis test, D: Growth in 2% NaCl medium (Positive), E: 5% NaCl (Weak positive), F: 8% NaCl (Negative), G: KOH test (*Xaj*-3), H: HR test in tomato (*Xaj*-1), I: HR test in tobacco

Pathogenicity and Virulence Test Results

Isolates that showed positive reactions in the HR tests were subjected to pathogenicity and virulence tests on walnut seedlings. Among the isolates, three showed positive results in the pathogenicity test, but the *Xaj*-2 isolate was more effective than the others. After 7 days, the isolates caused brown-black spots on the leaves, and these spots grew and merged after 14 days, leading to the formation of necrotic tissue. After 28 days, these necrotic formations expanded further, causing drying at the leaf edges and wilting throughout the leaf. No necrotic tissue formation was observed in the control application.

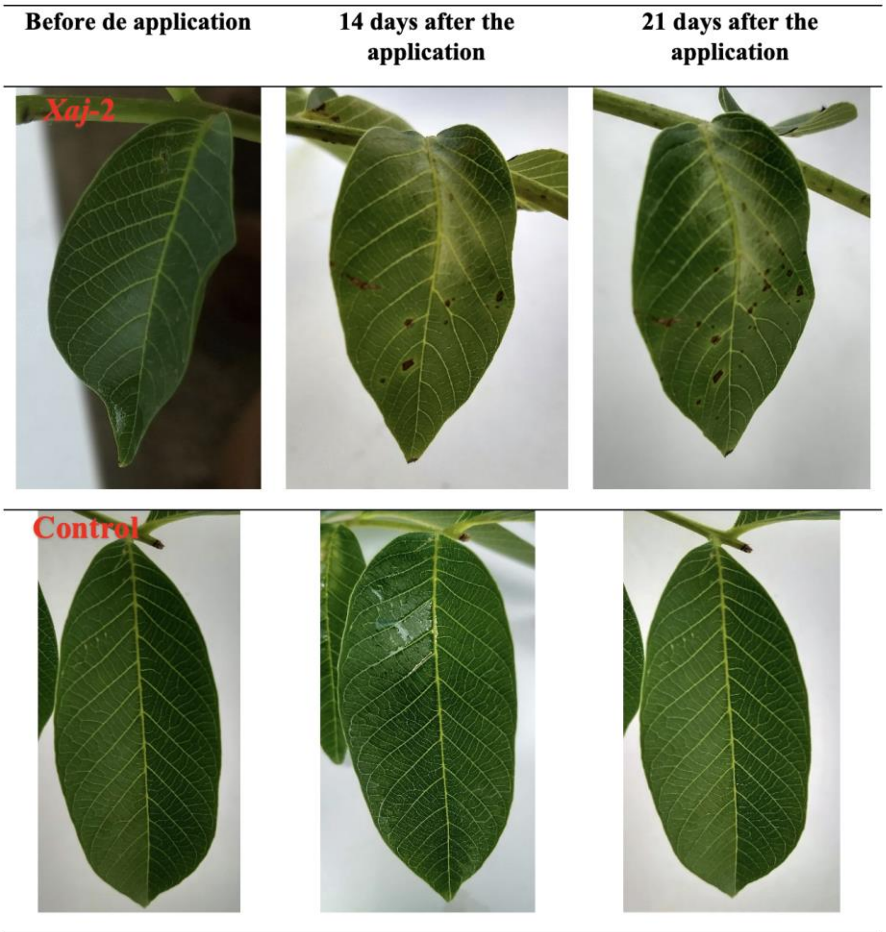

Figure 4. Pathogenicity and Virulence Test of *Xaj*-2 Isolate on Walnut Leaves

In the pathogenicity test conducted on fruits, superficial black spots appeared at the *Xaj*-2 inoculation sites after 7 days. No spots were observed at the inoculation sites in the control group. The *Xaj*-2 isolate showed the highest virulence, as it induced the formation of small spots on the fruits after 48 hours, and by the end of the 7th day, the average diameter of the necrotic lesion was measured as 2.91 mm. No necrotic tissue formation was observed in the control application. According to the scale of Erdal & Özaktan (20111), the *Xaj*-2 isolate exhibited 73% effectiveness.

Table 2. Pathogenicity and Virulence Test Results on Walnut Fruits

Bacterial isolate	**Pathogenicity test**	**Spot diameters at day 7 (mm) - Virulence test**			**Average spot diameter at day 7 (mm)**	**Effect (%)**
		1	**2**	**3**		
***Xaj*-2**	+	2,75	2.75	3,25	2,91	73
Control (H2O)	-	0	0	0	0	0

Before de application	7 days after the application

Figure 5. Pathogenicity and Virulence Test of *Xaj*-2 Isolate on Walnut Fruits

DISCUSSION AND CONCLUSION

According to FAO 2019 walnut production data, Turkey ranks fourth in the world with 215,000 metric tons of walnut production, accounting for approximately 6% of the global walnut production (FAO, 2019). *Xanthomonas arboricola* pv. *juglandis*, which causes bacterial blight in walnuts, leads to significant quality and quantity losses. It is one of the common bacterial diseases affecting walnut (*Juglans regia* L.) plant production (Yılmaz, et al., 2017). Controlling *Xaj*'s disease is challenging due to its dormant stage during winter. The yellow pigments (Xanthomonadines) of *Xanthomonas* species serve as distinctive characteristics for their identification. For diagnosing walnut bacterial blight agent *Xaj* and other *Xanthomonas* species, various morphological, biochemical, molecular, and pathogenicity tests are employed. Apart from classic tests containing biochemical and physiological features, protein and fatty acid analyses have been used in recent years to differentiate *Xanthomonas* species into pathovars (Lelliot and Stead, 1987; Klement, et al., 1990; Erdal and Özaktan, 2011).

In this study, colony morphology, certain biochemical tests (KOH, growth in YDCA, growth in 2%-5% and 8% NaCl, and starch hydrolysis), HR tests in tobacco and tomato, and pathogenicity tests in walnut plants and fruits were performed to identify the bacterial isolates used as pathogens. The results obtained from these tests showed similarities with the outcomes of previous studies conducted in Turkey by Erdal & Özaktan (2011) and Yörük and Mirik

(2018). Similar to the findings in Erdal and Özaktan (2011), our study revealed that pathogenicity tests on tomatoes were easier to assess compared to the pathogenicity tests on tobacco.

By reviewing the literature on *Xaj* disease, attempts have been made to identify walnut varieties that are susceptible to *Xaj* disease. According to various studies, in Greece, Tsiantos et al. (2007) determined that Payne and Serr were moderately susceptible, while Chandler was highly susceptible. In France, Tamponi (1990) reported that Serr and Payne varieties suffered significant losses due to *Xaj* disease in walnut fruits. In Spain, Moragrega et al. (2011) stated that Chandler and Hartley were the most affected varieties by *Xaj* disease. In Turkey, it was indicated that the commercially grown walnut varieties, Chandler and Hartley, showed high susceptibility to this disease (Erdal and Özaktan, 2011). Therefore, in this study, considering the above-mentioned results and Chandler's high susceptibility to *Xaj*, the Chandler variety was used.

REFERENCES

Aysan, Y., Benlioğlu, K., Çentinkaya, R., Karahan, A., Kotan, R., Özaktan, H., . . . Şahin, F. (2013). *Bitki Bakteri Hastalıkları.* İzmir: Çurkurova Üniversitesi.

Erdal, M., & Özaktan, H. (2011). *Batı Anadolu' da Ceviz Bakteriyel Yanıklığı Etmeni Xanthomonas arboricola pv. juglandis' in Tanısı ve Entegre Mücadele Olanakları Üzerine Bir Araştırma.* Bornova-İZMİR: Ege Üniversitesi Fen Bilimleri Enstitüsü (PhD Thesis).

FAO. (2019). *Food and Agriculture Organization of the United Nations.* FAOFAST: http://www.fao.org/faostat/es/

FIrat. (2006). *Ceviz yetiştiriciliği Raporu ve Yaturum Rehberi.* Tunceli

Klement, Z., & Goodman, R. N. (1967). The Hypersensitive Reaction to Infection by Bacterial Plant Pathogens. *Annual review of Phytopathology* (5), 17-44.

Lelliot, R. A., & Stead, D. (1987). *Methods for the Diagnosis of Bacterial Diseases of Plants.* Oxford, USA: Black Well Scientific Publication.

Moragrega, C., Matias, J., Aletà, N., Montesinos, E., & Rovira, M. (2011). Apical necrosis and premature drop of Persian walnut fruit caused by *Xanthomonas arboricola pv. juglandis*. *Plant dis., 95*, 1565-1570.

Schaad, N., Jones, J., & Chun, W. (2001). *Laboratory Guide for Identification of Plant Pathogenic Bacteria* (3nd ed.). St paul, USA: APS Press.

Sinavimo, (2018). *Descripción de Xanthomonas arboricola pv. juglandis.* Sistema Nacional de Vigilancia y Monitoreo de Plagas: https://www.sinavimo.gov.ar/plaga/*Xanthomonas-arboricola*-pv-*juglandis*

Swings, J., Vautrerin, L., & Kersters, K. (1993). The bacterium *Xanthomonas*. En: *Xanthomonas*. *Chapman & Hall, London, United Kingdom*, 121-156.

Tamponi, G. (1990). Walnut cultivars performance in central Italy. *Acta Hortic.*, *284*, 183-184.

Tsiantos, J., Vagelas, I. K., Rumbos, C., Chatzaki, A., Rouskas, D., & Gravanis, F. (2007). Evaluation of resistance of cultivated walnut varieties and selections to *Xanthomonas arboricola pv. juglandis* in Greece. *NAGREF Plant Protection Institute*.

Vauterin, L., Hoste, B., & Kersters, K. S. (1995). Reclassification of *Xanthomonas*. International. *Journal of Systematic Bacteriology, 45*, 472-489.

Yılmaz, A., Aydar, A., Özdem, A., Yeğül, M., Karacaoğlu, M., Öztürk, A., . . . Uzunok, S. (2017). *Ceviz entegre mücadele teknik talimatı.* Adana. Retrieved from T.C. Gıda Tarım ve Hayvancılık Bakanlığı.: www.tarim.gov.tr/TAGEM

Yörük, B., & Mirik, M. (2018). *Ceviz Bakteriyel Yanıklık Hastalık Etmeni Xanthomonas arboricola pv. juglandis'e Karşı Antagonist Bakteriyel İzolatların in vitro Koşullarda Biyokontrol Etkinliklerinin Belirlenmesi.* Tekirdağ: Namik Kemal Üniversitesi (Master Thesis).

Estimation of Pistachio Production Amount Between 2022-2030 with ARIMA Model, TURKEY

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ABSTRACT: This study aims to predict how the production amount of pistachios in Turkey will change between 2022-2030. The pistachio production amount (tons) for the study was obtained from FAO between 1977-2021. ARIMA model was used in the study. As a result of the analysis, forecast results were obtained for the specified years by choosing the ARIMA (1,1,1) model. As a result of the analysis, it was found that there was a constantly increasing amount of production between 1977 and 2021, and that production would show a similarly increasing trend between 2022 and 2030, and various policy recommendations were offered to preserve continuity.

Keywords: *Pistachios*, Production amount, ARIMA model

INTRODUCTION

The pistachio, which has a production history dating back to ancient times in Turkey, is a shelled fruit belonging to the cashew family (Aygün and Gürsoy, 2020). It is a species that particularly thrives in arid regions, especially between the parallels of 30° and 45° worldwide. Despite its high tolerance to stony, rocky areas, and dry conditions, it requires careful cultivation (Ayfer, 1990; Tilkat, 2006). Pistachio production is carried out in approximately 21 countries worldwide, with two main genetic centers identified in Central Asia and the Middle East (Öztep and Işın, 2023). The pistachio was first cultivated in the United States in 1957 by Yavuz et al. (2016). Leading pistachio-producing countries globally include the United States, Iran, China, Turkey, and Syria. In 2021, the distribution of pistachio production among countries was as follows: The United States at 57.2%, Iran at 14.7%, Turkey at 13%, China at 8.6%, and Syria at 4.7% (FAO, 2023). Accordingly, the United States holds the highest share in global pistachio production.

Analyzing yield values per hectare over the past six years, the top three countries are the United States at 2996 kg/ha, China at 2957 kg/ha, and Turkey at 1351 kg/ha (Anonymus, 2022).

Pistachio production in Turkey is predominantly concentrated in the Southeastern Anatolia region. Gaziantep, Şanlıurfa, Adıyaman, Siirt, Kahramanmaraş, and Kilis are significant contributors to production. In terms of their respective shares in production for the year 2022, Şanlıurfa holds 44.7%, Gaziantep 23.4%, Siirt 12.8%, Adıyaman 11.3%, Kahramanmaraş 1.6%, and Kilis 1.1% (TUİK, 2023).

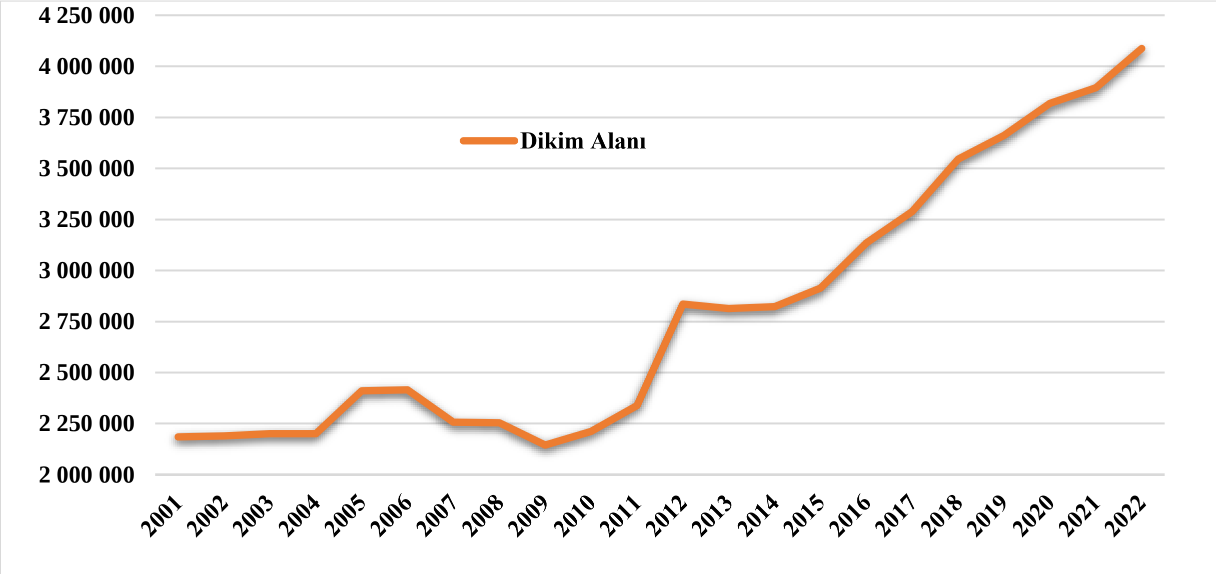

Figure 1. Türkiye pistachio planting areas (decares) (TUIK, 2023)**.**

Between 2001 and 2022, the planting areas for pistachios in Turkey have generally increased, with occasional declines in some years. The most significant increase in planting areas occurred, particularly in recent years, reaching 4,087,086 hectares in 2022 (Figure 1).

In Turkey, especially in recent years, the increase in the number of kunefe and baklava shops in every province has boosted domestic demand for pistachios. Consequently, this development has led to an increase in pistachio prices. With the rise in domestic demand, there has been an increase in the number of enclosed gardens practicing modern cultivation, especially in Gaziantep and Şanlıurfa provinces (Temel and Aksoy, 2020; Avcioglu and Aksoy, 2021).

On the other hand, the average pistachio yield in Turkey from 2001 to 2022 is 40.1 kg/ha. By province, Gaziantep with 35.8 kg/ha, Şanlıurfa with 38.4 kg/ha, Adıyaman with 37.2 kg/ha, and Kilis with 38.3 kg/ha fall below the national average, while Siirt with 50.4 kg/ha and Kahramanmaraş with 71.0 kg/ha surpass the national average (TUİK, 2023).

Pistachios play a significant role in international trade, with global exports reaching approximately \$3.2 billion. The United States leads the way with \$1.47 billion in pistachio exports (Anonymus, 2022). Over the 22-year period, Turkey has experienced fluctuations in exports, with a slight increase in the final years after a trend of losing export share each year. The increasing use of pistachios in the sweet sector in Turkey, particularly in recent years, has resulted in almost the entire produced quantity being consumed domestically. The rise in domestic consumption and the ongoing conflict in Syria have led to the importation of a certain amount of pistachios produced in Syria (Table 1).

Table 1. Foreign trade of pistachios

	İhracat	İthalat	Üretim	İhracat/Üretim	İthalat/Üretim
Yıllar	ton	ton	ton	%	%
2001	4947	28	30000	16.5	0.1
2002	1936	107	35000	5.5	0.3
2003	1038	68	90000	1.2	0.1
2004	757	120	30000	2.5	0.4
2005	823	37	60000	1.4	0.1
2006	864	79	110000	0.8	0.1
2007	975	84	73416	1.3	0.1
2008	2621	84	120113	2.2	0.1
2009	2376	197	81795	2.9	0.2
2010	717	14	128000	0.6	0.0
2011	1160	10	112000	1.0	0.0
2012	2328	0	150000	1.6	0.0
2013	3948	266	88600	4.5	0.3
2014	821	15	80000	1.0	0.0
2015	3154	15	144000	2.2	0.0
2016	4710	29	170000	2.8	0.0
2017	2706	8	78000	3.5	0.0
2018	4777	98	240000	2.0	0.0
2019	15951	15365	85000	18.8	18.1
2020	15614	13414	296376	5.3	4.5
2021	23390	16163	119355	19.6	13.5
2022	14509	9468	239289	6.1	4.0

(TUİK, 2023 and TradeMap 2023)

MATERIAL AND METHOD

In the study, data on global pistachio production from 2015 to 2020 were used for evaluating world pistachio production. For time series analysis, data on Turkey's pistachio production quantity from 1961 to 2021 were included in the assessment.

In the time series, the first step for selecting the most suitable model involves checking whether the series is stationary, and various unit root tests are applied to non-stationary series. The Box-Jenkins model assumes that time series are stationary. If a series is stationary, it has a constant mean, constant variance, and constant autocorrelation. Once stationarity is achieved, the next step is to determine the values of p and q in the ARIMA (p, I, q) model, where I indicates how many differences need to be taken to obtain a stationary series (Anonim, 2022).

The ARIMA (p, d, q) Box-Jenkins Model, proposed by Box and Jenkins, is a widely used method for constructing a univariate time series forecasting model (Mensah, 2015).

The Box-Jenkins ARIMA model approach was defined in a book published by statisticians George Box and Gwilym Jenkins in the 1970s. An ARIMA process is a mathematical model used for prediction. The Box-Jenkins modeling involves identifying an appropriate ARIMA process, fitting it to the data, and then using the suitable model for forecasting. One attractive feature of the Box-Jenkins approach for forecasting is that ARIMA processes encompass a rich class of models and often provide adequate explanations for the data (Hyndman, 2001). According to Box and Jenkins (1976), a non-seasonal ARIMA model is represented by ARIMA (p, d, q), where d represents the difference, p represents autoregressive coefficients, and q represents moving average coefficients (Dasyam et al., 2015).

These values are determined using autocorrelation functions (ACF) and partial autocorrelation functions (PACF) and the Dickey Fuller (ADF) test (Awal and Sıddıque, 2011). ACF and PACF shapes are used as tools to determine the stationarity of variables and the lag length of the ARIMA model when predicting ARIMA models. The Dickey Fuller (ADF) test is applied to non-stationarity during stationarity testing. PACF or partial correlogram is used to determine the appropriate number of lags for the AR model. The number of non-zero relationships in PACF determines where AR lags should be included. The ACF correlogram is used to determine the number of lags for the MA model, where again, non-zero relationships indicate where lags should be included (Anonim, 2019).

An AR model function is as follows:

$$Yt = c + Ø1\ Yt\text{-}1 + Ø2\ Yt\text{-}2 + \ldots + Øp\ Yt\text{-}p + \varepsilon_t$$

Here, c is a constant term, Øp p-otoregressive parameter and ε_t is the error term at time t.

The MA model function is:

$$Yt = c + \varepsilon_t - Q1\ \varepsilon_t\text{-}1 - Q2\ \varepsilon_t\text{-}2 - \ldots\ldots - Qq\ \varepsilon_t\text{-}q$$

Here, c is a constant term, θq, q-th moving average parameter, and et-k, is the error term at time t.

Generally, the ARIMA model is as follows:

$$\Delta d\ Zt = c + (Ø1\ \Delta d\ Zt\text{-}1 + \ldots + Øp\ \Delta d\ Zt\text{-}p) - (\theta 1\ \varepsilon_t\text{-}1 + \ldots + \theta q\ \varepsilon_t\text{-}q) + \varepsilon_t$$

Here, Δ denotes the difference as shown below: Zt-1, Zt-p, are the values of the past series with delays of 1, p, respectively.

$$\Delta\ Zt = Zt - Zt\text{-}1$$

$$\Delta 2\ Zt\text{-}1 = \Delta Zt - \Delta Zt\text{-}1$$

RESULTS AND DISCUSSION

In this stage of the study, descriptive statistics, unit root tests, ARIMA model results, and forecasts for the future, along with control tests, are presented. Firstly, descriptive statistics indicate that the average pistachio production quantity in Turkey is 69,876.78 tons (Table 2). Examining the highest and lowest production values over the years in Turkey, it can be stated that production varies from year to year. In Turkey, the total pistachio planting areas increased by 77.02% from 2.20 million hectares in 2004 to 3.89 million hectares in 2021. The pistachio production quantity in Turkey, which was 30 thousand tons in 2004, increased by 297.85% to 119.36 thousand tons in 2021. During the analyzed period, significant fluctuations occurred in the production quantity and the average yield per pistachio tree due to the impact of periodicity (Öztep and Işın, 2023).

Table 2. Descriptive Statistics Values

Descriptive İstatistic	Turkey (tons)
Mean	69876
Median	50000
Maximum	296376
Minimum	63000
Std. Dev.	59978.85
Skewness	1.814904
Kurtosis	6.776827
Jarque-Bera	51.44988
Probability	0.000000
Sum	3144455.
Sum Sq. Dev.	1.58E+11
Observations	45

The stationary nature of the production series, the presence of structural breaks, and whether it exhibits a normal distribution have been tested. The series was examined using autocorrelation (ACF) and partial autocorrelation (PACF) correlograms, and the stationarity of the series was tested using Augmented Dickey-Fuller (ADF) and Phillips-Perron unit root tests. The unit root test was conducted at both the level and the first difference. The analysis results indicate that, according to the PP test, the series became stationary at both the level and the first difference for models with a constant, a constant trend, and no trend. In the ADF unit root test, it was observed that the series became stationary at the first difference (Table 3). Therefore, it was decided that the production series is suitable for forecasting.

Table 3. Unit Root Test Results (ADF-PP)

UNIT ROOT TEST TABLE (PP)

At Level		
TURKIYE		
With Constant	t-Statistic	-4.2910
	Prob.	***0.0014*** ***
With Constant & Trend	t-Statistic	-8.9935
	Prob.	***0.0000*** ***
Without Constant & Trend	t-Statistic	-1.7777
	Prob.	***0.0718*** *
At First Difference		
d(TURKIYE)		
With Constant	t-Statistic	
	Prob.	***0.0001*** ***
With Constant & Trend	t-Statistic	-33.6053
	Prob.	***0.0000*** ***
Without Constant & Trend	t-Statistic	-25.0096
	Prob.	***0.0000*** ***

UNIT ROOT TEST TABLE (ADF)

At Level

		TURKIYE
With Constant	t-Statistic	2.0384
	Prob.	***0.9998***
		n0
With Constant & Trend	t-Statistic	-2.3329
	Prob.	***0.4080***
		n0
Without Constant & Trend	t-Statistic	3.5090
	Prob.	***0.9998***
		n0

At First Difference

		d(TURKIYE)
With Constant	t-Statistic	-8.6640
	Prob.	***0.0000***
		***
With Constant & Trend	t-Statistic	-7.0928
	Prob.	***0.0000***
		***
Without Constant & Trend	t-Statistic	-20.9825
	Prob.	***0.0000***
		***

Notes: (*)Significant at the 10%; (**)Significant at the 5%; (***) Significant at the 1%. and (no) Not Significant

*MacKinnon (1996) one-sided p-values.

This Result is The Out-Put of Program Has Developed By:

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In the study, the most suitable ARIMA (1,1,1) model was employed. The findings related to the ARIMA (1,1,1) model are presented in Table 4. When considering the prob. values of the AR(1) and MA(1) coefficients, statistical significance was observed at the 1% significance level. The R-squared value indicates that the model is statistically significant as a whole, and the high Log likelihood value, along with the Durbin-Watson test falling within the standard value range, demonstrates the significance of the model.

Table 4. ARIMA Model Results.

Dependent Variable: D(TURKIYE)
Method: Least Squares
Date: 11/06/23 Time: 16:02
Sample (adjusted): 1979 2021
Included observations: 43 after adjustments
Convergence achieved after 8 iterations
MA Backcast: 1978

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	4153.913	1326.407	3.131703	0.0032
AR(1)	-0.780809	0.132295	-5.902025	0.0000
MA(1)	-0.527005	0.169116	-3.116222	0.0034

R-squared	0.772625	Mean dependent var	2629.186
Adjusted R-squared	0.761256	S.D. dependent var	64371.56
S.E. of regression	31452.88	Akaike info criterion	23.61758
Sum squared resid	3.96E+10	Schwarz criterion	23.74046
Log likelihood	-504.7780	Hannan-Quinn criter.	23.66290
F-statistic	67.96024	Durbin-Watson stat	2.031793
Prob(F-statistic)	0.000000		

Inverted AR Roots	-.78
Inverted MA Roots	.53

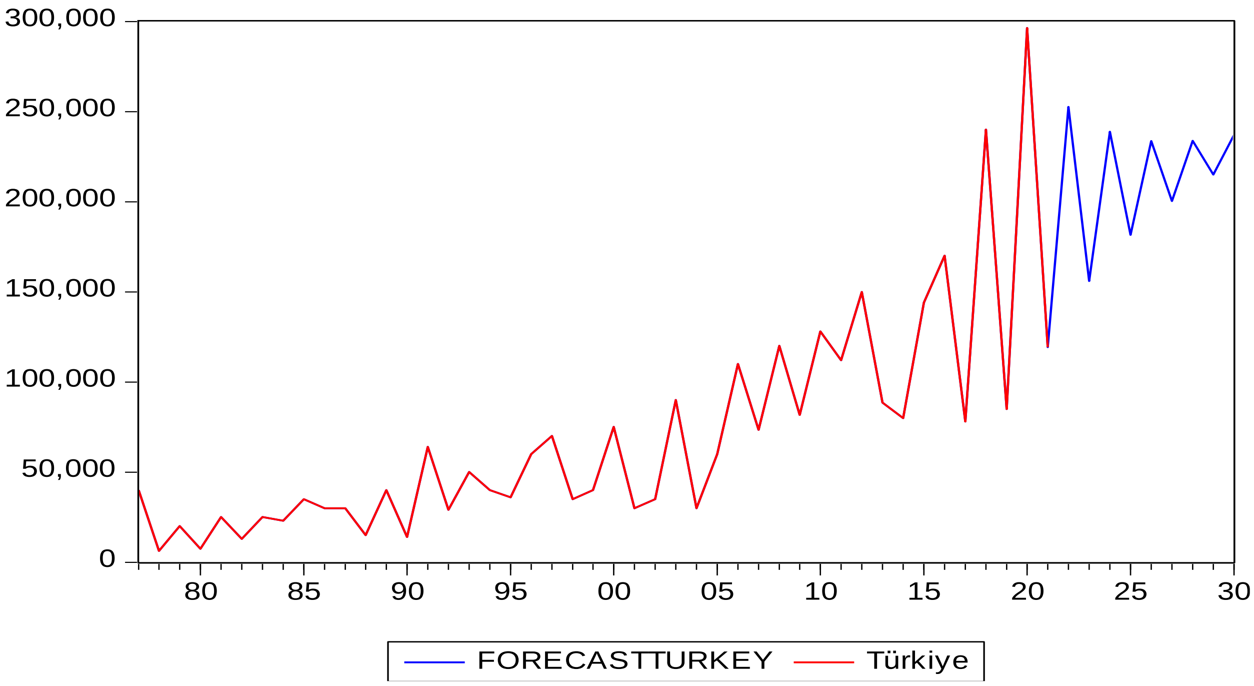


Figure 2. Production Amount Change between 1977-2030 and 2022-2030 Forecast Chart

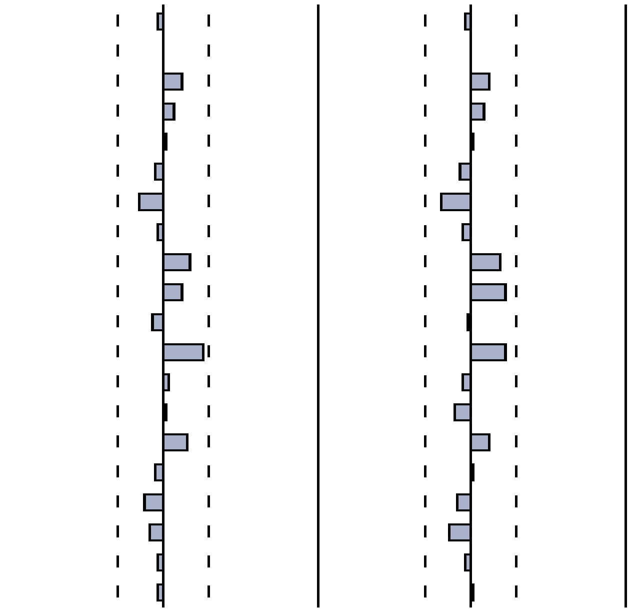
After determining the adequacy of the model, production forecasts were made. Both dynamic and static prediction methods can be used for pistachio production using the selected model. Dynamic forecasting calculates multi-step forecasts for subsequent examples starting from the first example. The forecast value of the previous year is preferred for making predictions. Therefore, for future years, dynamic forecasting method (ex-ante) has been used. An increase in production is observed in Turkey between the years 2022 and 2030 (Figure 2). The increase in planting areas (Figure 1) is expected to directly impact the production quantity. On the other hand, this situation may provide producers with more opportunities for product sales and consumers with the possibility of reaching pistachios at a more reasonable price. However, this situation may also lead to a decrease in pistachio prices due to an oversupply in the market compared to the existing domestic demand, resulting in a reduction in the prices received by producers.

The autocorrelation and partial correlation prob. values associated with the model's forecast reject the null hypothesis (H0), indicating that these model results are significant (Figure 3).

Correlogram of RESID

Date: 11/06/23 Time: 15:59
Sample: 1977 2030
Included observations: 43

Autocorrelation | Partial Correlation



	AC	PAC	Q-Stat	Prob
1	-0.025	-0.025	0.0294	0.864
2	0.006	0.005	0.0309	0.985
3	0.122	0.122	0.7515	0.861
4	0.079	0.087	1.0628	0.900
5	0.019	0.023	1.0805	0.956
6	-0.061	-0.078	1.2761	0.973
7	-0.170	-0.201	2.8212	0.901
8	-0.034	-0.066	2.8847	0.941
9	0.175	0.204	4.6284	0.865
10	0.120	0.232	5.4677	0.858
11	-0.078	-0.013	5.8388	0.884
12	0.264	0.224	10.199	0.599
13	0.036	-0.061	10.284	0.671
14	0.013	-0.101	10.296	0.740
15	0.171	0.128	12.308	0.656
16	-0.060	0.012	12.562	0.704
17	-0.126	-0.086	13.735	0.686
18	-0.082	-0.152	14.258	0.712
19	-0.027	-0.041	14.318	0.765
20	-0.033	0.011	14.409	0.809

Figure 3. Autocorrelation and partial autocorrelation plot of residuals

CONCLUSION

In this research, using data from the period 1977-2021 and the ARIMA (1,1,1) model, the pistachio production quantity in Turkey for the period 2022-2030 has been predicted. According to the research results, the forecast data for the upcoming years from 2022 to 2030 indicate a fluctuating production model for pistachios in Turkey. On the other hand, it is projected that the lowest production will occur in the year 2023 (155,970 tons), and the highest production will be in the year 2030 (237,083 tons). The main reason for this increase is believed to be the rise in the number of newly established pistachio orchards in recent years. Additionally, due to the periodicity feature of pistachios, fluctuations in pistachio production are expected to occur from year to year between 2022 and 2026.

The increase in production is expected to have a positive impact on exports; otherwise, the increases in production capacity in previous years have not been sufficiently reflected in exports. Therefore, careful attention to various issues is crucial to bring about changes in the export situation. Given the low level of productivity in production, it is advisable to prefer varieties that will increase productivity. Opting for irrigated production instead of production in arid and rocky areas can be preferable. In this regard, engineers, economists, academics specializing in this field, and farmers should be brought together through educational programs in various institutions and organizations. Additionally, the higher local market price compared to the international price encourages producers to market their products in the domestic market. Therefore, government efforts, training, new policies, and various R&D studies aimed at increasing productivity can guide producers to introduce their products from the domestic market to the international market and contribute to an increase in exports.

Statement of Conflict of Interest

There is no conflict of interest between the author(s).

Authors' Contributions

All authors contributed to the writing of the article, participated in the publication process, and read and approved it.

REFERENCES

Anoymus, 2021. Global statistical review 2020-2021. International Nut and Dried Fruits Council Foundation, Spai

Awal, M. A., & Siddique, M. A. B. 2011. Rice production in Bangladesh employing by ARIMA model. *Bangladesh Journal of Agricultural Research*, *36*(1), 51-62.

Avcioglu, Ü., & Aksoy, A. 2021. Analysis of Correlation of Pistachio Production and Income with the Koyck Models in Turkey. *Alinteri J. of Agr. Sci. 36*(1): 71-76

Ayfer, M. 1990. Antepfıstığının dünü bugünü geleceği. *Türkiye*, *1*, 11-12.

Aygün, M., & Gürsoy, S. 2020. Antep Fıstığı (Pistacia vera L.) Üretimi Yapan İşletmelerin Tarımsal Mekanizasyon Düzeylerinin Belirlenmesi: Türkiye, Siirt İli Örneği. *Türkiye Tarımsal Araştırmalar Dergisi*, *7*(2), 136-142.

FAO, Birleşmiş Milletler Gıda ve Tarım Örgütü İnternet Sayfası, 2023 https://www.fao.org/faostat/en/#data/QI

Hyndman, R. J. 2001. ARIMA processes. *Datajobs: Data science knowledge*.

Mensah, E. K. 2015. Box-Jenkins modelling and forecasting of Brent crude oil price.

Öztep, R., & Ferruh, I. Ş. I. N. 2023. ARMA Modeli ile Türkiye Antep Fıstığı Üretimi Tahmini. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *26*(4), 878-887.

Temel, O., Aksoy, A. 2020. Türkiye Antepfıstığı Sektörünün Eşanlı Model Yöntemiyle Tahmini. *Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi*, *30*(1), 80-89.

Tilkat, E., Onay, A., Yıldırım, H., & Ayaz, E. 2009. Direct plant regeneration from mature leaf explants of pistachio, Pistacia vera L. *Scientia Horticulturae*, *121*(3), 361-365.

Trade Map, 2023. https://www.trademap.org/Country_SelProduct_TS.aspx?nvpm=1%7c%7c%7c%7c%7c080252%7c%7c%7c6%7c1%7c1%7c1%7c2%7c1%7c2%7c2%7c1%7c1

TUİK, 2023. Türkiye istatistikkurumu internet sayfası, https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr

Yavuz, M. A., Yıldırım, H., & Ahmet, O. N. A. Y. (2016). Dünya antepfıstığı üretiminde son on yılın değerlendirilmesi. *Batman Üniversitesi Yaşam Bilimleri Dergisi*, *6*(2/2), 22-31.

Cytotoxic and Genotoxic Effects of Copper Nanoparticles Synthesis and Characterized from Black Grass (*Lavandula stoechas L.*) by Green Synthesis Method on Rtg-2 Cell Line

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ABSTRACT: The aim of this study is to synthesize and characterize copper oxide nanoparticles (CuO NPs) using lavandula (*Lavandula stoechas* L.) extract and to determine the cytotoxic and genotoxic effects of these nanoparticles on the RTG-2 cell line. CuO NPs were synthesized by the green synthesis method with black pepper extract and were characterized by scanning electron microscopy (TEM), UV-Vis spectroscopy, Fourier transform infrared (FTIR) spectroscopy and X-Ray Diffraction method (XRD). Then, Sulforhadamine B (SRB) and comet tests were performed, respectively, to determine the cytotoxic and genotoxic effects of CuO NP on the RTG-2 cell line. Approximately 5 nm in size and spherical in shape, it has a maximum absorbance value at 211 nm, exhibiting (111) and (110) in the FCC phase, exhibiting a corresponding band corresponding to the metal-oxygen (C-O) vibration supporting the monoclinic phase at the 514.9 cm-1 position. CuO NP, a stable structure with indexed planes, has been successfully synthesized. CuO NPs showed cytotoxic and genotoxic effects on RTG-2 cells at high doses after 48 hours of exposure (LD50 = 50 µg/mL). Considering the doses in the literature at which commercially available CuO NPs synthesized by chemical means show their cytotoxic effects in vitro, the possibility of the green synthesis method having a toxicity-reducing effect has been demonstrated.

Keywords: CuO nanoparticle, Green synthesis, Cytotoxicity, Genotoxicity

INTRODUCTION

Nanotechnology is a field that conducts research on the manipulation of substances at the atomic level and the obtaining and usability of nano-sized materials with superior properties and useful structures. Nanotechnology products, which have been produced and used commercially since the early 2000s, have been used in many different sectors that serve social life with the advantages they provide (Bayda et al. 2019). Nanoparticles, according to the European Commission definition; Materials with unique properties and dimensions, with at least one dimension in the range of 1-100 nm (Kohl et al. 2020). Due to their large surface area and volume ratio, their chemical activities increase due to the increased number of atoms on the nanoparticle surface. The increase in this chemical activity is one of the features that make nanoparticles preferred in many areas (Gatoo et al. 2014). Metal nanoparticles differ from other nanoparticles due to their nanoscale potential and large surface area/volume ratio, as well as their melting-freezing points and improved conductivity (Roduner 2006). Copper oxide nanoparticles (CuO NPs) attract great attention due to their wide range of uses, low cost, and properties similar to noble metals. CuO NPs are widely used in sensors, heat transfer systems, electronics (fuel cell and solar cell), as catalysts in many reactions, and as antimicrobial agents in hospital equipment (Imran et al. 2017).

The increasing use of CuO NPs also increases the synthesis studies of these nanoparticles. Nanoparticles can be synthesized by physical, chemical and biological methods using two main approaches, "top-down" or "bottom-up". However, most physical and chemical synthesis methods are not preferred due to reasons such as high cost, multi-step, use of toxic solvents and pollution of the environment. Recently, especially environmentally friendly, low-cost biological synthesis methods have come to the fore. (Nagar et al. 2018; Nasrollahzadeh et al. 2019). Green synthesis method, which is one of the biological synthesis methods, is an environmentally friendly approach in which biological materials such as plant materials, microorganisms, proteins and enzymes are used. Due to the disadvantage of high contamination risk in syntheses using microorganisms, The green synthesis methods using plant materials are more commonly used. Although CuO NPs have many functions, a serious concern arises due to their possible adverse effects on human, animal and plant organisms. Nanoparticles easily pass through the defense mechanisms of living organisms and can cause genotoxicity, cytotoxicity, oxidative stress, inflammation and apoptosis in the organism as a result of interaction with organs and genetic material (Halıcı et al. 2021). The toxicity mechanisms of nanoparticles are still not fully understood. Researchers have suggested that the mechanism of toxicity is generally related to the reduced size of the material. Nanoparticles penetrating tissues and cell membranes, reacting with biological molecules, changing the potential charge, creating oxidative stress, interacting with DNA and causing accumulation can constitute the steps of toxicity mechanisms (Scherer et al. 2019). Studies on the cytotoxicity and genotoxicity of CuO NPs are very few. Among the organisms used in toxicity studies, aquatic creatures, which are the best indicators of pollution, stand out. Aquatic organisms have been widely used in toxicity studies of nanoparticles (Ray et al. 2009). However, in vitro systems (cell culture) provide the best preliminary experimental system to examine toxic mechanisms at cellular levels due to their advantages (controlled environment, cost-effective, reproducible, ethically non-problematic, standardized) (Castaño et al. 2003). . Since DNA repair rates in fish cells are lower than in mammalian cells, these cells are very important for genotoxicity studies. In 1962, the RTG-2 cell line obtained from the gonads of juvenile rainbow trout (Oncorhynchus mykiss) was first used to study fish viruses (Žegura and Filipič 2019). There are also studies on the toxicity of nanoparticles in RTG-2 cells. Although there are studies on the cytotoxic and genotoxic effects of commercially purchased CuO NPs on RTG-2 cells, no study has been found on the toxicity and genotoxicity of CuO NPs obtained by green synthesis (Işık, 2022).

Nanoparticles are solid particles with at least one dimension referred to as nano (Wanget al. 2009). Nanoparticles have a large surface area/volume ratio due to their small size. Additionally, nanoparticles are under the influence of quantum gravitational forces. Compared to micro and macro particle forms, nanoparticles exhibit more effective and desirable electrical, thermal and optical properties with the increase in the number of atoms on their surfaces (Saygılı et al. 2021). Thanks to these properties, nanoparticles are used in many industrial fields such as energy, construction, medicine, agriculture, biomedical, cosmetics, chemistry, electronics, communication and information storage (Sengul and Asamutlu 2020). Nanoparticles can be examined in four classes: carbon, inorganic, organic and composite based. Inorganic-based nanoparticles include metals such as gold (Au), silver (Ag), copper (Cu), platinum (Pt) and iron (Fe) and metal oxide nanoparticles, which are the oxidized forms of these metals (Yaprak 2019). Copper and copper oxide nanoparticles (Cu NP and CuO NP) are used in heat transfer systems, antibacterial materials, super strong materials, sensors, drug delivery, food packaging, remediation, due to their advantages such as unique physical and chemical properties and low production costs compared to other metal nanoparticles. has attracted the attention of scientists in wide applications such as construction materials and catalysts (Shiravand et al. 2017; Tamilvanan et al. 2017). The basic top-down and bottom-up approaches used in the synthesis of nanoparticles are shown in (Figure 1). (Sepeur et al. 2008).

In the top-down synthesis approach, it is essential to divide macro- and micro-sized matter into nano-sized pieces by transferring energy from the outside through physical, mechanical or chemical intervention. Mechanical or physical disintegration, abrasion, and grinding are top-down synthesis methods. In bottom-up synthesis, which is another approach, substances with very small molecular or atomic structures are enlarged in volume and mass using various chemical and biological methods and nanoparticles are formed (Tamilvanan et al. 2017; Mittal et al. 2014). Chemical reduction, sonochemical reduction, microemulsion techniques, electrochemical, hydrothermal, sol gel synthesis, polyol process and microwave-assisted techniques are used in the preparation of Cu NPs and CuO NPs by chemical methods. There are also physical methods such as laser ablation, vapor phase synthesis, mechanical milling and pulsed wire discharge (Tamilvanan et al. 2017).

Due to the presence of toxic chemicals in chemical synthesis methods, many negative effects and toxic by-products occur (Hasan 2015). The yields of physical and chemical methods are lower than the biological synthesis method. The green synthesis method is an environmentally friendly, cost-effective, non-toxic approach for the production of nanoparticles. Pharmacologically important compounds and parts of plants (leaves, roots, stems, barks and fruits), enzymes and microbial extracts are used for the reduction and stabilization of metal nanoparticles (Gnanajobitha et al. 2013). The biological compounds in the materials used in this synthesis have the feature of reducing and stabilizing metal salts into metal ions (Santhoshkumar et al. 2019). Green synthesis using plant parts has a higher potential than microorganisms because it is safe, simple and environmentally friendly (Moteriya et al. 2017).

The most important reason for using plants instead of bacteria or fungi in nanoparticle production is the lack of pathogenicity. Biosynthesis of nanoparticles is very easy with plant parts. It does not require special conditions as in chemical and physical methods. Plant parts have higher reducing properties than microbial culture media with the phytocompounds they contain, therefore it requires less time for the formation of nanoparticles (Zangeneh et al. 2019). However, the formation rate, quality and other properties of nanoparticles synthesized using plant parts also depend on factors such as the nature of the plant material, metal salt concentration, extract concentration, temperature, pH and reaction time (El-Seedi et al. 2019). The chemical synthesis methods used to obtain CuO NPs have disadvantages such as the use of multi-step reactions, formation of intermediate chemical products, and excessive energy consumption. For this reason, studies on the green synthesis of copper-based metal nanoparticles come to the fore. Saranyaadevi et al. (2014). reported that Cu NPs were synthesized using the leaf extract of the Capparis zeylanica plant. The synthesized Cu NP was confirmed by the color change after adding the leaf extract to the copper sulfate solution. It has been reported that the sizes of the synthesized Cu NPs are in the range of 50-100 nm and have a cubic structure. Nagar et al. (2018) synthesized Cu NPs using leaf juice of the Azadirachta indica plant and analyzed the effect of different reaction parameters such as precursor metal salt concentration, leaf water percentage, ambient temperature and pH on the transformation rate and morphology of Cu NPs. Researchers have reported that plant biomolecules induce the reduction of $Cu2+$ ions to Cu NPs and act as stabilizing agents. According to characterization studies using SEM, TEM, It has been reported that the optimum conditions for green synthesis are 20% leaf water percentage, $7.5 \times 10{-}3$ M $CuCl_2$ metal salt concentration, 6.6 pH and 85°C temperature. Mukhopadhyay et al. (2018) reported that the flower extract of Quisqualis indica was used to synthesize Cu NPs. They stated that the synthesized Cu NP had a cytotoxic effect on B16F10 melanoma cells and caused apoptosis through induction of oxidative stress, leading to upregulation of caspase-dependent and caspase-independent (AIF) apoptotic genes.

Hasheminya et al. (2020) reported the synthesis of Cu NPs with spherical shape and dimensions less than 40 nm with the aqueous extract of the Eryngium (Eryngium caucasicum trautv) plant. FTIR spectra of Cu NPs showed that the presence of flavonoids and phenolic functional groups could reduce Cu^{2+} ions into Cu NPs. Researchers reported that the aqueous extract of E. caucasicum trautv has significant potential to develop a clean, easy, cost-effective and efficient method for the green synthesis of Cu NPs. Lavandula stoechas is a well-known plant species and is widely used in the Mediterranean Region for its medicinal properties, such as treating rheumatic diseases and nephrotic syndromes, as an antispasmodic agent, and reducing pain and inflammatory problems, mainly due to its essential oil content. Studies on L. stoechas essential oils and extracts have revealed the antibacterial, antifungal, anti-leishmanial, antioxidant anti-inflammatory properties of this plant. However, no study has yet been conducted on the synthesis of Cu and CuO NPs with this plant and its extracts (Ez Zaubi et al. 2020). As the usage areas of CuO NPs develop, their release into the environment also increases. Therefore, living creatures in the ecosystem are exposed to nanoparticles. For this reason, concerns about the effects of CuO NPs on the ecosystem are increasing (Sengul and Asamutlu 2020). Studies have shown that CuO NPs have a higher toxic effect compared to other carbon and metal oxide nanoparticles (Karlsson et al., 2008, Wang et al., 2011; Perreault et al., 2012). In the aquatic environment, nanoparticles can accumulate, aggregate, and then form bottom sediments. A study reported that Cu NP and Ag NP concentrations in some rivers reached levels of 0.06 and 0.04 mg L^{-1}, respectively (Ostaszewskaet al. 2018). Metal-based nanoparticles have a higher potential for inhalation into the organism than bulk materials. In addition, since these nanoparticles are difficult to reduce and have a long half-life, they become difficult to excrete once they enter the organism. Although the mechanism of the toxic effect of metal nanoparticles on cells has not been fully explained, there are some suggestions. Due to their high surface area/volume area, metal nanoparticles show higher chemical reactivity, leading to the formation of reactive oxygen species (ROS), creating oxidative stress, increasing inflammation, affecting membrane membrane potential, weakening the antioxidant defense system, causing lipid peroxidation in tissues. It has been reported that it causes damage to tissues and organs as a result of accumulation in and organs (Kumari et al., 2009; Saygılı et al. 2021). Due to their small size, nanoparticles can pass through biological barriers to reach different organs and accumulate in these organs. It is known that CuO NP application causes disruption of the blood-brain barrier in mice and rats (Sharma et al. 2010).

It has been reported that nanoparticles, after entering the cell through passive diffusion, receptor-mediated endocytosis, can enter the nucleus by diffusion across the nuclear membrane or through nuclear pore complexes and damage DNA (Asare et al. 2012; Alarabyet al. 2020).

Studies on the toxicity of CuO NPs have been conducted on bacteria, algae, rat, human cell lines and aquatic creatures, which are the best indicators of environmental pollutants (Naz et al. 2020). Sadiq et al. (2015) evaluated the cytotoxic and genotoxic effects of Cu NPs using the Ames test, in vitro (Escherichia coli and Bacillus subtilis) cytotoxicity test, micronucleus test and comet test. They performed the Ames assay using two Salmonella typhimurium bacterial strains, TA98 and TA100. The genotoxicity of Cu NPs was evaluated on the monkey kidney cell line CHS-20. Cu NPs had mutagenic and dose-dependent cytotoxic effects on TA98 and TA100. Cu NPs led to a significant ($p < 0.01$) increase in the number of cells with micronuclei (96.6 ± 5.40) at the highest concentration (25 µg/mL). Researchers reported that 10 mg/mL Cu NPs caused DNA chain breaks and 5-10 mg/mL Cu NPs caused oxidative DNA damage. Ostaszewska et al. (2018) revealed dilated sinusoids, shrunken hepatocytes, nuclear necrosis and an increased number of Kupffer cells in the hepatocytes of rainbow trout (*Oncorhynchus mykiss*) exposed to 0.15 mg L-1 Cu NPs for 28 days. Ultrastructural studies have shown mitochondrial edema and crystolysis, enlarged and relaxed endoplasmic reticulum, cytoplasm vacuolation, lipid droplet accumulation, glycogen depletion and formation of myelin-like bodies. Griffitt et al. (2007) reported that the toxicities of copper sulfate and 50-80 nm sized CuO NPs in female zebrafish (*Danio rerio*) were different, and their LC50 values were 0.25 mg Cu/L and 1.56 mg Cu/L, respectively, at 48 hours of exposure. They reported that, as a result of exposure to both copper suspensions, dose-dependent damage was observed in the proliferation of epithelial cells and gill tissues in fish. They also stated that vacuoles formed in the livers of fish in CuO NP suspensions (0.25 mg/L-1.5 mg/L), but there was no histological damage in their internal organs. It has been reported that the epithelial cells between the gill lamellae increased by 40%, the filament width increased by 3 to 1.9 times compared to the control, and the gills stood out as the primary target organ in female zebrafish where CuO NP caused acute toxicity. The same researchers, in their study in 2008, found that the LC50 value of CuO NP (26.7 ± 7.1 nm) was 0.94 mg l−1 in adult zebrafish (Danio rerio) and 0.71 mg l−1 in juveniles after 48 hours of exposure, and in another study they conducted in 2009, They reported that application of 100 µg l−1 Cu NP for 48 hours increased the gill filament by 3.5 and 1.5 times compared to control and dissolved Cu applications, respectively (Griffitt et al. 2008; 2009). In vitro systems based on fish-derived cells are considered an alternative to in vivo assays. Cells provide an excellent experimental system to study toxic mechanisms at the molecular level. Additionally, because cultured cell lines are genetically homogeneous, they can contribute to a better understanding of genotoxic effects (Castano and Becerril, 2004). RTG-2 cells, an in vitro rainbow trout gonad cell line, are one of the fish cell cultures that stand out especially in toxicity studies because they have a high genetic similarity index with in vivo rainbow trout (Oncorhynchus mykiss), do not require an exogenous metabolic system, and maintain their ability to metabolize toxic substances. (Kocan et al. 1979; Bols et al. 1985; Kammann et al. 2001; Nehls et al. 2001; Castano et al. 2003; 2004; Caminada et al. 2006; Žegura and Filipič 2019).

Several aspects of CuO NP toxicity in cellular systems remain unknown. The genotoxicity of nanoparticles is of great concern as altering genetic material can promote cancer development (Singh et al. 2009). Nanoparticles have been shown to induce DNA changes in two basic ways: Nanoparticles with a size of 1-2 nm can come into direct contact with DNA chains, or DNA damage can occur through oxidative stress induced by nanoparticles. Previous studies using human lung epithelial cells A549 have shown that CuO NPs may have detrimental effects on DNA integrity (Karlsson, 2010; Ahmed et al. 2010). However, further research is needed to provide a better understanding of the genotoxicity risk of CuO NPs.

MATERIAL AND METHOD

Preparation of Plant Extract

Dry forms of commercially supplied black pepper were washed with distilled water to remove dirt and dust, and were laid on blotting paper and left to dry. After the drying process was completed, the black-headed herb was divided into small pieces by hand and 15 g of black-headed herb was added to 400 ml of distilled water. The samples were covered with aluminum foil to prevent light exposure. It was mixed in a magnetic stirrer (1000 rpm) for 24 hours for homogenization and dispersion of the biological components of black pepper into distilled water. Then, the plant extract was filtered and transferred to falcon tubes (50 ml). Falkon tubes were centrifuged 4 times at 5000 rpm at 24°C for 5 minutes. In each centrifugation process, the supernatant part of the samples was transferred to new tubes and the pellet part was discarded. The pH values of the resulting plant extract were measured as 6.6. The prepared plant extract was stored at +4°C to be used in CuO NP synthesis.

Copper Oxide Nanoparticle (CuO NP) Synthesis

Copper acetate solution was prepared by adding 36 g of copper acetate ($Cu(CH3COO)^2$) to 500 mL of distilled water in a magnetic stirrer (20°C). Then, 25 ml of copper acetate solution (10:1 ratio) was added to 250 ml of plant extract, covered with aluminum foil to prevent light, and left on a magnetic stirrer at 60-70°C for 2 hours. Color changes were observed over a 2-hour period. The resulting color change was accepted as a sign of the conversion of copper (Cu) ions into copper nanoparticles (CuO NPs). Samples were centrifuged at 10000 rpm at 24°C for 20 minutes. Then, the supernatant was discarded and the pellet was washed once with distilled water. Finally, CuO NPs were transferred to beakers and dried in the oven at 80°C for 24 hours. After the drying process was completed, CuO NPs were scraped from the beakers and stored in sterile tubes, covered with aluminum foil to prevent light and at room temperature (Iravani et al. 2011).

Characterization of CuO NPs

Characterization of CuO NPs synthesized from black pepper by the green synthesis method, using Transmission electron microscopy (TEM), Ultraviolet and visible light absorption spectroscopy (UV-Vis), X-Ray Diffractometer (XRD) and Fourier Transform Infrared Spectroscopy (FTIR), Atatürk University It was built by the Eastern Anatolia High Technology Center (DAYTAM).

In this study, the synthesized CuO NPs were imaged with a Hitachi HighTech HT7700 TEM device and characterized in terms of size, shape, aggregation, absorption and distribution.

CuO NPs were sonicated to show good dispersion and were characterized by UV-Vis spectroscopy analysis at wavelengths of 200-875 nm.

It was characterized by determining the numerical values of the functional groups present in CuO NPs by Fourier transform infrared spectroscopy (FTIR) analysis. The XRD pattern of CuO NPs synthesized using the PANalytical Empyrean XRD device was analyzed in the 2θ range and between 10° and 90° with a step size of 0.02.

Preparation of RTG-2 Cells

RTG-2 cell line was obtained from the Turkish Foot and Mouth Institute (Ankara) as passage 10. Medium for RTG-2 cells was prepared by adding 500 µL Penicillin-Streptomycin, 5 mL Fetal Bovine Serum and 44.5 mL Eagle's Minimal Essential Medium (EMEM) to 50 mL sterile falcon tubes. RTG-2 cells were incubated with 5 mL of medium in accordance with the protocol given by the Turkish Foot and Mouth Institute, in 25 cm2 sterile filter-free capped cell growth flasks, in an oven set at 23.7°C, without CO2 respiration. Every two days, the flasks were examined under an inverted microscope for contamination, cellular waste, medium color, and surface coverage of the cells, and the medium was changed for the cells that were sure to be free of contamination (Işık, 2022).

Cell Standardization Process

Cells can be of different sizes depending on their type. Standardization was carried out by taking into account the areas covered by the cells and their growth rates, depending on the cells being of different sizes. Standardization process applications were carried out by taking into account the 48 hours, which was the dosing period in the study. During the process, the number of cells covering 80% of the plate surface was taken into account. 25,000 cells/well (h/k) for 48 hours of application in a 96-well plate is accepted as the standard for the study (Işık, 2022).

Preparation of Stock Solution and Positive Control Solution of CuO NP

For the stock solution, 200 µg of CuO NPs were weighed and dissolved in 1 mL of medium. It was mixed in an ultrasonicator for 10 minutes to ensure homogenization. For positive control, 10% DMSO was prepared.

Cell Viability Test (Sulforhodamine B)

Cell transplantation (n=6) was performed on 96-plate plates in a sterile cabin at the specified standards. After 24 hours, the cells adhered to the bottom of the plate and proliferated by dividing. Then, the medium was removed from the wells and doses were applied. At the end of the 48-hour dosing period, 100 µL of 10% Trichloroacetic acid (TCA) solution was added to each well to fix the cell proteins formed in the plaques. The plates were then incubated at +4°C for 1.5 hours. After incubation, the plate was inverted and the TCA solution was removed and washed 5 times with distilled water. After each wash, the plate was inverted and distilled water was removed. The plates were dried in a sterile cabinet with ventilation. SRB solution was added to each well as 50 µL and incubated for 30 minutes in the dark at room temperature. After incubation, the SRB solution was removed from the plate by inverting and washed 5 times with 1% acetic acid to separate the unbound SRB. After each acetic acid wash, the plate was inverted to remove acetic acid. The plates were then left to dry in a sterile cabinet. After ensuring that there were no water drops left in the wells of the plate, 150 µL of 10 mM Tris base was added to each well and the plates were kept on a shaker at 150 rpm for 10 minutes for homogenization. Absorbance was measured at 564 nm in a microplate reader (Orellana and Kasinski 2016).

Comet Test

Comet test was performed to examine the genotoxic effect (strand breaks) in DNA under a fluorescent microscope at the end of the exposure periods. One day before the experiment day, the slides were first sterilized by wiping with methanol and burning with a lighter, then they were covered with 1% normal melting point agarose and incubated for 24 hours at room temperature in a closed box. On the day of the experiment, 99 ml of the stock lysis solution was taken and 1 ml of Triton-X 100 was added and incubated at +4 ºC for 30 minutes. On the day of the experiment, trypsinization steps were applied to the cells on the plates. Then, 600 µL of fresh medium was added onto the cells and pipetting was performed to ensure homogenization. 10 µL of cell solution and 90 µL of 0.5% low-melting point agarose were added to 2 mL Eppendorf tubes and pipetted (0.5% low-melting point agarose was kept in a 37ºC water bath in the beaker. In this way, the agarose was frozen. blocked). 80 µL of cells with 0.5% low melting point agarose was added to the slides covered with 1% normal melting point agarose the previous day. Coverslips were wiped with methanol on the slides, covered so that no air bubbles remained, and incubated at +4°C for 15 minutes. The coverslips on the slide were removed and 75 µL of 0.5% low-melting point agarose was added and covered with the coverslips again. It was incubated at +4°C for 15 minutes. After the agarose was frozen, the coverslips on the slides were removed and the slides were placed vertically in the chalk containing lysis solution containing Triton-X 100. Incubated at +4°C for 24 hours. The slides were removed from the lysis solution and placed on a flat surface and left uncovered for 15 minutes to dry. The slides were placed in the electrophoresis tank so that no air bubbles remained and were kept in cold electrophoresis buffer for 20 minutes. Electrophoresis was carried out at 24 V, 300 mA for 30 minutes. The slides were removed from the electrophoresis tank and dried for 10 minutes. It was kept in neutralization buffer for 5 minutes to remove the electrophyse buffer. This process step was repeated 3 times. After neutralization, the slides were dried.

80 µL of 1X EtBr was added to the slide and covered with a coverslip. It was left to dry for 5 minutes. It was washed once with dH2O to remove excess EtBr, and after a 20-minute fixation

process with methanol at -20°C, it was kept in an oven set at 50°C for 30 minutes. The slides were stained again with 80 µL EtBr and dried. Imaging was performed under a fluorescence microscope at 40X magnification and at least DNA images were taken from each group and these photographs were analyzed with Tritek Comet ScoreTM image analysis software (Dhawan et al. 2003).

Statistical Analysis

UV-Vis spectra, FTIR and XRD analysis results of CuO NPs were evaluated in the Origin Lab data analysis program and their graphs were drawn. For the cytotoxicity of CuO NPs, the viability of the cells in the undosed control group was assumed to be 100%, and the viability rate in the application groups was calculated as % Viability=[100×(average cell absorbance in the application group-blind average)/(average cell absorbance in the control group-blind average)] has been calculated. Cytotoxicity results Tukey Pairwise Comparison analysis of One-Way ANOVA test and cytotoxicity graph were performed using GraphPad Prism Software 9.00 (La Jolla California USA). For the % Tail DNA calculation in the Comet test, the average values of the images obtained from 50 cells from each group were calculated. These group averages and standard deviations were then determined. One-way analysis of variance (ANOVA) was performed using SPSS 20 (IBM USA). Statistical significance level is given as $p>0.05$ (insignificant), $p<0.05$ (important) and $p<0.01$ (very important).

RESULTS

Synthesis of CuO NPs

The biological synthesis of CuO NPs was confirmed by macroscopic observation when the plant extract and copper acetate solution changed color on a magnetic stirrer for 2 h (Figure 2).

Figure 2. Biological synthesis of CuO NPs.

Characterization of CuO NPs

The morphology and dimensions of CuO NPs synthesized by the green synthesis method were examined using TEM analysis. According to TEM analysis, CuO NPs of different sizes (<5nm) and spherical shapes were observed (Figure 3).

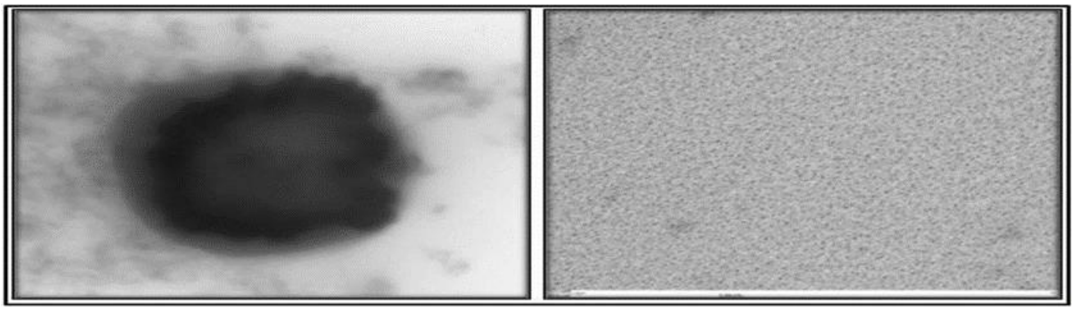

Figure 3. TEM images of CuO NPs.

According to Uv-Vis Spectroscopy analysis, it is seen that the maximum absorbance peak value of CuO NPs obtained from black pepper by the green synthesis method is in the range of 211-220 nm (Figure 4).

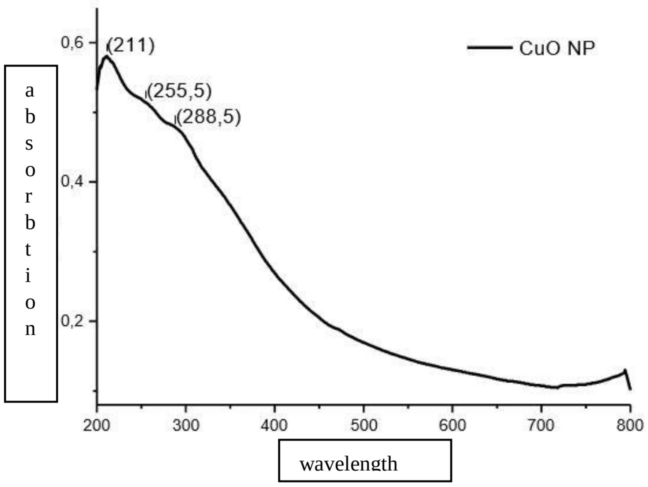

Figure 4. UV-Vis spectrum of CuO NPs. A.u/nm

By FTIR analysis, peaks showing the numerical values of the functional groups present in CuO NPs were detected (Figure 5). The peak values in the FTIR spectrum were obtained at wave numbers of 310, 514, 738, 757, 898, 1153, 1199, 1332, 2156, 3662 and 3791 cm^{-1}.

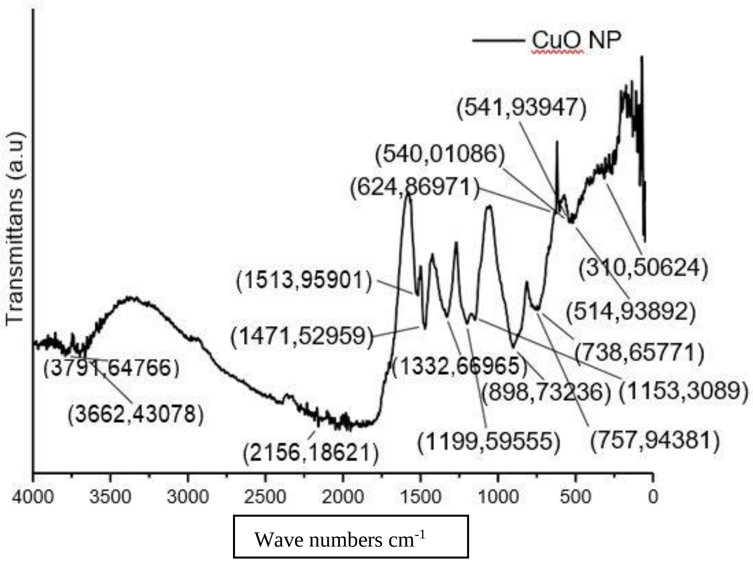

Figure 5. FTIR spectrum of CuO NPs.

The crystal structure and elemental composition of the synthesized CuO NPs were examined by XRD analysis. XRD analysis of CuO NPs was measured in the range of 2θ = 20–80°. It was observed that distinct crystal phase formation peaks of CuO NPs occurred (Figure 6).

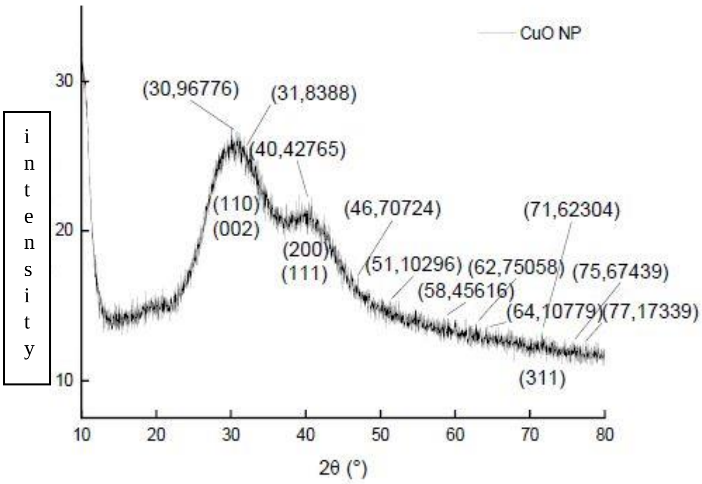

Figure 6. XRD spectrum of CuO NPs.

Cytotoxicity Analysis

For the determination of the cytotoxicity of CuO NPs, RTG-2 cells were exposed to CuO NP application doses of 3.125 µg/ml, 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml for 48 hours. SRB test was performed after exposure for a short period of time. 10% DMSO was used for positive control. Figure 7 shows the images of RTG-2 cells after 48 hours of exposure to CuO NPs at the doses used in the study. The % viability rates and standard deviation graphs determined according to the SRB analysis results are given in Figure 8.

At the end of 48 hours of exposure, a CuO NP dose of 50 µg/ml, which killed approximately 50% of RTG-2 cells compared to the control group, was accepted as the LD50 value.

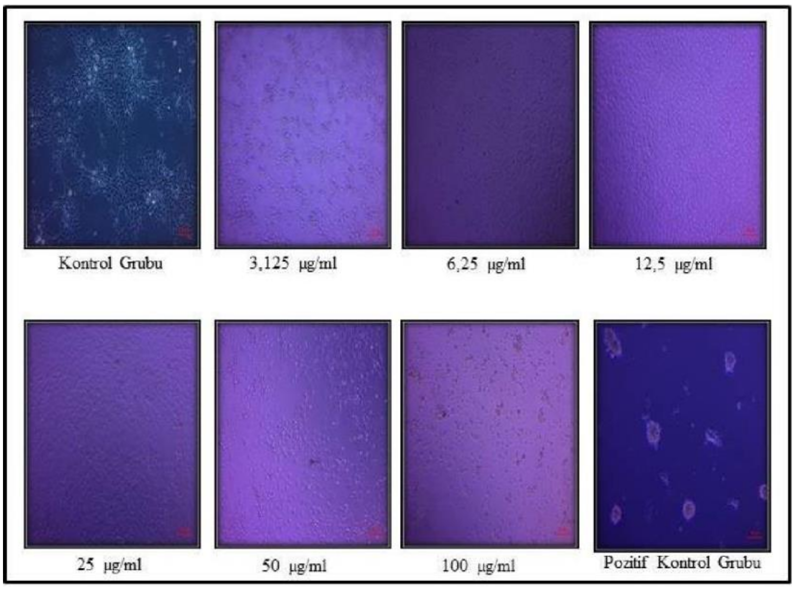

Figure 7. CuO NP exposure of RTG-2 cells at determined doses for 48 hours.

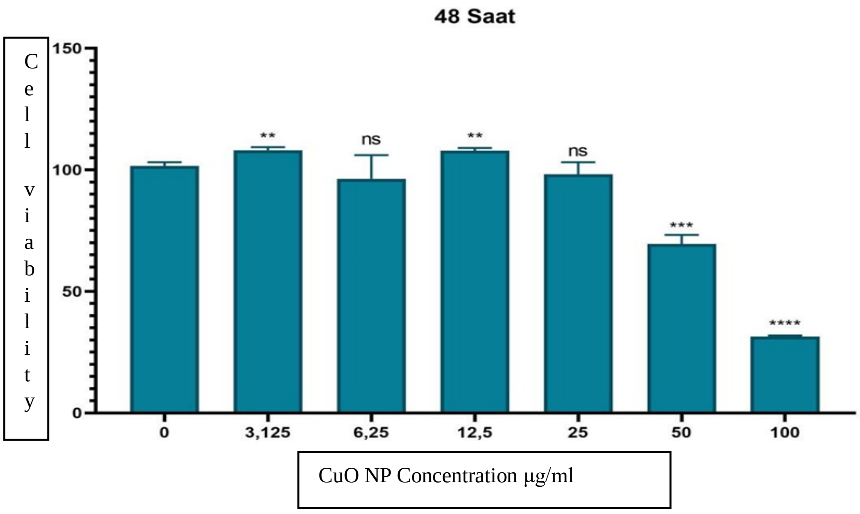

Figure 8. % survival rates of RTG-2 cells after 48 hours of CuO NP exposure.

Comet Analysis

To detect DNA damage, comet test was applied to RTG-2 cells exposed to 50 µg/ml CuO NPs for 48 hours. The damage caused by CuO NPs to DNA was examined by imaging under a fluorescence microscope at 40X magnification (Figure 9). Additionally, statistical results are given as % Tail DNA (Figure 10).

It was determined that vulgare essential oil did not have any antibacterial effect. The MIC values were determined as 7.8 µl/ml in the nanoemulsion form of O. onites essential oil, 15.6 µl/ml in O. onites essential oil and 1000 µl/ml in the nanoemulsion form of F. vulgare essential

Control	50 µg/mL CuO NP Concentration	Positive Control

oil (Table 1). Due to the low MIC value of O. onites essential oil and nanoemulsion form, it was determined that the sensitivity of A. hydrophila bacteria was higher than the other oil.

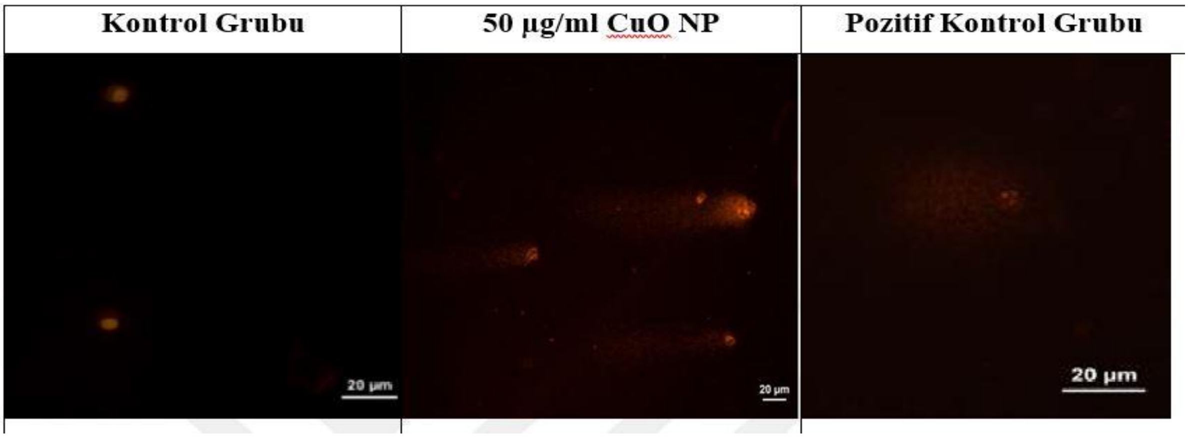

Figure 9. Damage caused by CuO NPs to DNA after 48 hours of exposure.

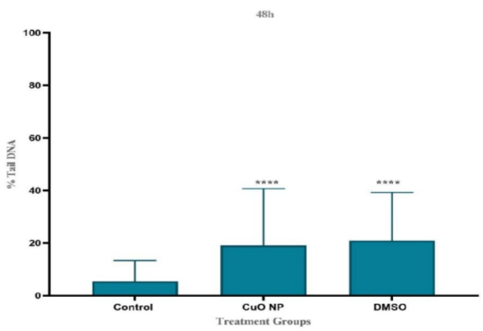

Figure 10. % Tail DNA amount for 48 hours.

CONCLUSION AND RECOMMENDATIONS

In this study, spherical CuO NPs with dimensions of approximately 5 nm were synthesized by the green synthesis method using black pepper. The maximum absorbance peak value of the synthesized CuO NPs was obtained at 211 nm in the UV-vis spectrophotometer. According to the FTIR spectrum, a band was observed at the 530-490 cm-1 position (514.9 cm-1 position), which corresponds to the metal-oxygen (C-O) vibration that supports the monoclinic phase of CuO NPs. According to the XRD spectrum, it is understood that CuO NPs exhibit a stable structure in the FCC phase, having planes with indexes 111 and 110. It has been shown that CuO NPs obtained by green synthesis have a cytotoxic effect on RTG-2 cells after 48 hours of exposure to high doses (LD50 = 50 µg/mL). However, considering the doses at which commercially available CuO NPs synthesized by chemical means in the literature show their cytotoxic effect in vitro, the possibility of the green synthesis method to have a toxicity-reducing effect has been demonstrated. Additionally, it was determined that CuO NPs caused genotoxicity in RTG-2 cells and increased % tail DNA.

In subsequent studies, the cytotoxicity and genotoxicity of CuO NPs synthesized using black pepper and chemically synthesized CuO NPs were compared in RTG-2 cells at the same dose, time and environmental conditions, and the changes in gene expressions related to the antioxidant defense system were examined in order to prove oxidative stress-induced cell death. It is recommended to examine.

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REFERENCES

Ahamed, M., Lateef, R., Akhtar, M. J., and Rajanahalli, P. 2022. Dietary Antioxidant Curcumin Mitigates CuO Nanoparticle-Induced Cytotoxicity through the Oxidative Stress Pathway in Human Placental Cells. Molecules, 27(21), 7378.

Ahamed, M., Siddiqui, M. A., Akhtar, M. J., Ahmad, I., Pant, A. B., Alhadlaq, H. A. 2010. Genotoxic potential of copper oxide nanoparticles in human lung epithelial cells. Biochemical and Biophysical Research Communications, 396(2), 578-583.

Akintelu, S. A., Folorunso, A. S., Folorunso, F. A., and Oyebamiji, A. K. 2020. Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation. Heliyon, 6(7), e04508.

Alaraby, M., Demir, E., Domenech, J., Velázquez, A., Hernández, A., Marcos, R. 2020. In vivo evaluation of the toxic and genotoxic effects of exposure to cobalt nanoparticles using Drosophila melanogaster. Environmental Science: Nano, 7(2), 610-622

An, K., and Somorjai, G. A. 2012. Size and shape control of metal nanoparticles for reaction selectivity in catalysis. Chem Cat Chem, 4(10), 1512-1524.

Anonim, 2022. https://www.sigmaaldrich.com/TR/en/technical-documents/technical-article/analytical-chemistry/photometry-and-reflectometry/ir-spectrum-table (23.12.2022).

Asare, N., Instanes, C., Sandberg, W. J., Refsnes, M., and Schwarze, P. 2012. Cytotoxic and genotoxic effects of silver nanoparticles in testicular cells. Toxicology, 291 (1-3), 65-72.

Bayda, S., Adeel, M., Tuccinardi, T., Cordani, M., and Rizzolio, F. (2019). The history of nanoscience and nanotechnology: from chemical–physical applications to nanomedicine. Molecules, 25(1), 112.

Berra, D., Laouini, S. E., Benhaoua, B., Ouahrani, M. R., Berrani, D., and Rahal, A. 2018. Green synthesis of copper oxide nanoparticles by Pheonix dactylifera L leaves extract. Digest Journal of Nanomaterials and Biostructures, 13(4), 1231-1238.

Bols, N. C., Boliska, S. A., Dixon, D. G., Hodson, P. V., and Kaiser, K. L. E. 1985. The use of fish cell cultures as an indication of contaminant toxicity to fish. Aquatic Toxicology, 6(2), 147-155.

Caminada, D., Escher, C., and Fent, K. 2006. Cytotoxicity of pharmaceuticals found in aquatic systems: comparison of PLHC-1 and RTG-2 fish cell lines. Aquatic Toxicology, 79(2), 114-123.

Carmona, E. R., Inostroza-Blancheteau, C., Obando, V., Rubio, L., and Marcos, R. 2015. Genotoxicity of copper oxide nanoparticles in Drosophila melanogaster. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 791, 1-11.

Castano, A. and Becerril, C. 2004. In vitro assessment of DNA damage after short- and long-term exposure to benzo(a)pyrene using RAPD and the RTG-2 fish cell line. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 552(1-2), 141–151.

Castaño, A., Bols, N., Braunbeck, T., Dierickx, P., Halder, M., Isomaa, B., and Segner, H. 2003. The use of fish cells in ecotoxicology: the report and recommendations of Ecvam Workshop 47. Alternatives to Laboratory Animals, 31(3), 317-351. 30

Dhawan, A., Bajpayee, M. M., Pandey, A. K., Parmar, D. 2003. Protocol for the single cell gel electrophoresis/comet assay for rapid genotoxicity assessment. Sigma, 1077(1), 1-10.

El-Seedi, H. R., El-Shabasy, R. M., Khalifa, S. A., Saeed, A., Shah, A., Shah, R., and Guo, W. 2019. Metal nanoparticles fabricated by green chemistry using natural extracts: Biosynthesis, mechanisms, and applications. RSC advances, 9(42), 24539-24559.

Ez Zoubi, Y., Bousta, D., and Farah, A. 2020. A Phytopharmacological review of a Mediterranean plant: Lavandula stoechas L. Clinical Phytoscience, 6(1), 1-9.

Gallo, A., Manfra, L., Boni, R., Rotini, A., Migliore, L., and Tosti, E. 2018. Cytotoxicity and genotoxicity of CuO nanoparticles in sea urchin spermatozoa through oxidative stress. Environment international, 118, 325-333.

Gatoo, M. A., Naseem, S., Arfat, M. Y., Mahmood Dar, A., Qasim, K., and Zubair, S. 2014. Physicochemicalproperties of nanomaterials: implication in associatedtoxicmanifestations. Bio Med Research nternational, volüme 2014.

Gnanajobitha, G., Paulkumar, K., Vanaja, M., Rajeshkumar, S., Malarkodi, C., Annadurai, G., & Kannan, C. 2013. Fruit-mediated synthesis of silver nanoparticles using Vitis vinifera and evaluation of their antimicrobial efficacy. Journal of Nanostructure in Chemistry, 3(1), 1-6.

Griffitt, R. J., Hyndman, K., Denslow, N. D., & Barber, D. S. 2009. Comparison of molecular and histological changes in zebrafish gills exposed to metallic nanoparticles. Toxicological Sciences, 107(2), 404-415.

Griffitt, R. J., Luo, J., Gao, J., Bonzongo, J. C., and Barber, D. S. 2008. Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. Environmental Toxicology and Chemistry: An International Journal, 27(9), 1972-1978.

Griffitt, R. J., Weil, R., Hyndman, K. A., Denslow, N. D., Powers, K., Taylor, D., and Barber, D. S. 2007. Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (Danio rerio). Environmental science & technology, 41(23), 8178-8186.

Halıcı, A., Seyrek, A., Aykan, K., Ünal, F., and Yüzbaşıoğlu, D. 2021. Nanopartiküllerin Genotoksik Etkileri. Gazi Üniversitesi Fen Fakültesi Dergisi, 2(2).

Hasan, S. 2015. A review on nanoparticles: their synthesis and types. Journal of Recent Sciences, 2277, 2502.

Hasheminya, S. M., and Dehghannya, J. 2020. Green synthesis and characterization of copper nanoparticles using Eryngium caucasicum Trautv aqueous extracts and its antioxidant and antimicrobial properties. Particulate Science and Technology, 38(8), 1019-1026.

Hernández-Moreno, D., Li, L., Connolly, M., Conde, E., Fernández, M., Schuster, M., Navas, J. M., Fernández-Cruz, M. L. 2016. Mechanisms underlying the enhancement of toxicity caused by the coincubation of zinc oxide and copper nanoparticles in a fish hepatoma cell line. Environmental Toxicology and Chemistry, 35(10), 2562-2570.

Ibrahim, A. M., Munshi, G. H., and Al-Harbi, L. M. 2018. Copper (II) oxide nanocatalyst preparation and characterization: green chemistry route. Bulletin of the National Research Centre, 42(1), 1-4.

Imran Din, M., and Rani, A. 2016. Recent advances in the synthesis and stabilization of nickel and nickel oxide nanoparticles: a green adeptness. International journal of analytical chemistry, 2016. 31

Imran, M., Baig, A. Q., Azhar, M. R., Farahani, M. R., and Zhang, X. 2017. Eccentricity based geometric-arithmetic and atom-bond connectivity indices of copper oxide CuO. International Journal of Pure and Applied Mathematics, 117(3), 481-502.

Iravani, S. 2011. Green synthesis of metal nanoparticles using plants. Green Chemistry, 13(10), 2638-2650.

Işık, S., 2022. Investigation of the Effects of Resveratrol on Copper II Oxide (CuO) Nanoparticle Toxicity in the RTG-2 Cell Line in terms of Cytotoxicity, Biochemical, and Genotoxicity, Master Thesis, graduate school of natural and applıed scıence, Erzurum.

Jillani, S., Jelani, M., Hassan, N. U., Ahmad, S., Hafeez, M. 2018. Synthesis, characterization and biological studies of copper oxide nanostructures. Materials Research Express, 5(4), 045006.

Kammann U, Bunke M, Steinhart H, Theobald N. 2001. A permanent fish cell line (EPC) for genotoxicity testing of marine sediments with the comet assay. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 498(1-2), 67-77.

Karlsson, H. L. 2010. The comet assay in nanotoxicology research. Analytical and Bioanalytical Chemistry, 398 (2), 651-666.

Karlsson, H. L., Cronholm, P., Gustafsson, J., and Moller, L. 2008. Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. Chemical research in toxicology, 21(9), 1726-1732.

Karlsson, H. L., Di Bucchianico, S., Collins, A. R., and Dusinska, M. 2015. Can the comet assay be used reliably to detect nanoparticle-induced genotoxicity? Environmental and molecular mutagenesis, 56(2), 82-96.

Karlsson, H.L., Cronholm, P., Gustafsson, J., Moller, L., 2008. Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. Chem. Res. Toxicol. 21(9), 1726-1732.

Katsumiti, A., Thorley, A. J., Arostegui, I., Reip, P., Valsami-Jones, E., Tetley, T. D., and Cajaraville, M. P. 2018. Cytotoxicity and cellular mechanisms of toxicity of CuO NPs in mussel cells in vitro and comparative sensitivity with human cells. Toxicology in Vitro, 48, 146-158.

Kaur, S., Singh, J., Rawat, R., Kumar, S., Kaur, H., Venkateswara Rao, K., and Rawat, M. 2018. A smart LPG sensor based on chemo-bio synthesized MgO nanostructure. Journal of Materials Science: Materials in Electronics, 29(14), 11679-11687.

Kocan, R. M., Landolt, M. L., and Sabo., K. M. 1979. In vitro toxicity of eight mutagens/carcinogens for three fish cell lines. Bull. Environ. Contam. Toxicol, 23(1), 269-274.

Kohl, Y., Rundén-Pran, E., Mariussen, E., Hesler, M., El Yamani, N., Longhin, E. M., and Dusinska, M. 2020. Genotoxicity of nanomaterials: advanced in vitro models and high throughput methods for human hazard assessment—a review. Nanomaterials, 10(10), 1911.

Kumari, M., Mukherjee, A., and Chandrasekaran, N. 2009. Genotoxicity of silver nanoparticles in Allium cepa. Science of the total environment, 407(19), 5243-5246.

Liz-Marzán, L. (Ed.). 2020. Colloidal Synthesis of Plasmonic Nanometals. CRC Press. 32

Mihailovic, V., Katanic Stankovic, J. S., Selakovic, D., & Rosic, G. 2021. An overview of the beneficial role of antioxidants in the treatment of nanoparticle-induced toxicities. Oxidative Medicine and Cellular Longevity, 2021.

Mittal, J., Batra, A., Singh, A., and Sharma, M. M. 2014. Phytofabrication of nanoparticles through plant as nanofactories. Advances in Natural Sciences: Nanoscience and Nanotechnology, 5(4), 043002.

Moteriya, P., and Chanda, S. 2017. Synthesis and characterization of silver nanoparticles using Caesalpinia pulcherrima flower extract and assessment of their in vitro antimicrobial, antioxidant, cytotoxic, and genotoxic activities. Artificial cells, nanomedicine, and biotechnology, 45(8), 1556-1567.

Mukhopadhyay, R., Kazi, J., and Debnath, M. C. 2018. Synthesis and characterization of copper nanoparticles stabilized with Quisqualis indica extract: evaluation of its cytotoxicity and apoptosis in B16F10 melanoma cells. Biomedicine Pharmacotherapy, 97, 1373-1385.

Nagar, N., and Devra, V. 2018. Green synthesis and characterization of copper nanoparticles using Azadirachta indica leaves. Materials Chemistry and Physics, 213, 44-51.

Nagaraj, E., Karuppannan, K., Shanmugam, P., and Venugopal, S. 2019. Exploration of bio-synthesized copper oxide nanoparticles using pterolobium hexapetalum leaf extract by photocatalytic activity and biological evaluations. Journal of Cluster Science, 30(4), 1157-1168.

Naseer, S., Hussain, S., Naeem, N., Pervaiz, M., Rahman, M. 2018. The phytochemistry and medicinal value of Psidium guajava (guava). Clinical Phytoscience, 4(1), 1-8.

Nasrollahzadeh, M., Sajadi, S. M., and Maham, M. 2015. Tamarix gallica leaf extract mediated novel route for green synthesis of CuO nanoparticles and their application for N-arylation of nitrogen-containing heterocycles under ligand-free conditions. RSC Advances, 5(51), 40628-40635.

Nasrollahzadeh, M., Sajadi, S. M., Sajjadi, M., and Issaabadi, Z. 2019. An introduction to nanotechnology. In Interface science and technology (Vol. 28, pp. 1-27). Elsevier.

Naz, S., Gul, A., Zia, M. 2020. Toxicity of copper oxide nanoparticles: a review study. IET Nanobiotechnology, 14(1), 1-13.

Nehls, S., and Segner, H. 2001. Detection of DNA damage in two cell lines from rainbow trout, RTG-2 and RTL-W1, using the comet assay. Environmental Toxicology: An International Journal, 16(4), 321-329.

Nikdehghan, N., Kashiri, H., & Hedayati, A. A. 2018. CuO nanoparticles-induced micronuclei and DNA damage in Cyprinus carpio. Aquaculture, Aquarium, Conservation & Legislation-International Journal of the Bioflux Society (AACL Bioflux), 11(3).

Orellana, E. A., Kasinski, A. L. 2016. Sulforhodamine B (SRB) assay in cell culture to investigate cell proliferation. Bio-protocol, 6(21), e1984-e1984.

Ostaszewska, T., Śliwiński, J., Kamaszewski, M., Sysa, P., and Chojnacki, M. 2018. Cytotoxicity of silver and copper nanoparticles on rainbow trout (Oncorhynchus mykiss) hepatocytes. Environmental Science and Pollution Research, 25(1), 908-915.

Perreault, F., Melegari, S. P., da Costa, C. H., Rossetto, A. L. D. O. F., Popovic, R., and Matias, W. G. 2012. Genotoxic effects of copper oxide nanoparticles in Neuro 2A cell cultures. Science of the total environment, 441, 117-124. 33

Rafique, M., Shaikh, A. J., Rasheed, R., Tahir, M. B., Bakhat, H. F., Rafique, M. S., Rabbani, F. 2017. A review on synthesis, characterization and applications of copper nanoparticles using green method. Nano, 12(04), 1750043.

Ray, P. C., Yu, H., and Fu, P. P. 2009. Toxicity and environmental risks of nanomaterials: challenges and future needs. Journal of Environmental Science and Health Part C, 27(1), 1-35.

Rehana, D., Mahendiran, D., Kumar, R. S., and Rahiman, A. K. 2017. Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts. Biomedicine & Pharmacotherapy, 89, 1067-1077.

Roduner, E. 2006. Size matters: why nanomaterials are different. Chemical Society Reviews, 35(7), 583-592.

Sadiq, R., Khan, Q. M., Mobeen, A., and Hashmat, A. J. 2015. In vitro toxicological assessment of iron oxide, aluminium oxide and copper nanoparticles in prokaryotic and eukaryotic cell types. Drug and chemical toxicology, 38(2), 152-161.

Saif, S., Tahir, A., Asim, T., and Chen, Y. 2016. Plant mediated green synthesis of CuO nanoparticles: comparison of toxicity of engineered and plant mediated CuO nanoparticles towards Daphnia magna. Nanomaterials, 6(11), 205.

Santhoshkumar, J., Agarwal, H., Menon, S., Rajeshkumar, S., and Kumar, S. V. 2019. A biological synthesis of copper nanoparticles and its potential applications. In Green Synthesis, Characterization and Applications of Nanoparticles (pp. 199-221). Elsevier.

Saranyaadevi, K., Subha, V., Ravindran, R. E., and Renganathan, S. 2014. Synthesis and characterization of copper nanoparticle using Capparis zeylanica leaf extract. Int J Chem Tech Res, 6(10), 4533-4541.

Saygılı, Y., Yüzbaşıoğlu, D., ve Ünal, F., 2021. Metal oksit nanopartiküllerin genotoksik etkileri. International Journal of Advances in Engineering and Pure Sciences, 33(3), 429-443.

Scherer, M. D., Sposito, J. C., Falco, W. F., Grisolia, A. B., Andrade, L. H., Lima, S. M., and Caires, A. R. 2019. Cytotoxic and genotoxic effects of silver nanoparticles on meristematic cells of Allium cepa roots: A close analysis of particle size dependence. Science of the Total Environment, 660, 459-467.

Sengul, A. B., and Asmatulu, E. 2020. Toxicity of metal and metal oxide nanoparticles: a review. Environmental Chemistry Letters, 18(5), 1659-1683.

Sepeur, S. 2008. Nanotechnology: technical basics and applications. Vincentz Network GmbH and Co KG.

Sharma, H. S., Hussain, S., Schlager, J., Ali, S. F., Sharma, A. 2010. Influence of nanoparticles on blood–brain barrier permeability and brain edema formation in rats. In Brain edema XIV (pp. 359-364). Springer, Vienna.

Sharmila, G., Thirumarimurugan, M., and Sivakumar, V. M. 2016. Optical, catalytic and antibacterial properties of phytofabricated CuO nanoparticles using Tecoma castanifolia leaf extract. Optik, 127(19), 7822-7828.

Shi, L. B., Tang, P. F., Zhang, W., Zhao, Y. P., Zhang, L. C., and Zhang, H. 2017. Green synthesis of CuO nanoparticles using Cassia auriculata leaf extract and in vitro evaluation of their biocompatibility with rheumatoid arthritis macrophages (RAW 264.7). Tropical Journal of Pharmaceutical Research, 16(1), 185-192. 34

Shiravand, S., and Azarbani, F. 2017. Phytosynthesis, characterization, antibacterial and cytotoxic effects of copper nanoparticles. Green Chemistry Letters and Reviews, 10(4), 241-249.

Shukla, A. K., and Iravani, S. (Eds.). 2018. Green synthesis, characterization and applications of nanoparticles. Elsevier.

Singh, A., Singh, N. B., Hussain, I., Singh, H., Yadav, V. 2017. Synthesis and characterization of copper oxide nanoparticles and its impact on germination of Vigna radiata (L.) R. Wilczek. Tropical Plant Research, 4(2), 246-253.

Singh, N., Manshian, B., Jenkins, G. J., Griffiths, S. M., Williams, P. M., Maffeis, T. G., Doak, S. H. (2009). NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials. Biomaterials, 30(23-24), 3891-3914.

Singh, S., Kumar, N., Kumar, M., Agarwal, A., and Mizaikoff, B. 2017. Electrochemical sensing and remediation of 4-nitrophenol using bio-synthesized copper oxide nanoparticles. Chemical Engineering Journal, 313, 283-292.

Tamilvanan, A., Balamurugan, K., Ponappa, K., Kumar, B. M. 2014. Copper nanoparticles: synthetic strategies, properties and multifunctional application. International Journal of Nanoscience, 13(02), 1430001.

Wang, F., Gao, F., Lan, M., Yuan, H., Huang, Y., and Liu, J. 2009. Oxidative stress contributes to silica nanoparticle-induced cytotoxicity in human embryonic kidney cells. Toxicology in vitro, 23(5), 808-815.

Wang, F., Li, H., Yuan, Z., Sun, Y., Chang, F., Deng, H., and Li, H. 2016. A highly sensitive gas sensor based on CuO nanoparticles synthetized via a sol–gel method. RSC Advances, 6(83), 79343-79349.

Wang, Y., Aker, W. G., Hwang, H. M., Yedjou, C. G., Yu, H., Tchounwou, P. B. 2011. A study of the mechanism of in vitro cytotoxicity of metal oxide nanoparticles using catfish primary hepatocytes and human HepG2 cells. Science of The Total Environment, 409(22), 4753-4762.

Wang, Z., Li, N., Zhao, J., White, J. C., Qu, P., Xing, B., 2012. CuO nanoparticle interaction with human epithelial cells: cellular uptake, location, export, and genotoxicity. Chem. Res. Toxicol. 25 (7), 1512-1521.

Yaprak, E., 2019. Yeşil sentez metoduyla karabaş otundan (Lavandula stoechas L.) bakır nanopartikül sentezi, karakterizasyonu ve antimikrobiyal aktivitesinin değerlendirilmesi. Atatürk Üniversitesi Fen Bilimleri Enstitüsü, Yüksek lisans tezi.

Zangeneh, M. M., Ghaneialvar, H., Akbaribazm, M., Ghanimatdan, M., Abbasi, N., Goorani, S., and Zangeneh, A. 2019. Novel synthesis of Falcaria vulgaris leaf extract conjugated copper nanoparticles with potent cytotoxicity, antioxidant, antifungal, antibacterial, and cutaneous wound healing activities under in vitro and in vivo condition. Journal of Photochemistry and Photobiology B: Biology, 197, 111556.

Žegura, B., and Filipič, M. 2019. The application of the Comet assay in fish cell lines. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 842, 72-84.

Zivari Fard, M., Fatholahi, M., Abyadeh, M., Bakhtiarian, A., Mousavi, S. E., and Falahati, M. (2020). The Investigation of the Cytotoxicity of Copper Oxide Nanoparticles on Peripheral Blood Mononuclear Cells. Nanomedicine Research Journal, 5(4), 364-36.

Drought Stress in Plants

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ABSTRACT: Drought, which is one of the most important abiotic stress factors that adversely affect yield and quality in crop production, is defined as a meteorological concept that lasts long enough to endanger the life of the plant with the limited amount of water contained in the soil and is lower than the average rainfall. Drought seriously affects crop production depending on the water holding capacity of the soil, morphological (age, root structure, etc.) and physiological (evapo-transpiration rate, transpiration coefficient) structure of plants. Drought, especially under arid and semi-arid conditions, can affect plant growth and even survival. With drought stress, leaf area in the plant decreases and photosynthesis slows down and decreases accordingly. While stomatal size decreases in drought stress, stomatal number is positively correlated with stomatal permeability, CO_2 assimilation and water use efficiency. Plants react to drought stress depending on their genetic structure and climatic factors in order to survive. These responses can be listed as escape from drought stress, prevention of water loss and tolerance to water loss.

Keywords: Abiotic stress, Drought, Plant, Photosynthesis

INTRODUCTION

Climate change due to global warming and environmental impacts has led to the emergence of the concept of stress affecting yield and quality in crop production. The concept of stress in crop production is defined as any situation or substance that occurs continuously or periodically in an environment and causes a negative effect on the growth, development and even productivity of plants or plant organs. In general, stress is an important force that causes various damages in plants Mahajan and Tuteja (2005), Hale and Orcutt (1987), Kocaçalışkan (2004).

Stress factors of biotic (insects, microorganisms, etc.) and abiotic (drought, light, radiation, temperature, etc.) origin cause many morphological, physiological and biochemical damages in plants and cause a decrease in yield and quality.

Plants suffer the most from biotic and abiotic stress factors because of their lack of mobility. Plants can change their growth and development mechanisms to prevent damage due to stress conditions. Plant species that survive in the same climatic conditions for a long time have developed the ability to adapt in such a way that they are least affected by biotic and abiotic stress factors Dolferus (2014), and Ergen, NZ., Thimmapuram, J., Bohnert, HJ., Budak, H. (2009).The fact that plants belonging to the same species can grow in regions of the world with different climatic characteristics is a proof of the adaptation ability they have developed (Dolferus, 2014).

Drought, which is one of the most important abiotic stress factors that negatively affect yield and quality in crop production, is defined as a meteorological concept that is lower than the average amount of precipitation, which lasts long enough to jeopardize the life of the plant with the limited amount of water contained in the soil (Kalefetoğlu and Ekmekçi 2005). According to another definition, drought is the presence of moisture in the soil at the wilting point of the plant (Çırak and Esendal 2003). Drought seriously affects crop production depending on the water holding capacity of the soil, morphological (age, root structure, etc.) and physiological (evapo-transpiration rate, transpiration coefficient) structure of plants. Drought, especially under arid and semi-arid conditions, can affect plant growth and even survival (Yang et al. 2009).

Possible Effects of Drought Stress on Plants

The morphological changes that occur in plants under drought conditions are generally directed at reducing the amount of water lost by transpiration in the leaves and at absorbing water from the soil with a higher force in the roots. Under drought stress, plants first develop a rapid root system and the ratio of root to stem increases. Under drought conditions photosynthesis slows down and as a consequence shoot development is weakened. Most of the photosynthesis products are transported to the roots for root development (Öztürk and Seçmen, 1992). In drought conditions, photosynthesis slows down and seedling development slows down as a result of this slowdown. In plants due to drought, cell growth slows down and seed germination, seedling growth and the transmission of substances in the phloem and xylem due to the presence of a small amount of water in the plant body are adversely affected, resulting in immaturity of fruits and seeds in plants and decreases in the quality of these products.

In response to drought stress, some plants develop hairs on leaves and stems. These hairs usually show an arid character and cause the plant surface to cool down, reducing the rate of transpiration (Göksoy and Turan, 1991). Again, plants form a waxy layer on the leaf surface as a different way of reducing the transpiration rate to avoid arid conditions. The accumulation of a waxy layer on the leaf surface, which leads to the formation of a thicker cuticle, reduces water loss through the epidermis. This also reduces carbon dioxide uptake but does not affect leaf photosynthesis. Because epidermal cells under the cuticle are not photosynthetic (Özcan, 2020).

Drought-induced changes in plants in general;

- Root structure that goes deeper and increases in proportion to the stem
- Changes that reduce leaf and stem transpiration (such as feather and waxy layer formation)
- Reduction in leaf area, curling/rolling of leaves and reduction in the number of leaves
- Shortening of plant height, early maturity and low quality crop can be listed as.

With drought stress, leaf area in the plant decreases and photosynthesis slows down and decreases accordingly (Çırak and Esendal, 2006). While stomatal size decreases in drought stress, stomatal number is positively correlated with stomatal permeability, net CO2 assimilation and water use efficiency (Xu and Zhou, 2008).

A decrease in photosynthesis occurs in plants due to drought stress. This decrease in photosynthesis is generally due to stomatal limitations due to stomatal closure under moderate water deficit conditions and non-stomatal limitations that usually occur under longer and more severe drought stresses (Kalefetoğlu and Ekmekçi, 2005). The initial reduction in photosynthesis under drought stress is caused by stomatal closure and reduced CO2 absorption. When the plant closes its stomata to prevent water loss, the uptake of CO2 required for photosynthesis is also prevented (İpek, 2015).

Photosynthesis can also be reduced due to non-stomatal limitations. These are mostly chloroplast-related factors. Chloroplasts, especially in the so-called stroma, contain enzymes such as RuBPCase (RuBPCase), which flips and reduces CO2 into organic compounds. As a result of biochemical reactions with water loss, the RuBPCase enzyme is reduced, thus disrupting CO2 fixation. Initially, photosynthesis is reduced by stomatal factors, but as drought stress persists or increases in severity, chloroplast and enzyme activity is depressed, so photosynthesis is reduced by factors other than stomata (Özcan, 2020).

Plants are divided into two groups, C3 and C4, in terms of their photosynthetic pathways and their responses to drought stress are different. C4 plants are more resistant to drought than C3 plants and can photosynthesize at very low CO2 concentration even if their stomata are closed in drought stress, in addition to these plant groups, there is a CAM plant group and these plants are scullent plants belonging to the Crassulaceae family and plants belonging to this group are known as the most resistant plants to drought stress (Kocaçalışkan 2008).

Drought Stress Resilience

Drought is an important phenomenon among abiotic stress factors that negatively affects crop production, yield and quality. In the life cycle of plants, important physiological processes such as the formation of above-ground (leaf, stem, fruit, etc.) and below-ground (root, tuber, etc.) parts, the opening and closing of stomata and photosynthesis can be affected by water availability. While the lack of rainfall in approximately 43% of the world's soils and in the majority of agricultural lands causes drought problems, irregular rainfall regimes cause stress on plant growth and development and negatively affect plant growth and development. In these and similar cases, changes due to drought stress that occur in the plant body can be reduced to some extent by irrigation.

Drought Stress Tolerance; refers to the ability of a plant to show adequate growth in areas where water deficit is observed. According to Turner (1986), drought stress tolerance mechanism is divided into 3 parts as drought escape, prevention of water loss and tolerance to water loss in plants.

The ability of the plant to complete vital activities before water loss occurs is referred to as 'Drought Escape'. In this way, plants can survive in arid environments due to their rapid development and ability to adjust their growth well. Plants such as desert ephemerals, which are annual plants whose seeds remain dormant during dry periods, can avoid drought in this way. Their seeds can germinate thanks to the rains that fall on the soil to a certain extent and they form at least one seed by maturing before the moisture in the soil is exhausted (Salisbury & Ross, 1992).

Since the germination requirements of plants that produce seeds with morphologically and physiologically different characteristics will be different, germination of these plants can be spread over a wide environmental range and up to several years. Thus, drought avoidance can occur. As a result, drought avoidance is realized (Salisbury & Ross, 1992). On the other hand, some desert plants living in arid environments can survive drought by shedding their leaves when water stress occurs. After rainfall, these plants leaf out again. This cycle repeats two or more times a year (Tietz & Tietz, 1982).

By using early varieties in areas where drought is observed, drought can be avoided in the late stages of plant development. In this way, plant cultivation can be shifted towards drier areas (Turner, 1986). Cereals such as wheat and barley with early flowering varieties are less affected by drought. However, these varieties give less yield than other varieties when rainfall is sufficient, which is seen as a disadvantage (Bidinger & Mahalakshmi, 1987).

A second method developed by plants against drought stress is *'Prevention of Water Loss'*. Plants can survive by minimizing water loss with the following factors.

1) Stimulation of Root Growth: When plants are exposed to water stress, they carry the assimilates they produce to the root zone, allowing the plant roots to extend to the soil layers where water is available. Thus, the leaf water potential is maintained by utilizing the water in the deeper layers through the roots (Wright and Smith, 1983; Morgan and Condon, 1986). Another way of protection from water stress is the ability of plants such as cacti to absorb surface moisture through their dense superficial root systems (Salisbury and Ross, 1992).

2) Reducing Water Loss: There are some mechanisms to reduce water loss. One of these mechanisms is the reduction of leaf area (Turner, 1986). The second mechanism to reduce water loss is stomatal closure. Stomatal closure varies depending on leaf water potential, stomatal conductance and water uptake and transport by plant roots (Turner, 1986; Harris, 1992). Stomatal closure due to water stress varies among plant species. For example, stomata of desert plants and tree species close more quickly in water deficit (Camacho-B et al., 1974). The fact that stomata in CAM plants are open at night and closed during the daytime is another adaptation of these plants to water stress. The fact that the stomata are embedded in the cuticle layer also causes low water loss. An impermeable cuticle layer is another way to reduce water loss (Taiz & Zeiger, 1991). This cuticle layer prevents water loss from the epidermis (Jordan et al., 1984). Reducing the radiation effect by leaf movement or increased reflection and leaf curling is another way to compensate for water loss (Innes & Blackwell, 1987). Paraheliotropic plants (plants that keep their leaves away from the sun) can be given as an example. Depending on the amount of water stress, changes in leaf position may occur in some plants such as soybean (Stevenson & Shaw, 1971). Leaf hairs are another factor in the elimination of water loss. Leaf surfaces covered with dense hairs reflect light and keep the leaf cool (Baldocchi et al., 1983), thus reducing water loss through transpiration due to reduced heating.

3) High Water Use Efficiency: Some plants store the water they receive in their tissues. Since water loss due to morphological changes is low in these plants, they use the available water efficiently (Hanscom and Ting, 1978; El-Sharkawy et al., 1984).

4) Making the Most of Available Moisture: Some plants growing in desert environments benefit from dew. These plants secrete salt from their leaves. The salt absorbs moisture from the air, which is then taken into the leaf. In addition, competition is often seen in desert plants. The plant prevents the germination and growth of the rival plant for water with the substances it secretes and uses the available water only by itself.

5) Adjustment of Osmotic Pressure: Osmotic adjustment or osmoregulation is an important form of adaptation seen in many organisms exposed to water and other stresses (Morgan, 1984). In osmotic adjustment, there is no decrease in turgor with an increase in solutes in the cell (Turner, 1986). This form of adjustment is due to increased concentrations of sugars, organic acids (especially proline) and ions such as K+ in the cell (Wright et al., 1977; Munns et al., 1979; Hanson and Hitz, 1982). Through osmotic adjustment, plants also utilize water that is tightly held in small pores in the soil.

6) Elasticity of Tissues: As it is known, cells start to shrink when water stress occurs. If the cells are elastic, the cell structure does not deteriorate during shrinkage and can return to its previous state with water intake. In this way, the plant is not damaged much by drought (Turner, 1986).

In addition, some plants are tolerant to water loss due to their membrane structure and enzyme activity (Turner, 1986). In such plants, the concept of 'Water Loss Tolerance' emerges in the plant against drought stress. While creosote (Larrea divaricata), which shows a shrub form, loses its vitality when its wet weight falls below 30%, this rate can be lethal even at 50-75% in many plants. The ability of these plants to dry and become metabolically active after dehydration depends on their special characteristics. Although this is not clear enough, it is attributed to mechanically damaged cells, disruption of membrane structure and the ability of proteins in the cytoplasm to withstand denaturation (Gaff, 1980).

CONCLUSION

The concept of stress, which occurs due to changing climatic conditions, has an important place in crop production. When the available areas in the world are evaluated according to the stress factors they are exposed to, it is reported that drought stress is effective in 26% of the area, mineral stress in 20% of the area and cold and frost stress in 15% of the area. It has been determined that other stresses cover an area of 29% and only 10% of the area is not exposed to any stress factor (Blum, 1986).

The response of plants to drought stress varies depending on many factors (plant species, other stress factors, etc.). However, in general, plants respond to drought stress first by maintaining water status, then by performing vital activities even under low water conditions, and finally by restoring vital functions and water status after drought stress (Kutlu 2010).

It should also be taken into account that drought and other plant stressors (temperature, salinity, etc.) can occur simultaneously in nature and therefore plants can be exposed to all these stressors at the same time, and it is important to note that the mechanisms to combat these stressors cannot be separate from each other (Suzuki et al., 2014). Therefore, studies on plant stressors should be conducted not only by considering a single stressor but also by considering all other stressors and the interactions between them.

REFERENCES

Baldocchi, D. D., Verma, S. B., Rosenberg, N. J., Blad, B. L., Garay, A., & Specht, J. E. (1983). Leaf Pubescence Effects on the Mass and Energy Exchange Between Soybean Canopies and the Atmosphere 1. Agronomy Journal, 75(3), 537-543.

Bidinger, F. R., Mahalakshmi, V., & Rao, G. D. P. (1987). Assessment of drought resistance in pearl millet (Pennisetum americanum (L.) Leeke). II. Estimation of genotype response to stress. Australian Journal of Agricultural Research, 38(1), 49-59.

Blum-Kulka, S. (1986). Shifts of Cohesion and Coherence in Translation Shoshana Blum-Kulka, Hebrew University of Jerusalem. Interlingual and intercultural communication: Discourse and cognition in translation and second language acquisition studies, 272, 17.

Camacho-B, S. E., Hall, A. E., & Kaufmann, M. R. (1974). Efficiency and regulation of water transport in some woody and herbaceous species. Plant Physiology, 54(2), 169-172.

Çırak, C., & Esendal, E. (2006). Soyada kuraklik stresi. Anadolu Tarım Bilimleri Dergisi, 21(2), 231-237.

Dolferus, R. 2014. To grow or not to grow: A stressful decision for plants. Plant Sci., 2229: 247-261.

El-Sharkawy, M. A., Cock, J. H., & Held K, A. A. (1984). Water Efficiency of Cassava. II. Differing Sensitivity of Stomata to Air Humidity in Cassava and Other Warm-Climate Species 1. Crop Science, 24(3), 503-507.

Ergen, NZ., Thimmapuram, J., Bohnert, HJ., Budak, H. 2009. Transcriptome pathways unique to dehydration tolerant relatives of modern wheat. Funct Integr Genomics., 9: 377-396.

Furr, B. J. A., & Jordan, V. C. (1984). The pharmacology and clinical uses of tamoxifen. Pharmacology & therapeutics, 25(2), 127-205.

Gaff, J. G. (1980). Avoiding the Potholes: Strategies for Reforming General Education. Educational Record, 61(4), 50-59.

Göksoy AT, Turan Z.M (1991) Kuraklığın Bitki Fizyolojisi ve Morfolojisi Üzerine Etkileri. U.Ü.Z.F. Dergisi, No: 8, s. 189-199, Bursa.

Hale, M. G. AND ORCUTT, D. M., The Physiology of Plants under Stress, John Wiley & Sons Inc., 1-4 and 206. pp., New York, 1987.

Hanscom III, Z., & Ting, I. P. (1978). Responses of succulents to plant water stress. Plant physiology, 61(3), 327-330.

Hanson, A. D., & Hitz, W. D. (1982). Metabolic responses of mesophytes to plant water deficits. Annual Review of plant physiology, 33(1), 163-203.

Harris, T. (1992). Performance assessment measures for univariate feedback control. The Canadian Journal of Chemical Engineering, 70(6), 1186-1197.

Hungr, O., Morgan, G. C., & Kellerhals, R. (1984). Quantitative analysis of debris torrent hazards for design of remedial measures. Canadian Geotechnical Journal, 21(4), 663-677.

Innes, S., Lavigne, D. M., Earle, W. M., & Kovacs, K. M. (1987). Feeding rates of seals and whales. The Journal of Animal Ecology, 115-130.

Kalefetoğlu, T. & Ekmekçi, Y. (2005). THE EFFECTS OF DROUGHT ON PLANTS AND TOLERANCE MECHANISMS . Gazi University Journal of Science , 18 (4) , 723-740

Keyser, H. H., & Munns, D. N. (1979). Tolerance of rhizobia to acidity, aluminum, and phosphate. Soil Science Society of America Journal, 43(3), 519-523.

Kocaçalışkan, Bitki Fizyolojisi, 126. sy. Kütahya, 2004.

Kocaçalışkan, İ.,2008. Bitki Fizyolojisi. Nobel Basımevi, 1338, Ankara.

Mahajen, S., Tuteja, N., Cold, salinity and drought stresses: an overview, Arch. Biochem. Biophys., 444, 139-158, 2005.

Morgan, J. M., & Condon, A. G. (1986). Water use, grain yield, and osmoregulation in wheat. Functional plant biology, 13(4), 523-532.

Özcan, M. (2020). Bahçe Bitkilerinde Stres Fizyolojisi.

Öztürk, M. A. ve Seçmen, Ö., 1992, Bitki Ekolojisi, Ege Üniv. Fen Fak. Yayınları No: 141, İzmir.

Qin, J., Yang, K., Liang, S., & Guo, X. (2009). The altitudinal dependence of recent rapid warming over the Tibetan Plateau. Climatic Change, 97(1-2), 321-327.

Salisbury FB, Ross CW 1992. Plant physiology. California, Wadsworth Publishing Comp. 682.

Stevenson, K. R., & Shaw, R. H. (1971). Effects of Leaf Orientation on Leaf Resistance to Water Vapor Diffusion in Soybean (Glycine max L. Merr) Leaves 1. Agronomy Journal, 63(2), 327-329.

Taiz, S. L. and Taiz, L. (1991). Ultrastructural comparison of the vacuolar and mitochondrial H+-ATPases of Daucus carota. Botánica Acta 104, 117–121.

Turner, N. C. (1986). Adaptation to water deficits: a changing perspective. Functional Plant Biology, 13(1), 175-190.

Wright, T. L., & Okamura, R. T. (1977). Cooling and crystallization of tholeiitic basalt, 1965 Makaopuhi lava lake, Hawaii (Vol. 1004). US Government Printing Office.

Wright, G. C., Smith, R. C. G., & McWilliam, J. R. (1983). Differences between two grain sorghum genotypes in adaptation to drought stress. I. Crop growth and yield responses. Australian Journal of Agricultural Research, 34(6), 615-626.

Xu, Z. and Zhou, G, 2008, Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass, Journal Exper. Botany., 59(12): 3317- 3325.

Overview of Organic Fertilizers

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ABSTRACT: Ensuring balanced and adequate nutrition for the rapidly growing world population is only possible by increasing agricultural production and productivity. Increasing productivity in agricultural production is possible through the use of quality seeds, control of diseases and pests, irrigation, fertilization and sustainable and efficient use of natural resources such as soil. It is important to use soil, which is a determining and exhaustible resource in agricultural production, in a sustainable manner. Fertilizers, one of the important inputs in agricultural production, determine yield and quality. Today, it is known that soil sustainability and human health are negatively affected by some reasons. Among these, unconscious and excessive use of inorganic (chemical) fertilizers to obtain high yields and continuous use of inorganic fertilizers cause water and soil pollution. Therefore, considering the negative effects of chemical fertilizers today, organic fertilizers have become very important as an alternative to chemical fertilizers used worldwide to ensure agricultural sustainability. Organic fertilizers stand out with the nutrients they contain. Organic fertilizers containing nitrogen (N), phosphorus (P), potassium (K) and other nutrients at different rates according to the source of organic matter, meet the need for plant nutrients, which have an important place in plant production, as well as improving the physical, chemical and biological properties of the soil. Organic fertilizers commonly used in our country are barn and poultry manure, green manure and composts.

Keywords: Agricultural production, Organic fertilizer, Chemical fertilizer

INTRODUCTION

Ensuring balanced and adequate nutrition for the rapidly growing world population is only possible by increasing agricultural production and productivity. Increasing productivity in agricultural production is possible through the use of quality seeds, control of diseases and pests, irrigation, fertilization and sustainable and efficient use of natural resources such as soil.

It is important to use the soil, which is a determining and exhaustible resource in agricultural production, in a sustainable manner. Fertilizers, one of the important inputs in agricultural production, determine yield and quality. Inorganic (chemical) fertilizers are used all over the world to increase agricultural productivity (Karaman and Turan, 2012). Today, it is known that soil sustainability and human health are negatively affected by some reasons. Among these; unconscious and excessive use of inorganic (chemical) fertilizers to obtain high yields and continuous use of inorganic fertilizers are negatively affected by many reasons such as water and soil pollution, soil sustainability and human health. Nowadays, deficiency in soil organic matter content causes a decrease in soil microbial activities, heavy metal accumulation, decrease in soil fertility, nitrate accumulation and significant health problems (Savcı, 2012). Therefore, considering the negative effects of chemical fertilizers today, organic fertilizers have become very important as an alternative to chemical fertilizers used worldwide to ensure agricultural sustainability. Organic fertilizers should be preferred for the reconstruction and protection of the deteriorated natural order. Since organic fertilizers contain plant nutrients, organic matter and a wide variety of microorganisms, they have a positive effect on the biological, physical and chemical properties of agricultural soils (Kacar and Katkat, 2007).

Organic fertilizers are products that contain nutrients in the form of organic compounds, made from plant, animal, etc. wastes or by-products, improving the chemical and physical structure of the soil and facilitating the uptake of plant nutrients. Organic fertilizers facilitate the warming and aeration of the soil, help the plant nutrients in the soil to transform into plant-available form, are a source of nutrients for plants, improve the structure of the soil and positively affect plant growth (Soyergin, 2003; Mercik and Stepien, 2006; Adiloğlu and Eraslan, 2012). Organic fertilizers alter soil physicochemical properties as well as different soil enzyme activities, thus reducing the negative impact of long-term over-application of a single inorganic fertilizer on soil quality (Chen et al., 2020, Qaswar et al., 2020, Samuel et al., 2018). Organic fertilizer application not only increases nutrients such as total phosphorus and nitrogen, but also increases the nutrients available in the soil (available phosphorus, available potassium, alkaline nitrogen) (Qaswar et al., 2020). Organic fertilizers are composed of substances of animal and plant origin.

Organic fertilizers stand out with the nutrients they contain. Organic fertilizers containing nitrogen (N), phosphorus (P), potassium (K) and other nutrients at different rates according to the source of organic matter, meet the need for plant nutrients, which have an important place in plant production, as well as improving the physical, chemical and biological properties of the soil. Organic fertilizers commonly used in our country are barn and poultry manure, green manure and composts.

1) Barn and Poultry Manure

In general, the solid and liquid excreta of barnyard and poultry animals and the material laid as bedding under these animals are defined as 'barnyard and poultry manure'. These fertilizers improve the soil structure by increasing the amount of organic matter and microbiological activity of the soil while providing the nutrients necessary for plants to agricultural soils. As with other organic fertilizers, the amount of plant nutrients contained in barnyard and poultry manure is different. This difference varies depending on the source of the organic manure as well as the type of animal, its age, the feed used in feeding and the storage of the manure obtained. The nutrient content of various barnyard and poultry manures is given in the table 1 below.

Table 1. Nutrient content of various barnyard and poultry manures (Sezen, 1984)

Organik Gübre Kaynağı	Su içeriği (%)	Kuru Madde İçeriği (%)	% N	% P_2O_5	% K_2O
Sığır Gübresi	83,2	16,2	0,29	0,17	0,10
Koyun Gübresi	65,5	34,8	0,55	0,31	0,15
At Gübresi	75,7	24,3	0,44	0,35	0,35
Kaz, Ördek Gübresi	75	25,0	0,80	1,00	0,80
Güvercin, Tavuk	62,0	38,0	1,70	1,60	0,90

According to the table given above, pigeon and chicken manure stands out with 1.70% N, 1.60% P_2O_5 and 0.90% K_2O among barnyard and poultry manures in terms of plant nutrient content, which has an important place in agricultural production. Organic fertilizers of cattle and sheep origin, which are widely used in our country, have an important place in agricultural systems with their high content of plant nutrients (0.29-0.55% N, 0.17-0.31% P_2O_5, 0.10-0.15% K_2O).

Barn and poultry manures affect the soil and thus crop production in various ways. These effects can be listed as follows;

- It increases the water holding capacity of the soil and provides aeration.
- Prevents soil and water erosion.

- It provides the soil to be tempered.
- It warms the soil and maintains this heat for agricultural production.
- It is effective on the pH of soils.
- Microorganism activities and biological processes accelerate and increase with the decomposition of organic materials given to the soil.
- As a result of microorganism activities in the soil, the amount of macro (nitrogen, phosphorus, potassium) and micro (iron, zinc, manganese, etc.) plant nutrients required for plant production increases.

In order to obtain optimum benefit from barn and poultry manure; storage of manure, maturation period, amount to be used, time and method of application, etc. should be given due attention.

2) Green Fertilizer

Plants that are grown in order to provide the necessary organic matter needed by the soil and that are plowed and buried in the soil during certain periods of their development (especially in the green phase) are called 'green manure' and this process is called 'green fertilization'. In general, green manure plants have the characteristics of rapid growth, abundant vegetative parts and the ability to grow easily in many soil types.

Although many plants can be used as green manure plants, legume plants come to the fore. While legume crops such as cowpea, soybean, vetch, clover are frequently preferred as green manure plants, rye, oat, barley, rapeseed come to the forefront from wheatgrasses, and legume plants as green manure plants bind more nitrogen to the soil than wheatgrass plants.

The most important and basic feature of green manure application is to enrich the organic matter content of the soil. With green fertilization, soil microorganisms by providing nutrient source, positive development is provided on microorganism activities. With this positive development in the soil structure, it is ensured that the soil is annealed more quickly and stays annealed for a long time. Again, in heavy textured soils, tillage due to green fertilization becomes easier and a soil with suitable soil properties is left for the plant to be planted after green fertilization. Green manure plants protect the soil surface against various factors and especially against erosion. Green manure plants increase infiltration by covering the surface of the soil, prevent soil erosion on sloping lands, and prevent frost damage to the roots of perennial plants by retaining falling snow in winter regions (Aygün and Acar 2019).

3) Composts

Composts are humus and are organic products obtained as a result of microbial decomposition of garbage, animal waste, kitchen waste, city dumps and some fabrication residues of organic origin in agricultural enterprises after various processes. These are organic fertilizers that are free from pathogens due to aerobic decomposition and have the smell and appearance of heather soil (Yetgin 2010). By providing organic matter to the soil with compost fertilization, the physical, chemical and biological properties of the soil are improved and a suitable growth and development environment is prepared for plants, which are the basis of agricultural production.

CONCLUSION

Today, the use of organic fertilizers has gained importance in sustainable agricultural production. Organic fertilizers ensure sustainable use of soil and increase yield and quality in crop production. In our country, various organic fertilizers are used in agricultural production and barnyard-poultry manure, green manure and composts come to the forefront in our country's agriculture. It is predicted that these fertilizers will increase the organic material content of the soil and contribute positively to the increase in soil fertility and crop production.

REFERENCES

Adiloğlu, A., & Eraslan, F. (2012). Gübreler ve gübreleme tekniği. M. R. Karaman (Ed.), Bitki Besleme Sağlıklı Bitki, Sağlıklı Üretim. (ss. 420-421). Gübretaş Rehber Kitaplar Dizisi, 2, Ankara.

Aygün, Y. and Acar, M., 2019. Organik Gübreler ve Önemi. https://www.researchgate.net/publication/330598198.

Chen, Z., Pang, X., Guo, S., Zhang, W., Geng, Z., Zhang, Z., Du, S. & Deng, Z. (2019). Chemical composition and bioactivities of alpinia katsumadai hayata seed essential oil against three stored product ınsects. Journal of Essential Oil Bearing Plants, 22(2), pp.504-515.

Kacar, B., & Katkat V. A. (2007). Gübreleme ve Gübreleme Tekniği. Nobel Basımevi, Ankara.

Karaman, M. R., & Turan, M. (2012). Bitki beslemede sürdürülebilir yönetim stratejisi ve gübre etkinlik parametreleri. Toprak Su Dergisi 1(1), 15-21.

Mercik, S., & Stepien, W. (2006). Crop yields and selected soil properties on manured and not manured fields at the period of many years. Nawozy Nawozenie (Fertilisers and Fertilization), 8(4), 141-149.

Savcı, S. (2012). An agricultural pollutant: Chemical fertilizer. International Journal of Environmental Science and Development, 3(1), 77-80.

Sezen, Y. 1984. Gübreler Ve Gübreleme, Atatürk Üniversitesi Ziraat Fakültesi Yayınları No:19; Sayfa:39-83. ERZURUM

Soyergin, S. (2003). Organik Tarımda Toprak Verimliliğinin Korunması, Gübreler ve Organik Toprak İyileştiricileri. Atatürk Bahçe Kültürleri Merkez Araştırma Enstitüsü, Yalova.

Yetgin, M., A.2010. Organik Gübreler ve Önemi. Samsun Tarım İl Müdürlüğü. Çiftçi Eğitimi ve Yayın Şubesi. SAMSUN.

Exopolysaccharides from Lactic Acid Bacteria: Insight into the Optimal Conditions for Production and Extraction*

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ABSTRACT: Herein, the effects of different production and extraction conditions on the yield and chemical structure of exopolysaccharide (EPS) were evaluated. Lactic acid bacteria were isolated from dairy products. The EPS-positive strains, selected using ruthenium red selective agar, were incubated under different incubation conditions (oxygen, shaking, and incubation time). Free and capsular EPS were extracted using cold ethanol precipitation. The EPS production efficiency was determined using the phenol-sulfuric acid method. The effect of different ethanol ratios on EPS yield was determined using ethanol at 1:1 and 1:2 ratios (v/v) during ethanol precipitation. The impact of applied conditions on the chemical structure of EPS was determined by FTIR spectroscopy, identifying the functional groups of EPS. The results showed that the effect of oxygen on the yields of sEPS and kEPS varied from strain to strain. Longer incubation times increased EPS production by 1.7-2.2 fold, depending on the strain. Using ethanol at a ratio of 1:2 almost doubled the extraction yield. No obvious peak shift was observed except for some differences in band intensities in the FTIR spectra, proving that the applied conditions had no effect on the chemical structure. The FTIR results demonstrated that fEPS and cEPS were structurally different, consisting of distinct functional groups.

Keywords: Free exopolysaccharide, Capsular exopolysaccharide, Ethanol precipitation, Exopolysaccharide yield

INTRODUCTION

Exopolysaccharides, also known as extracellular polymeric substances (EPS), are naturally occurring polymers made up of high-molecular-weight polysaccharides that are produced inside cells and secreted out of the cell by a variety of microorganisms (Ruas-Madiedo and De Los Reyes-Gavilán 2005). EPSs represent multiple functions, including shielding bacteria from desiccation, concentrating nutrients, lessening exposure to harmful substances, and improving bacterial adhesion to surfaces and survival throughout their epiphytic or saprophytic lives by facilitating the creation of biofilms (Tampakaki, Hatziloukas, and Panopoulos 2009). EPSs also exhibit diverse bioactivities such as antioxidant, antimicrobial, anticancer, antibiofilm, prebiotic, immunomodulatory, antifreeze, and anti-inflammatory activities. Moreover, they also possess functional properties like emulsifier, water binding, texture improver, stabilizer, and gelling. Owing to their functional diversities, EPSs have extensive commercial applications in a wide range of industries, especially in food, cosmetics, pharmaceuticals, textiles, petroleum, and agriculture (Kaur and Dey 2023).

One of the most important organisms that are considered the main sources of EPS production is lactic acid bacteria (LAB). Numerous LAB strains produce EPSs, which can be either bound to the bacterial surface as capsular polysaccharides (cEPS) or released into the surrounding medium as free polysaccharides (fEPS) (Angelin and Kavitha 2020; Cescutti 2010). Studies have shown that while the majority of EPS-producing LAB strains produce fEPS, some LAB strains can produce both cEPS and fEPS simultaneously (Yang et al. 2010). EPS-producing LAB can be isolated from a variety of sources and has a range of properties because of the available nutrients and ambient conditions. The EPS obtained from LAB represents several bioactivities that confer various health-promoting benefits to humans. LAB-EPSs also contribute techno-functional properties as thickeners, stabilizers, gelling agents, and emulsifiers in commercial products (Riaz Rajoka et al. 2020). The genera *Lactobacillus*, *Streptococcus*, *Lactococcus*, and *Leuconostoc* contain the majority of EPS-producing bacteria. *Lactococcus lactis*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Streptococcus thermophilus*, and *Lactobacillus rhamnosus* are the LABs that have been investigated the most for EPS generation (Kaur and Dey 2023).

Although many studies have been carried out on EPS, the discovery of EPS with superior properties that may have commercial value is still remarkable. In the research on EPS, different protocols are used for the production and purification of EPS. Herein, we aim to optimize both the incubation conditions of LAB and the ethanol ratio used in EPS precipitation to increase the EPS production yield of LAB. Moreover, the effects of the applied methods on the chemical structure of EPS were analyzed by Fourier-transformation infrared spectroscopy (FTIR), identifying functional groups of EPS.

MATERIAL AND METHOD

Sampling and isolation of LAB

Samples of traditional Turkish cheese from different animals (pot cheese with black cumin, pickled ewes cheese, fresh village cheese made from cow milk, and sheep/goat cave cheese) were purchased from local markets in various regions of Türkiye. Samples (10 g) were homogenized with 90 mL of physiologic peptone water, and serial dilutions were carried out. 100 µL of dilutions were surface-plated on MRS agar (Merck, Darmstadt, Germany), M17 agar (Merck, Darmstadt, Germany), and MSE agar (Bioneks, Türkiye) plates. MRS agar plates were incubated at 15, 30, and 40 °C under anaerobic conditions in a sealed jar using Anaerocoult A packs (Merck, Germany), while MSE agar plates were incubated at 30 °C under anaerobic conditions in a sealed jar using Anaerocoult A packs. M17 agar plates were incubated at 37 °C under aerobic conditions. All medium was supplemented with cycloheximide to a final concentration of 0.1 mg/mL. Several colonies with varying morphologies (a total of 242 isolates) were chosen after 72 hours of incubation, and they were subsequently streaked on the proper medium for purification. The isolates that were Catalase-negative and Gram-positive were chosen as LAB.

Screening of EPS-producing LAB strain

EPS-producing LAB strain was selected firstly with the detection of "ropy" phenotype and "mucoide non-ropy" phenotype and secondly using ruthenium-red milk agar plates. Ten EPS-producing LAB strains were chosen, which were successively cultivated (at least five times) to select those that maintained a stable phenotype and stored at −80 °C in a proper medium with 20% glycerol.

Optimization of incubation condition of LAB strains for EPS production

Fresh cultures were inoculated into the appropriate broth medium and incubated for 48 h under appropriate incubation conditions. The cells were harvested by centrifugation (8000xg, 20 min, 4 °C, Hanil Science Industrial Combi 514R, Korea). The cell pellets were resuspended using physiological saline, and the turbidity of the bacterial suspensions was adjusted to match that of 0.5 McFarland standards. The suspension was used to inoculate 100 mL of proper modified broth medium (without yeast extract) to a final density of 10^6 CFU/mL. To optimize growth conditions, each strain was prepared in quadruplicate, and incubation was carried out at different time intervals (48 or 72 hours) either aerobically or anaerobically.

Isolation of EPS

After incubation of LAB strains, cells were separated by centrifugation at 8000xg for 30 min at 4 °C. The supernatant that contained the free EPS (fEPS) was taken, and the trichloroacetic acid (Sigma-Aldrich, Germany) solution was added to the supernatant to a final concentration of 10% (w/v) and incubated at 4 °C for 12 h. The solutions were centrifuged at 10,000xg for 30 min at 4 °C to remove precipitated proteins. The supernatant was then mixed with either 1:1 or 1:2 volumes of cold ethanol (95%, Merch, Germany) and kept at 4 °C for 12 h. EPS were collected by centrifugation at 10,000xg for 40 min at 4 °C and lyophilized (FDU-8612, Operon Co., Ltd., Gimpo, Korea) for further study. To purify capsuler EPS (cEPS), the cell pellets were resuspended in 1 M NaCl and treated by a cell sonicator (Hielscher-UP200H, Germany) at 40 W for 3 min (Wang et al. 2014). Then, the cell suspension was centrifuged at 8000xg for 30 min at 4 ºC, the supernatant was collected, and the protocol carried out for fEPS was repeated (Rajoka et al. 2019).

Determination of total sugar content of EPS

The total sugar content of EPS was determined by the phenol–sulfuric acid method suggested by Dubois (DuBois et al. 1956). 200 µL of 5% phenol were added to 200 µL of the sample, and then rapidly, 1000 µL of 95% H2SO4 was added. The mixture was vortexed and left to stand for at least 60 minutes. The absorbance was read at 490 nm (Shimadzu UV-1208, Japan) against the blank. The total sugar content of EPS was determined using the equation of the calibration curve for glucose.

Fourier-transform infrared spectroscopy

The FTIR spectrum as an average of 32 scans of the EPS was recorded in the region of 4,500–500 cm^{-1} on an ATR FTIR system (Perkin Elmer Spektrum 100, USA) by subtracting both the background and atmospheric water at resolution.

Statistical analysis

The findings were presented as mean values with standard errors from the three replications. The level of significance between different conditions ($p < 0.05$) was computed using one-way analysis of variance (ANOVA) using SPSS statistics software version 22.00 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

EPSs have gained increasing attention due to their functional and beneficial properties for novel applications. However, a major barrier to using EPSs in applications is their low production yield. This study was aimed at maximizing EPS production by optimizing the incubation conditions and ethanol ratio that are used for ethanol precipitation.

Isolation of LAB and selection of EPS-producing LAB strain

A total of 242 LABs were isolated from four different traditional cheese samples. The selection of EPS-producing strains was carried out in two steps: i) strains with mucoid or ropy colonies were identified; ii) from those, strains exhibiting Ruthenium red positive colony properties were selected. Ten EPS-producing strains among 242 isolates were chosen for further analysis.

Optimization of incubation condition of LAB strains for EPS production

Three strains that were isolated from MRS medium at 40 °C were chosen to determine the effect of the presence of oxygen and incubation time on EPS production. The characteristics of the selected strains are presented in Fig. 1.

Strain No	Source	Colony Morphology	Microscopic Morphology	Ruthenium Red
21.2	Fresh village cheese	small, mucoid	Rod	Positive-strong
22.1	Sheep/goat cave cheese	Small, shiny, mucoid	Rod	Positive-strong
22.4	Sheep/goat cave cheese	Very small, mucoid	Rod	Positive-strong

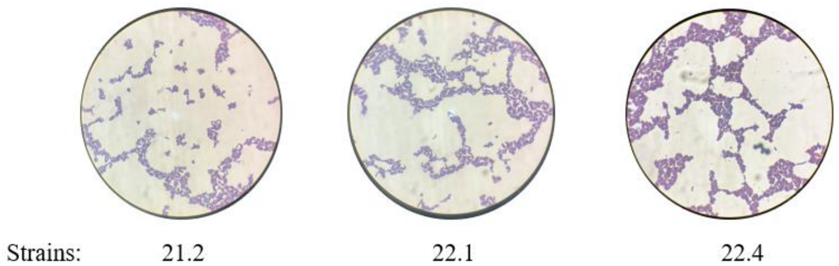


Figure 1. The properties of the selected strains isolated from MRS media

The selected isolates were incubated in modified MRS broth at different time intervals (48 or 72 hours) either aerobically or anaerobically. Both fEPS and cEPS were extracted, and the resulting EPS were lyophilized. The total sugar content of isolated EPS was determined by the phenol-sulfuric acid method, and the results are presented in Fig. 2.

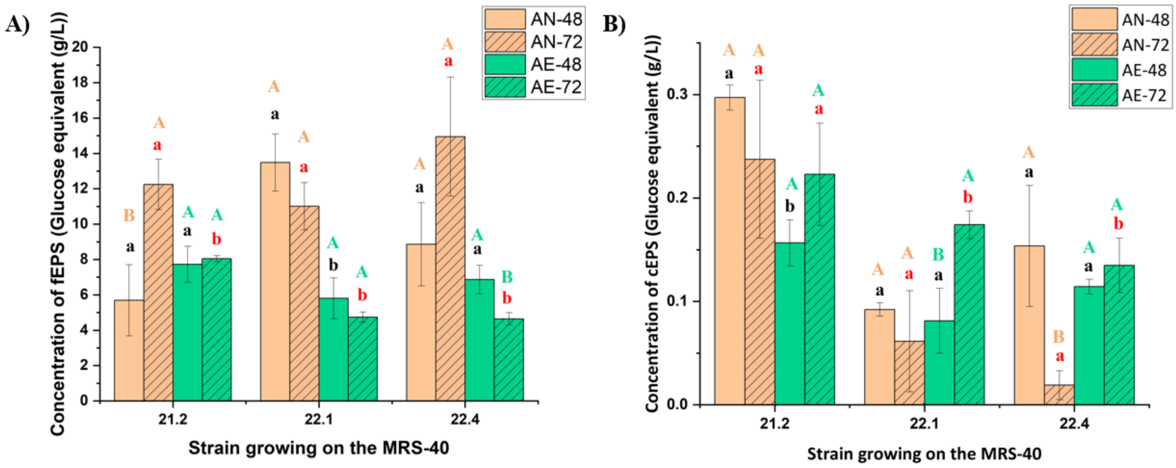


Figure 2. The effect of incubation conditions on fEPS (A) and cEPS (B) production of LAB

Different black lowercase letters indicate a significant difference between the anaerobic and aerobic groups at 48 hours of incubation (fEPS: $p < .05$, cEPS: $p < .001$), while different red lowercase letters indicate a significant difference between the anaerobic and aerobic groups at 72 hours of incubation. Different orange uppercase letters indicate a significant difference between the 48 and 72 hours of incubation groups for anaerobic conditions ($p < .05$), while different yellow uppercase letters indicate a significant difference between the 48 and 72 hours of incubation groups for aerobic conditions ($p < .05$)

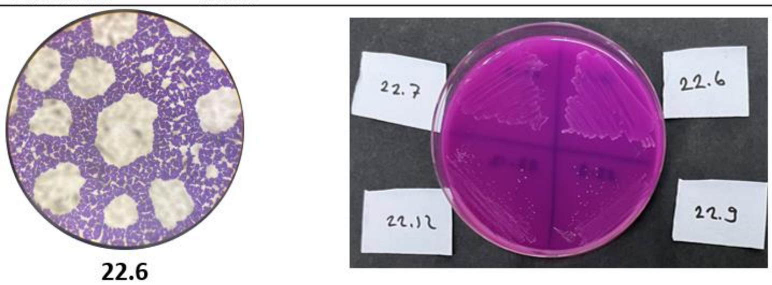
Since most LABs are generally anaerobic or facultatively anaerobic, the oxygenated atmosphere may put them under stress. There is a wide range of oxygen tolerance among LAB species, from those that are extremely sensitive to oxygen to those that can survive for long periods of time in the presence of oxygen (Yan et al. 2018). The results showed that the use of an oxygenated atmosphere as a stress source during the same incubation periods reduced or did not change the fEPS production efficiency. For cEPS, the effect of oxygen varied from strain to strain, and the cEPS production efficiency of 22.1 and 22.4 increased up to fourfold under an oxygenated atmosphere for 72 hours of incubation.

When the effect of incubation times on fEPS yield was examined, it was determined that the effect differed from strain to strain depending on anaerobic and aerobic conditions. Under anaerobic conditions, some strains (21.2 and 22.4) produced 1.7-2.2 times more fEPS with varying incubation times. On the other hand, for cEPS production, increasing the incubation time decreased cEPS production under anaerobic conditions and increased cEPS production under aerobic conditions.

To determine the effect of incubation time on EPS production of LAB strains isolated under aerobic conditions, four strains isolated from M17 agar were selected (Fig. 3). No statistical difference was observed in the production efficiency of fEPS with the exception of one strain, which showed a drop in production efficiency after 72 hours of incubation. While the cEPS production amount of strain 20.1

increased after 72 hours of incubation, no statistical difference was observed for strain 2.9. cEPS could not be extracted after 72 hours of incubation for strains 1.5 and 22.6.

Strain No	Source	Colony Morphology	Microscopic Morphology	Ruthenium Red
1.5	Cow's milk	Creamy white, shiny	Cocci	Positive-strong
2.9	Sheep pickled cheese	White, mucoid	Cocci	Positive-strong
20.5	Sheep pickled cheese	White, shiny, mucoid	Cocci	Positive-strong
22.6	Sheep/goat cave cheese	White, large, convex, mucoid	Cocci	Positive-strong



22.6

Figure 3. The properties of the selected strains isolated from M17 media

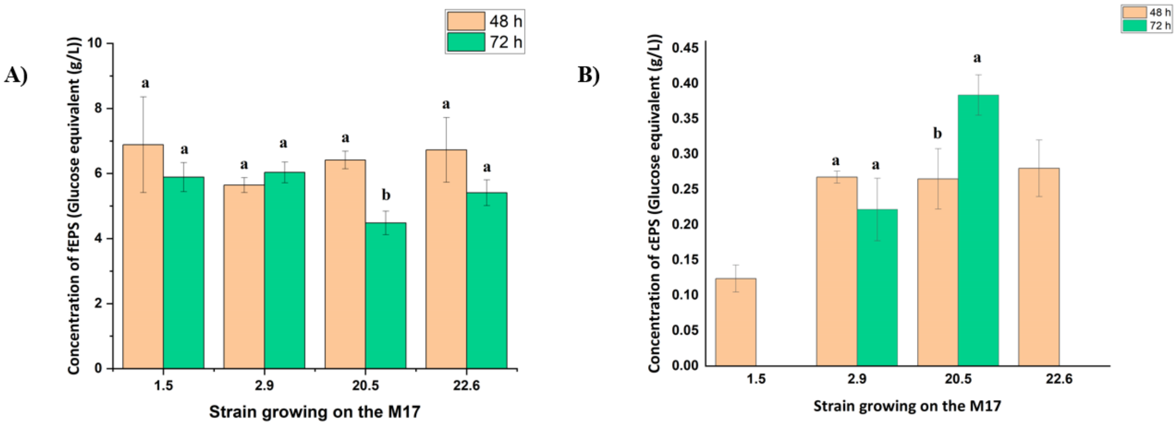


Figure 4. The effect of incubation time on fEPS (A) and cEPS (B) production of LAB.

Black lowercase letters indicate a significant difference between the groups ($p < .001$).

The effect of ethanol ratio used for ethanol precipitation on crude EPS extraction

Three strains isolated from MSE agar were selected to determine the effect of ethanol ratios on EPS extraction from LAB strains (Fig. 5). The results revealed a significant statistical difference ($p < 0.05$) in the extraction yield of EPS. Using ethanol at a ratio of 1:2 (v/v) almost doubled the extraction yield of EPS.

Strain No	Source	Colony Morphology	Microscopic Morphology	Ruthenuim Red
19.4	Pot cheese with black cumin	White, cream, shiny, mucoid	Rod	Positive-strong
20.1	Sheep pickled cheese	Shiny, water droplet, large, mucoid	Cocci	Positive-strong
20.6	Sheep pickled cheese	Very large, transparent	Cocci	Positive-strong

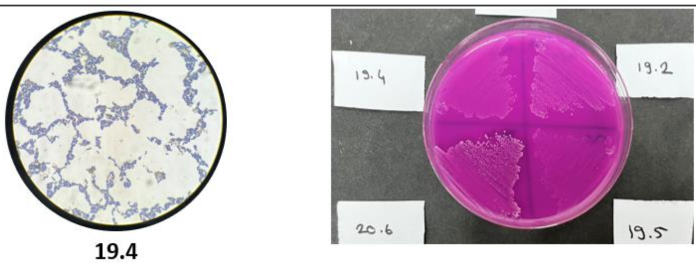


Figure 5. The properties of the selected strains isolated from MSE media

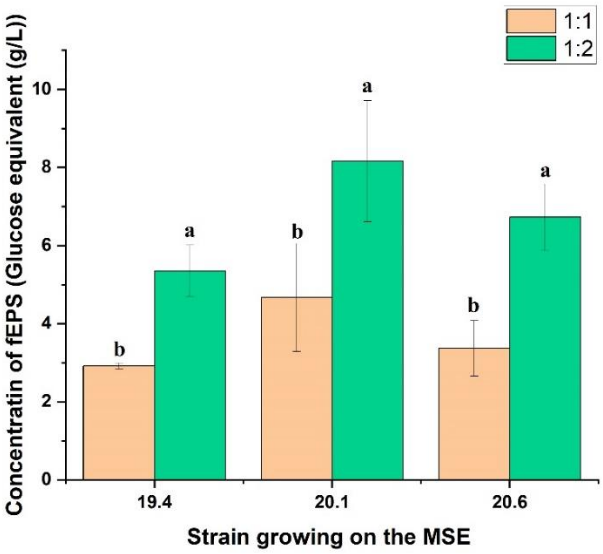


Figure 6. The effect of applied conditions of chemical structure of EPS

The effect of different conditions on the chemical structure of EPS

FTIR analysis was used to determine the effect of applied conditions on the chemical structure of EPS by identifying the functional groups of EPS. Except for some differences in peak intensities, the FTIR spectra of EPSs taken for both fEPSs and cEPSs showed similar characteristics, and this situation proved that applied conditions did not have any effect on the chemical structure of EPSs (Figs. 6 and 7). The EPSs spectra revealed functional groups that are characteristic of exopolysaccharides. A broad absorption peak in the region of 3,200 cm^{-1} indicated the stretching -OH stretching vibrations, and the peak related to C-H stretching of the aliphatic methylene group was at 2,900 cm^{-1} which confirmed the presence of polysaccharides. Another characteristic of polysaccharides was the presence of bands in the region of 1650–1550 cm^{-1}, which typically correspond to the C–O and carboxyl groups (Srinivash et al. 2023). An intense absorbance at 1,039 cm^{-1} strongly indicates the C-O-C stretching vibration, which indicated that the sugars exist mainly in the pyranose form (Chen et al. 2015). The band around 900 cm^{-1} demonstrated the presence of glycosidic linkages (Gawande et al. 2021). Also, the band around 810 cm^{-1} also indicated the possible presence of sulfated groups.

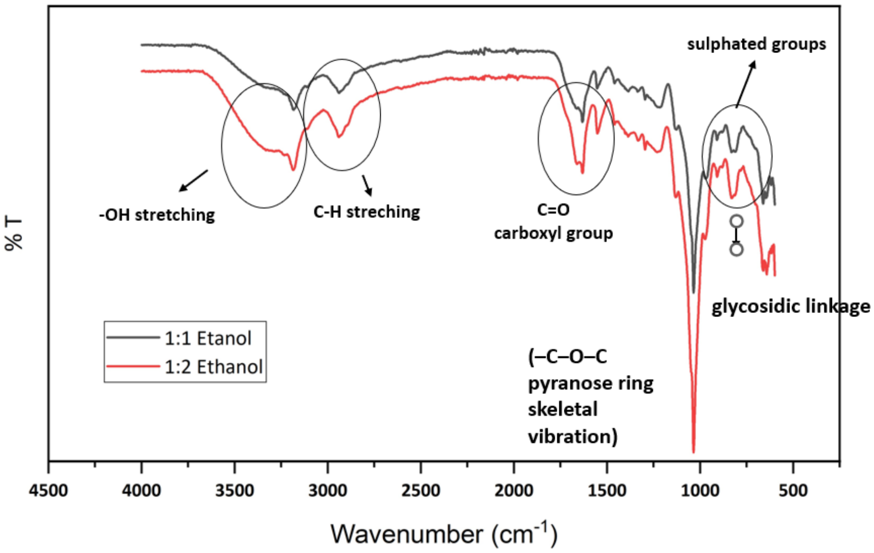


Figure 7. The effect of different ethanol ratios on the chemical structure of EPS from the strain 20.1, isolated from MSE.

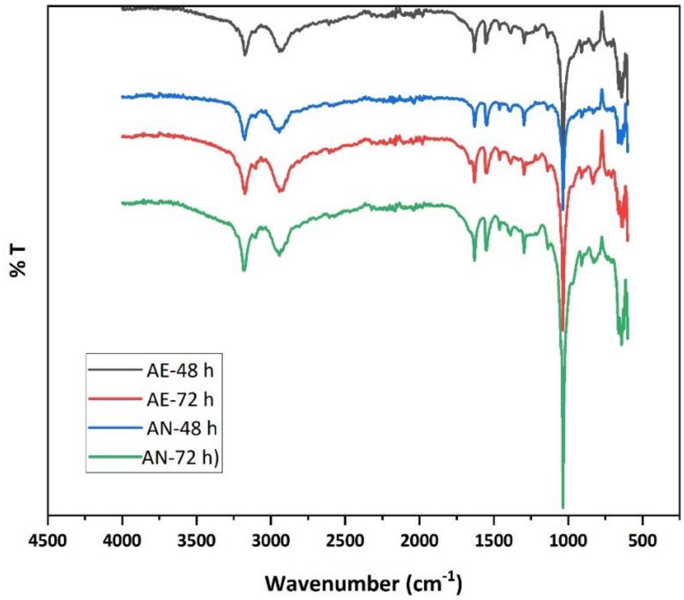
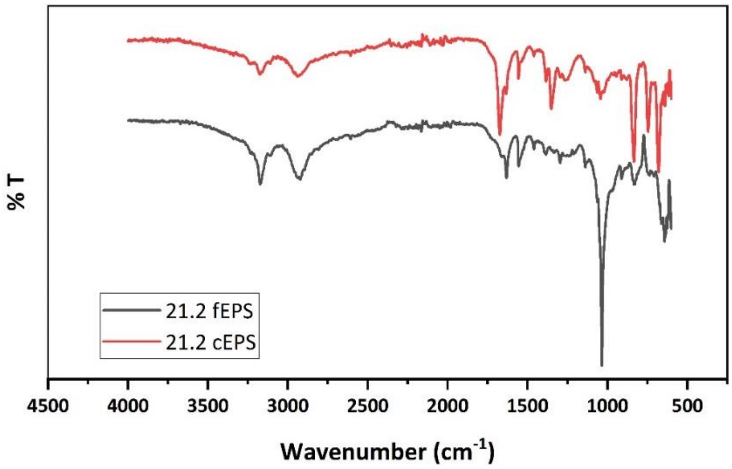


Figure 8. The effect of different incubation condition on the chemical structure of cEPS from the strain 22.4, isolated from MRS.

Comparing the FTIR spectra of cEPS and fEPS revealed that they have different chemical structures. The peak in the region of 1350 cm^{-1}of cEPS related to the sulfates wagging (Srinivash et al. 2023) was not observed in the spectrum of free EPS. Also, the peaks in the region of

740 and 675 cm^{-1}, might indicate that the bending of the OH group was not observed in the spectrum of free EPS. Moreover, the peak, which represented the pyranose ring, was stronger for fEPS. Also, the peak stand for glycosidic linkage was stronger for cEPS.



Figue 9. Differences between fEPS and cEPS isolated from the strain 21.2.

CONCLUSION

In this study, production and extraction conditions were optimized to produce EPS with maximum efficiency. The result showed that different LAB strains produced different amounts of EPS in response to different incubation conditions. Varying the incubation time compared to the presence of oxygen showed a greater effect on EPS production. Especially in some strains, increasing the incubation period to 72 hours doubled the EPS yield. In cold ethanol precipitation, using ethanol at a ratio of 1:2 (v/v) doubled the EPS extraction efficiency. All FTIR spectra obtained from different applications have proven that the treatments do not have any effect on the chemical structure, as there were no distinguishable differences other than intensities or peak areas.

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Statement of Conflict of Interest

The authors declare that they are no conflict of interest.

Authors' Contributions

Tareq DBL, Bahar Tuba Findik and Hilal Yildiz designed and analyzed the research, Bahar Tuba Findik and Hilal Yildiz worked on the preparation of pictures and tables. All authors contributed to the writing of the article and took part in the process of publication of the article and read and approved it.

REFERENCES

Angelin, J., and M. Kavitha. 2020. "Exopolysaccharides from Probiotic Bacteria and Their Health Potential." International journal of biological macromolecules 162: 853–65. https://pubmed.ncbi.nlm.nih.gov/32585269 (March 19, 2021).

Cescutti, Paola. 2010. "Bacterial Capsular Polysaccharides and Exopolysaccharides." In Microbial Glycobiology, Elsevier Inc., 93–108.

Chen, Zhina et al. 2015. "Chemical and Physical Characteristics and Antioxidant Activities of the Exopolysaccharide Produced by Tibetan Kefir Grains during Milk Fermentation." International Dairy Journal 43: 15–21.

DuBois, Michel. et al. 1956. "Colorimetric Method for Determination of Sugars and Related Substances." Analytical Chemistry 28(3): 350–56. https://doi.org/10.1021/ac60111a017.

Gawande, Krushna et al. 2021. "Lactic Acid Bacteria Based Purified Exopolysaccharide Showed Viscofying and Hypercholesterolemic Capabilites." Food Hydrocolloids for Health 1: 100042.

Kaur, Navneet, and Priyankar Dey. 2023. "Bacterial Exopolysaccharides as Emerging Bioactive Macromolecules: From Fundamentals to Applications." Research in Microbiology 174(4): 104024.

Rajoka, Muhammad Shahid Riaz et al. 2019. "Characterization, the Antioxidant and Antimicrobial Activity of Exopolysaccharide Isolated from Poultry Origin Lactobacilli." Probiotics and Antimicrobial Proteins 11(4): 1132–42. https://doi.org/10.1007/s12602-018-9494-8.

Riaz Rajoka, Muhammad Shahid et al. 2020. "Lactobacillus Exopolysaccharides: New Perspectives on Engineering Strategies, Physiochemical Functions, and Immunomodulatory Effects on Host Health." Trends in Food Science & Technology 103: 36–48.

Ruas-Madiedo, P., and C. G. De Los Reyes-Gavilán. 2005. "Invited Review: Methods for the Screening, Isolation, and Characterization of Exopolysaccharides Produced by Lactic Acid Bacteria." Journal of Dairy Science 88(3): 843–56.

Srinivash, Moovendran, Raman Krishnamoorthi, Pambayan Ulagan Mahalingam, and Balasubramanian Malaikozhundan. 2023. "Exopolysaccharide from Lactococcus Hircilactis CH4 and Lactobacillus Delbrueckii GRIPUMSK as New Therapeutics to Treat Biofilm Pathogens, Oxidative Stress and Human Colon Adenocarcinoma." International Journal of Biological Macromolecules 250: 126171.

Tampakaki, A. P., E. Hatziloukas, and N. J. Panopoulos. 2009. "Plant Pathogens, Bacterial." Encyclopedia of Microbiology, Third Edition: 655–77.

Wang, Kun et al. 2014. “Characterization of a Novel Exopolysaccharide with Antitumor Activity from Lactobacillus Plantarum 70810.” International Journal of Biological Macromolecules 63: 133–39.

Yan, Minghui et al. 2018. “Extrusion of Dissolved Oxygen by Exopolysaccharide from Leuconostoc Mesenteroides and Its Implications in Relief of the Oxygen Stress.” Frontiers in Microbiology 9. https://www.frontiersin.org/articles/10.3389/fmicb.2018.02467.

Yang, Zhennai et al. 2010. “Capsular and Slime-Polysaccharide Production by Lactobacillus Rhamnosus JAAS8 Isolated from Chinese Sauerkraut: Potential Application in Fermented Milk Products.” Journal of Bioscience and Bioengineering 110(1): 53–57.

Genetic Mapping and Quantitative Trait Loci in Chickpea

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ABSTRACT: Classical plant breeding is based on phenotypic selection of traits of interest with the goal of assembling desirable gene combinations in new varieties. These practices have been very effective in improving crop productivity over the past decades. However, traditional methods often face problems that can reduce the efficiency of phenotypic selection and make it difficult to identify superior genotypes. The development of genetic maps allows focusing on a few or single regions that explain a significant percentage of the total phenotypic variation of a given trait. The regions that control quantitative traits are known as quantitative trait loci (QTL). Chickpea (*Cicer arietinum* L.) genetic mapping was initiated in early 1990 to identify markers associated with simple phenotypic traits, isozymes and interspecific crosses between C. arietinum and each of C. reticulatum (wild ancestor) and C. echinospermum. QTL analysis in chickpea initially focused on resistance to biotic stresses, particularly anthracnose. Today, QTL studies have been extended to abiotic stresses.

Keywords: QTL, Chickpea, Genetic mapping

INTRODUCTION

Classical plant breeding is based on phenotypic selection of traits of interest with the objective of assembling desirable gene combinations in new varieties. These practices have been very effective in improving crop productivity over the past decades. However, traditional methods often face problems that can reduce the efficiency of phenotypic selection and make it difficult to identify superior genotypes (Torres, 2009). In recent years, molecular markers and highly detailed genetic maps have been developed in different species. These tools have made it possible to link different genes or genomic regions to the phenotypic variation of qualitative and quantitative traits.

Quantitative traits (also known as "polygenic", "multifactorial" or "complex" traits) are often controlled by different genes with small effects. These effects are more influenced by environmental conditions than qualitative traits and show continuous variation. Epistatic and genotype-environment interactions can also occur. The development of genetic maps allows focusing on a few or single regions that explain a significant percentage of the total phenotypic variation of a given trait. Therefore, they facilitate the selection of quantitative traits. The regions that control quantitative traits are known as quantitative trait loci (QTL). This term was proposed by Geldermann (1975).

Molecular markers tightly linked to QTLs facilitate marker-assisted selection (MAS). The MAS approach is not only a tool to speed up the process of gene transfer, but also allows pyramiding of desired QTLs from different genetic backgrounds. In addition, precise mapping of QTL regions facilitates a deeper understanding of plant genomics through candidate gene analysis and germplasm characterization allowing its efficient use (Asíns, 2002). Chickpea (*Cicer arietinum* L.) genetic mapping was initiated as early as 1990 with the aim of identifying markers associated with simple phenotypic traits, isozymes and interspecific hybridizations between *C. arietinum* and each of *C. reticulatum* (wild ancestor) and *C. echinospermum* (Gaur and Slinkard, 1990; Kazan et al., 1993). Later, Simon and Muehlbauer (1997) incorporated RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) markers into the chickpea map, establishing synthetic relationships with other legumes for the first time. The STMS (sequence labeled microsatellite region) markers developed by Hüttel et al. (1999) and Winter et al. (1999) started a new era in chickpea genetic maps. The first reference map was created by Winter et al. (2000). Furthermore, STMS enabled the transfer of linkage information between populations and provided linkage points for comparison with the genetic map of the model species Medicago truncatula (Millan et al., 2010; Nayak et al., 2010). More recently, next-generation sequencing (NGS) technologies in chickpea, large-scale transcriptome data combined with genomic markers based on single nucleotide polymorphisms (SNPs), have facilitated the development of highly saturated second-generation genetic maps (Gujaria et al., 2011; Thudi et al., 2011; Gaur et al., 2012; Hiremath et al., 2012). Accurate identification of QTLs in genetic maps requires suitable plant material and a thorough phenotypic evaluation to be able to identify candidate genes and tightly linked markers useful in MAS, as described in the following sections.

QTL Analysis in Chickpea

QTL analysis in chickpea initially focused on resistance to biotic stresses, particularly anthracnose. Nowadays, QTL studies have been extended to abiotic stresses. All available information together with new genomic tools provide great possibilities to improve the selection of complex traits using marker-assisted selection in chickpea breeding programs.

QTLs for Biotic Stresses

Drought and salt stress are two of the major abiotic stresses affecting crop productivity in chickpea in many parts of the world. Chickpea is known for its superior drought tolerance compared to most other cool climate legumes. However, drought reduces chickpea yields and can even lead to complete crop failure. As it is a complex trait and screening methods are laborious, the use of molecular markers is a powerful approach to study the genetic control of drought. QTLs for drought-related traits have been identified in several studies (Rehman et al., 2011; Hamwieh et al., 2013). However, their validation has not yet been reported. Rehman et al. (2011) identified QTLs associated with different traits affecting drought tolerance in ILC588 (drought tolerant) x ILC3279 (drought sensitive) RIL population. QTLs located on LG1 (Q1-1) and LG3 (Q3-1) were associated with several drought-related traits (yield components and phenological traits, plant height, drought tolerance score and canopy temperature difference). showed an effect on the growth of the plants.

The same RIL population was also used by Hamwieh et al. (2013). They detected 93 QTL (LOD 2) for 12 drought-related traits studied across the genome in different environments, although only 19 of them showed LOD 3 values. Eight QTL were observed in more than one environment. Two interesting regions are located on LG3 and LG4 and show pleiotropic effects for several traits. The first region on LG3 contains determinant markers (H4G-07 and H6C-07) associated with days to flowering and ripening, drought tolerance indices (D) and yield-related traits (Y). The second region on LG3 (markers H3G09, NCPGR-50 and TA179) was associated with phenological (Ph) and yield-related traits (Y). Similarly, two common QTL were observed on LG4 for different traits. The first region contains two markers (H1G-20 and H5G-01) associated with drought tolerance (D), phenological (Ph) and yield-related traits (Y). The second region on LG4 contains markers H1H-15 and H1B-17, indicative of a common significant QTL for phenological (Ph) and seed number (Y).

Varshney et al. (2014) reported 45 mitogenic QTL (M-QTL) and 973 epistatic QTL (E-QTL) explaining 58.20% and 92.19% of phenotypic variation for several targeted traits, respectively. The researchers used two RIL mapping populations [ICC 4958 (drought tolerant) x ICC 1882 (sensitive) and ICC 283 (sensitive) x ICC 8261 (tolerant)]. This study presents nine QTL clusters associated with root, morphological, phenological, yield-related traits and drought indices. Among these QTL clusters, QTL Cluster 5 (~29 cM and 7.74 Mb) on CaLG04, the so-called "QTL-hotspot", harbors stable and consistent QTLs for several drought tolerant traits. This cluster containing seven markers (ICCM0249, NCPGR127, TAA170, NCPGR21, TR11, GA24 and STMS11) is the most important region and is used in molecular breeding to increase yield under terminal drought conditions. This region was studied by Jaganathan et al. (2015) up to 14 cM in the genetic map corresponding to ~4 Mb in the physical map. The present analysis integrated 49 new SNP markers into the "QTL-hotspot" region, increasing the same region from 7 to 55 markers. Furthermore, Kale et al. (2015) used the SkimGBS approach to split the ~3 Mb "QTL-hotspot" region into two smaller regions, "QTL-hotspot_a and b". A QTL cluster on CaLG08 (Cluster 9) also seems to be an interesting genomic region to target drought tolerant molecular breeding.

Salinity is another complex abiotic stress and only a few studies have reported the presence of QTLs for salinity tolerance in chickpea. Saminemi (2010) identified QTLs associated with yield-related traits (seed yield, 100-seed weight and shoot biomass) on LG1, LG2, LG3 and LG7 under salinity conditions using the RIL population ICC6263 (salt sensitive) x ICC1431 (tolerant). Genomic regions on LG1 and LG7 were found to be significant, but these QTLs were not validated. Later, Vadez et al. (2012) identified QTLs on LG3 and LG6 for seed yield and its components under salinity conditions using the ICCV2 (salt sensitive) x JG62 (tolerant) RIL population. The population was also segregated for flowering time and lines were separated according to "early and late" phenology. Several QTLs with limited overlap were identified for seed yield and its components under saline conditions in each phenology group. However, when the analysis was performed on the entire RIL population, no significant QTL were identified, highlighting the importance of phenology in the genotypic response to salt stress.

Pushpavalli et al. (2015) identified a total of 46 QTLs including 19 QTLs for phenological traits and 27 QTLs for yield-related traits across years and treatments (control and salinity) in a RIL population derived from ICCV 2 (salt sensitive) x JG 11 (tolerant). QTLs were clustered in different genomic regions of LG5, LG7 and LG8. Genomic regions on CaLG05 (28.6 cM) and CaLG07 (19.4 cM) harbor QTLs for traits significantly associated with yield under salinity, explaining 12% and 17% of phenotypic variation, respectively.

QTLs for Adaptation and Yield Traits

One of the most important traits for plant adaptation to a particular environment is flowering days. Early flowering and thus early maturity in chickpea can be very important in semi-arid and Mediterranean environments. This is because it allows escape from terminal drought and high temperature at the end of the growing season, resulting in a positive effect on chickpea yield (Rubio et al., 2004; Gaur et al., 2007). Although flowering time can be seen as a relatively simple trait, it participates in a complex network of interactions with other developmental processes influenced by various loci related to light perception, photoperiod response, etc. (Weller and Ortega, 2015).

Classical genetic studies under both long and short day conditions have identified that flowering time in chickpea may be controlled by one or two genes (Gumber and Singh, 1996; Or et al., 1999; Kumar and van Rheenen, 2000; Anbessa et al., 2006). The first QTLs identified were associated with markers currently found on LG8 (Cho et al., 2002; Lichtenzveig et al., 2006). These were later confirmed by Vadez et al. (2012).

Differences in growth habit are not only related to plant height, but also to plant structure, which affects yield and yield stability (Rubio et al., 2004). Growth habit, an important adaptive trait, was thought to be controlled by a single gene (Hg) in interspecific crosses with tillers, semi-tillers or upright growth and was mapped in LG3 of the chickpea genetic map (Kazan et al, 1993; Cobos et al, 2009; Aryamanesh et al, 2010). However, phenotypic evaluations considering intermediate values (0 = tilted, 1 = semi-tilted and 2 = erect) revealed a genomic region in LG1 (QTLHg2) that overlapped with the location of a second gene (Hg2) involved in the control of this trait and responsible for semi-tilted and erect phenotypes (Ali et al., 2016).

QTLs for yield-related components have also been analyzed in several studies. Thus, regions associated with plant seed number were identified on LG1 and LG4 in a population derived from an intraspecific cross (Cho et al., 2002). A QTL for seed weight was co-localized in the same region of LG4 (TA130). These two traits are negatively correlated (r = -0.476) and, therefore, the co-localization of QTLs for both traits may indicate a pleiotropic effect of a single QTL (Cho et al., 2002). The QTL for seed weight on LG4 has been widely validated using wide and narrow crosses.

QTLs Associated with Quality Traits

Chickpea is mainly used in the human diet and its nutritional value is well known (Asif et al., 2013; Sánchez-Chino et al., 2015). However, only a few QTL associated with quality components in chickpea seeds have been published. Two QTLs for protein content were identified using a set of 187 genotypes including both international and exotic collections with protein content ranging from 13.25 to 26.77%. These QTLs were located on LG3 and LG5, but a larger number of markers and genotypes should be used to confirm these relationships (Jadhav et al., 2015). Abbo et al. (2005) investigated beta-carotene and lutein concentration variation in an F2 population derived from an interspecific cross (Hadas x *C. reticulatum*). He identified two QTL with LOD values >3 in two regions of LG3 linked to markers TA64, STMS28 and TS19.

REFERENCES

Abbo, S., Molina, C., Jungmann, R., Grusak, M.A., Berkovitch, Z., Reifen, R., Kahl, G., Winter, P., Reifen, R., 2005. Quantitative trait loci governing carotenoid concentration and weight in seeds of chickpea (*Cicer arietinum* L.). Theor Appl Genet 111:185–195.

Ali, L., Deokar, A., Caballo, C., Taran, B., Gil, J., Chen, W., Millan, T., Rubio, J., 2016. Fine mapping for double podding gene in chickpea. Theor Appl Genet 129:77–86.

Anbessa, Y., Taran, B., Warkentin, T.D., Tullu, A., Vandenberg, A., 2009. Genetic analyses and conservation of QTL for ascochyta blight resistance in chickpea (*Cicer arietinum* L.). Theor Appl Genet 119:757–765

Anbessa, Y., Warkentin, T., Vandenberg, A., Ball, R., 2006. Inheritance of time to flowering in chickpea in a short-season temperate environment. J Heredity 97:55–61

Anuradha, C., Gaur, P.M., Pande, S., Gali, K.K., Ganesh, M., Kumar, J., Varshney, R.K., 2011. Mapping QTL for resistance to botrytis grey mould in chickpea. Euphytica 182:1–9

Aryamanesh, N., Nelson, M.N., Yan, G., Clarke, H.J., Siddique, K.H.M., 2010. Mapping a major gene for growth habit and QTLs for ascochyta blight resistance and flowering time in a population between chickpea and Cicer reticulatum. Euphytica 173:307–319

Asif, M., Rooney, L.W., Ali, R., Riaz, M.N., 2013. Application and opportunities of pulses in food system: a review. Crit Rev Food Sci Nutr 53:1168–1179

Asíns, M.J., 2002. Present and future of quantitative trait locus analysis in plant breeding. Plant Breed 121:281–291

Bhardwaj, R., Sandhu, J.S., Kaur, L., Gupta, S.K., Gaur, P.M., Varshney, R., 2009. Genetics of ascochyta blight resistance in chickpea. Euphytica 171:337–343

Cho, S., Chen, W., Muehlbauer, F.J., 2004. Pathotype-specific genetic factors in chickpea (*Cicer arietinum* L.) for quantitative resistance to ascochyta blight. Theor Appl Genet 109:733–739

Cho, S., Kumar, J., Shultz, J.L., Anupama, K., Tefera, F., Muehlbauer, F.J., 2002. Mapping genes for double podding and other morphological traits in chickpea. Euphytica 128:285–292

Cobos, M.J., Fernández, M.J., Rubio, J., Kharrat, M., Moreno, M.T., Gil, J., Millán, T., 2005. A linkage map of chickpea (Cicer arietinum L.) based on populations from Kabuli _ Desi crosses: location of genes for resistance to fusarium wilt race 0. Theor Appl Genet 110:1347–1353

Cobos, M.J., Winter, P., Kharrat, M., Cubero, J.I., Gil, J., Millan, T., Rubio, J., 2009. Genetic analysis of agronomic traits in a wide cross of chickpea. Field Crops Res 111:130–136

Danehloueipour, N., Yan, G., Clarke, H.J., Siddique, K.H.M., 2007. Diallel analyses reveal the genetic control of resistance to ascochyta blight in diverse chickpea and wild Cicer species. Euphytica 154:195–205

Gaur, P.M., Gowda, C.L.L., Knights, E.J., Warketin, T., Acikgöz, N., Yadav, S.S., Kumar, J., 2007. Breeding achievements. In: Yadav S, Redden R, Chen W, Sharma B (eds) Chickpea breeding and management. CABI Publ, Wallingford, UK, pp 391–416

Gaur, P.M., Jukanti, A.K., Varshney, R.K., 2012. Impact of genomic technologies on chickpea breeding strategies. Agronomy 2:199

Gaur, P.M., Slinkard, A.E., 1990. Genetic control and linkage relations of additional isozyme markers in chickpea. Theor Appl Genet 80:648–656

Geldermann, H., 1975. Investigations on inheritance of quantitative characters in animals by gene markers I. Methods. Theor Appl Genet 46:319–330

Gowda, S.J.M., Radhika, P., Kadoo, N.Y., Mhase, L.B., Gupta, V.S., 2009. Molecular mapping of wilt resistance genes in chickpea. Mol Breed 24:177–183

Gujaria, N., Kumar, A., Dauthal, P., 2011. Development and use of genic molecular markers (GMMs) for construction of a transcript map of chickpea (*Cicer arietinum* L.). Theor Appl Genet 122:1577–1589

Gumber, R., Singh, S., 1996. Genetics of flowering time in chickpea. A preliminary report. Crop Improv 23:295–296

Halila, I., Cobos, M.J., Rubio, J., Millán, T., Kharrat, M., Marrakchi, M., Gil, J., 2009. Tagging and mapping a second resistance gene for Fusarium wilt race 0 in chickpea. Eur J Plant Pathol 124:87–92

Hamwieh, A., Imtiaz, M., Malhotra, R.S., 2013. Multi-environment QTL analyses for drought-related traits in a recombinant inbred population of chickpea (*Cicer arientinum* L.). Theor Appl Genet 126:1025–1038

Hiremath, P.J., Kumar, A., Penmetsa, R.V., 2012. Large-scale development of cost-effective SNP marker assays for diversity assessment and genetic mapping in chickpea and comparative mapping in legumes. Plant Biotechnol J 10:716–732

Hüttel, B., Winter, P., Weising, K., Choumane, W., Weigand, F., Kahl, G., 1999. Sequence-tagged microsatellite site markers for chickpea (*Cicer arietinum* L.). Genome 42:210–217

Jadhav, A.A., Rayate, S.J., Mhase, L.B., Thudi, M., Chitikineni, A., Harer, P.N., Jadhav, A.S., Varshney, R.K., Kulwal, P.L., 2015. Marker-trait association study for protein content in chickpea (*Cicer arietinum* L.). J Genet 94:279–286

Jaganathan, D., Thudi, M., Kale, S., 2015. Genotyping-by-sequencing based intra-specific genetic map refines a ‘‘QTL-hotspot” region for drought tolerance in chickpea. Mol Genet Genomics 290:559–571

Kale, S.M., Jaganathan, D., Ruperao. P., 2015. Prioritization of candidate genes in “QTL-hotspot” region for drought tolerance in chickpea (*Cicer arietinum* L.). Sc Rep 5:15296

Kazan, K., Muehlbauer, F.J., Weeden, N.E., Ladizinsky, G., 1993. Inheritance and linkage relationships of morphological and isozyme loci in chickpea (*Cicer arietinum* L.). Theor Appl Genet 86:417–426

Kumar, J., van Rheenen, H., 2000. Brief communication. A major gene for time of flowering in chickpea. J Heredity 91:67–68

Lichtenzveig, J., Bonfil, D.J., Zhang, H.B., Shtienberg, D., Abbo, S., 2006. Mapping quantitative trait loci in chickpea associated with time to flowering and resistance to Didymella rabiei the causal agent of ascochyta blight. Theor Appl Genet 113:1357–1369

Madrid, E., Rubiales, D., Moral, A., Moreno, M.T., Millán, T., Gil, J., Rubio, J., 2008. Mechanism and molecular markers associated with rust resistance in a chickpea interspecific cross (*Cicer arietinum* x *Cicer reticulatum*). Eur J Plant Pathol 121:43–53

Millán, T., Madrid, E., Imtiaz, M., Kharrat, M., Chen, W., 2013. Disease resistance in chickpea. Translational genomics for crop breeding. Wiley, Hoboken, pp 201–219

Millan, T., Winter, P., Jüngling, R., Gil, J., Rubio, J., Cho, S., Cobos, M.J., Iruela, M., Rajesh, P.N., Tekeoglu, M., Khal, G., Muehlbauer, F.J., 2010. A consensus genetic map of chickpea (Cicer arietinum L.) based on 10 mapping populations. Euphytica 175:175–189

Nayak, S.N., Zhu, H., Varghese, N., Datta, S., Choi, H.K., Horres, R., Jüngling, R., Singh, J., Kavi Kishor, P.B., Sivaramakrishnan, S., Hoisington, D.A., Kahl, G., Winter, P., Cook, D.R., Varshney, R.K., 2010. Integration of novel SSR and gene-based SNP marker loci in the chickpea genetic map and establishment of new anchor points with Medicago truncatula genome. Theor Appl Genet 120:1415–1441

Or, E., Hovav, R., Abbo, S., 1999. A major gene for flowering time in chickpea. Crop Science 39:315–322

Pande, S., Siddique, K.H.M., Kishore, G.K., Bayaa, B., Gaur, P.M., Gowda, C.L.L., Bretag, T., Crouch, J.H., 2005. Ascochyta blight of chickpea (*Cicer arietinum* L.): A review of biology, pathogenicity, and disease management. Aust J Agric Res 56:317–332

Pushpavalli, R., Krishnamurthy, L., Thudi, M., 2015. Two key genomic regions harbour QTLs for salinity tolerance in ICCV 2xJG 11 derived chickpea (*Cicer arietinum* L.) recombinant inbred lines. BMC Plant Biol 15:124

Rehman, A.U., Malhotra, R.S., Bett, K., Tar'an, B., Bueckert, R., Warkentin, T.D., 2011. Mapping QTL associated with traits affecting grain yield in Chickpea (*Cicer arietinum* L.) under terminal drought stress. Crop Sci 51:450–463

Rubio, J., Flores, F., Moreno, M.T., Cubero, J.I., Gil, J., 2004. Effects of the erect/bushy habit, single/double pod and late/early flowering genes on yield and seed size and their stability in chickpea. Field Crops Res 90:255–262

Sabbavarapu, M.M., Sharma, M., Chamarthi, S.K., 2013. Molecular mapping of QTLs for resistance to Fusarium wilt (race 1) and Ascochyta blight in chickpea (*Cicer arietinum* L.). Euphytica 193:121–133

Saminemi, S., 2010. Physiology, genetics and molecular mapping of salt tolerance in chickpea (*Cicer arietinum* L). The University of Western Australia

Sánchez-Chino, X., Jiménez-Martínez, C., Dávila-Ortiz, G., Álvarez-González, I., Madrigal-Bujaidar, E., 2015. Nutrient and nonnutrient components of legumes, and its chemopreventive activity: a review. Nutr Cancer 67:401–410

Sharma, K.D., Muehlbauer, F.J., 2007. Fusarium wilt of chickpea: Physiological specialization, genetics of resistance and resistance gene tagging. Euphytica 157:1–14

Simon, C.J., Muehlbauer, F.J., 1997. Construction of a chickpea linkage map and its comparison with maps of pea and lentil. J Heredity 88:115–119

Udupa, S.M., Baum, M., 2003. Genetic dissection of pathotype-specific resistance to ascochyta blight disease in chickpea (*Cicer arietinum* L.) using microsatellite markers. Theor Appl Genet 106:1196–1202

Thudi, M., Bohra, A., Nayak, S.N., 2011. Novel SSR markers from BAC-end sequences, DArT arrays and a comprehensive genetic map with 1291 marker loci for chickpea (*Cicer arietinum* L.). PLoS One 6

Torres, A.M., 2009. Application of molecular markers for breeding disease resistant varieties in crop plants. In: Jain SM, Brar DS (eds) molecular techniques in crop improvement. Springer, Netherlands, pp 185–205

Vadez, V., Krishnamurthy, L., Thudi, M., Anuradha, C., Colmer, T., Turner, N.C., Siddique, K.H.M., Gaur, P.M., Varshney, R.K., 2012. Assessment of ICCV 2 x JG 62 chickpea progenies shows sensitivity of reproduction to salt stress and reveals QTL for seed yield and yield components. Mol Breed 30:9–21

Varshney, R.K., Mohan, S.M., Gaur, P.M., 2014. Marker-Assisted backcrossing to introgress resistance to fusarium wilt race 1 and ascochyta blight in C 214, an elite cultivar of chickpea. The Plant Genome 7

Weller, J.L., Ortega, R., 2015. Genetic control of flowering time in legumes. Front Plant Sci 6

Winter, P., Benko-Iseppon, A.M., Hüttel, B., Ratnaparkhe, M., Tullu, A., Sonnante, G., Pfaff, T., Tekeoglu, M., Santra, D., Sant, V.J., Rajesh, P.N., Kahl, G., Muehlbauer, F.J., 2000. A linkage map of the chickpea (Cicer arietinum L.) genome based on recombinant inbred lines from a *C. arietinum* x *C. reticulatum cross*: localization of resistance genes for fusarium wilt races 4 and 5. Theor Appl Genet 101:1155–1163

Winter, P., Pfaff, T., Udupa, S.M., Hüttel, B., Sharma, P.C., Sahim, S., Arreguin-Espinoza, R., Weigand, F., Muehlbauer, F.J., Kahl, G., 1999. Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (Cicer arietinum L.) genome. Mol Gen Genet 262:90–101.

Classical and Modern Breeding Methods in Chickpea

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ABSTRACT: The decline in the amount of food per capita due to the rate of population growth seriously calls for increased productivity, especially in developing countries. According to the Food and Agriculture Organization of the United Nations (FAO), the human population will reach 9 billion by the end of 2050 and feeding such a large population will be a challenging task. In the past, the breeding of legumes, cereals and other important food crops, especially chickpea, has been carried out using traditional methods such as Bulk, Pedigree and crossbreeding, taking advantage of the available genetic diversity. However, these methods are currently insufficient to make a significant contribution to managing the world's rapidly increasing demand for food. The establishment of genetic variability, effective selection and subsequent evaluation of selected lines are the three basic steps of a breeding program. Genetic variability forms the basis of any crop improvement program and determines the success of a breeding program. Traditional chickpea breeding approaches have increased yields but have not achieved the desired production targets. Traditional breeding approaches tend to combine all the desired genes in a variety by performing phenotypic selection of traits. However, phenotypic selection of traits is laborious, requires complex screening of elite genotypes and is therefore very difficult to achieve with traditional approaches. This makes modern plant breeding approaches necessary.

Keywords: Classical Breeding, Chickpea, Modern Breeding

INTRODUCTION

Legume crops play an important role in reducing food insecurity and malnutrition in developing countries such as India, Pakistan and Bangladesh. The decline in the amount of food per capita due to the rate of population growth seriously calls for increased productivity, especially in developing countries. According to the Food and Agriculture Organization of the United Nations (FAO), the human population will reach 9 billion by the end of 2050 and feeding such a large population will be a challenge. With a rapidly growing population, diminishing arable land, depleting water resources, increasing urbanization and industrialization, hunger has become a haunting specter for millions of poor people around the world. Erratic climate change, abiotic and biotic stresses that significantly affect production and yields are other obstacles to achieving global food security (Ahmad et al., 2019a, b; Naikoo et al., 2019).

Breeding of legumes, cereals and other important food crops, especially chickpea, has been carried out in the past by utilizing the available genetic diversity using traditional methods such as Bulk, Pedigree and crossbreeding. However, these methods are currently insufficient to make a significant contribution to managing the world's rapidly increasing demand for food. Existing genetic variability in food crops, especially grain legumes, has been depleted, requiring the use of innovative breeding tools to generate new genetic variability in yield traits. Chickpea is an annual, self-pollinated, cool-climate legume crop and the second most important food legume crop after beans. Chickpea seeds are nutrient-rich and contain adequate amounts of carbohydrates, protein and fat, offering a good substitute for meat, especially among malnourished populations. The aim of this review is to describe the classical and modern breeding methods used in chickpea.

Classical Breeding Methodology

Chickpea is predominantly self-fertilizing and has very little genetic variability. Establishing genetic variability, efficient selection and subsequent evaluation of selected lines are the three basic steps of a breeding program. Genetic variability forms the basis of any crop improvement program and determines the success of a breeding program. The traditional breeding approach is used for the establishment of genetic variability, including plant selection and crossbreeding.

Hybridization

Hybridization is carried out to combine desirable traits from two or more parents, especially wild relatives, into a single variety. Wild relatives of the crop are recognized as critical resources for the improvement of many traits, especially seed yield, in various crops (Stalker, 1980). In chickpea, yield genes have been transferred from *Cicer reticulatum*, *C. echinospermum* (Singh et al., 2005; Singh and Ocampo, 1997) and *C. pinnatifidum* (Sandhu et al., 2006; Singh et al., 2012a, b, c). Successful interspecific hybridizations between *C. arietinum* and *C. reticulatum* were obtained and later hybridizations were also made between *C. arietinum* and *C. echinospermum* by various researchers (Ladizinsky and Adler, 1976; Pundir and Mengesha, 1995; Singh and Ocampo, 1993).

Selecting Parents

The selection of appropriate parents is important as it largely determines the success or failure of crossbreeding programs. The choice of parents varies depending on the main objectives to be achieved. If the goal is to create a superior variety, then varieties that are better adapted to the local environment are selected as the first parent and the second parent is selected only to complement the first parent. However, if the aim is to increase the degree of genetic variability, different varieties are selected as parents. For effective and efficient selection of parents, biometric approaches are used to analyze the combining ability of genotypes and diversity.

Single cross

In this type of crossing, only two parental species are involved in the development of varieties with improved traits. The traits to be improved are mainly resistance to biotic and abiotic stresses. Varieties developed through single crossing are resistant to fusarium and anthracnose, high yielding and widely adapted to diverse agro-ecosystems.

Three-way cross

In this type of crossbreeding, three parents, each with a particular desirable trait, are used to recombine the traits into a new variety. A cross is made between two parents to breed the F1 generation, followed by a backcross between the F1 and the other parent. Three-way cross are more beneficial than single crosses as they provide additional opportunities for gene interaction and lead to varieties with greater genetic variability. Three-way cross can be managed by pedigree or mass breeding methods. Therefore, it takes more time to isolate uniform progeny.

Multiple Crosses

In such crosses, more than three parents are used to improve a single trait of a superior variety. The main objective behind multiple crosses is to increase the degree of genetic variability. The resulting populations can be managed by pedigree or bulk breeding methods. Varieties developed from multiple crosses are better adapted to diverse agro-ecosystems.

Handling of Segregating Populations

The diversity and combining ability of the parents are two important aspects to consider when crossing. The use of opened populations is important to obtain better results as they correlate well with the heterosis of F1 hybrids. The most commonly used method to handle the opened population in chickpea is the pedigree selection method (Lal et al., 1973), but Byth et al. (1980) reported that this method is less suitable and suggested the bulk method. The development of varieties with improved resistance to biotic stresses is more suitable for the pedigree method; drought tolerance and winter hardiness are suitable for the bulk-pedigree method; abiotic stresses, seed size, earliness and plant type are suitable for the modified bulk method (Singh, 1987).

Basic Components of Mutation Breeding

The main components of mutation breeding are (a) mutation expression, (b) mutation detection, (c) mutant testing and (d) release of cultivars. Mutation application is carried out by exposing biological material to mutagens; it is fast, taking minutes or a few hours, while mutation detection takes several months or even years, although high-throughput screens are very useful to detect a mutation in less time. The release of mutant varieties takes on average 10 years but can be accelerated using marker-assisted selection and other new biotechnologies (Joung and Sander, 2013; Zheng et al., 2013). Gaul (1964) phenotypically categorized mutations into two groups:

(a) Macromutations: A mutation that is phenotypically visible and morphologically distinct and produces a phenotype that is well outside the range of variation that previously existed in the population.

(b) Micromutations: Mutations of small effect that can only be detected with the help of statistical analyses such as character mean, variance and heritability.

Most such mutations are in polygenically controlled traits; they are of greatest value to plant breeders because most economically useful traits are polygenically controlled.

Modern Breeding Methods

Conventional chickpea breeding approaches have increased yields but have not achieved the desired production targets. Traditional breeding approaches tend to combine all the desired genes in a variety by performing phenotypic selection of traits. However, phenotypic selection of traits is laborious, requires complex screening of elite genotypes and is therefore very difficult to achieve with traditional approaches (Torres, 2009). This necessitates modern plant breeding approaches.

Double Haploid Production

Further development of new and improved varieties through traditional breeding approaches is time-consuming and laborious. Anther culture represents an alternative approach and offers a rapid method for the recovery of homozygous inbred lines. The main benefits of doubled-haploid production are improving variety development, increasing homozygosity and responding to market demands. However, the success of anther embryogenesis and subsequent regeneration of full haploid plants in Fabaceae is limited to a few species such as forage pea and alfalfa (Croser et al., 2006). The resistant nature of legumes considerably slows progress in haploid plant production. The literature is scanty on haploid production protocols in any cool climate legume crop and only a few published literatures are available (Altaf and Ahmad, 1986; Croser, 2002; Huda et al., 2001). The first study on the development of double haploid chickpea cultivars such as Canadian CDC Xena (kabuli) and Australian Sonali (desi) through anther culture using some physical stresses such as anther centrifugation and electric shock was presented by Grewal et al. (2009).

Transgenic Approaches

To maintain the yield potential of the crop and address food security issues, crop production needs to be increased many times over to feed a rapidly growing population. The desired targets for chickpea production have remained consistently low due to its susceptibility to a number of pathogens and insect pests. Among insect pests, bruchids cause significant losses during storage (Singh et al., 1994). Traditional breeding methods have failed to create insect resistant varieties. Although insecticides play a positive role in reducing the impact of insect pests, they negatively affect the biota and its environment. Therefore, new approaches to pest management are needed to improve production, environment and health in order to achieve the desired production target and sustainability of agriculture. New techniques such as molecular breeding and genetic engineering need to be used to develop insect resistant varieties. Recently developed recombinant DNA technologies and genetically modified plants have been successful in managing pest and pathogen outbreaks (Gatehouse, 2008). The insertion of foreign genes into the plant genome that confer resistance to insects has been made possible by advances in recombinant DNA technology (Bennett, 1994). δ-endotoxin genes, protease inhibitors and plant lectins are the main genes that have been inserted and confer a broad resistance to various pests and pathogens.

The combination of recombinant DNA technology and plant tissue culture has paved the way for the creation of new options for biotic stress management, especially for insect pests. These technologies have led to a major reduction in losses caused by pests. Advances in biotechnology have created many different opportunities, such as plant transformation techniques, the identification of new and effective molecules and their role, changes in gene expression and the development of transgenics resistant to insect attack. In both developed and developing countries, transgenic crops have generated significant income. Since their first introduction in the mid-1990s, the use of such plants has revolutionized agriculture and allied sectors with their input traits for pest management, particularly insects and herbicide resistance. Pest-resistant transgenics play a critical role in substituting insecticides for pest management, thereby reducing yield losses due to pests. They also offer sources of resistance that cannot be obtained from natural plant sources and help reduce large investments in pest control beyond the basic requirements for growing a crop. This feature will be of unique importance in increasing dryland production if dryland crops are used effectively against pests.

Pests are responsible for major production and yield losses in pulse crops, including chickpea. Among them, pod borer (*Helicoverpa armigera* Hüberand) and aphid (*Aphis craccivora* C.L. Koch) are the most damaging, causing annual losses of US$ 200 million in chickpea in India alone (Zahid et al., 2008). The development of transgenic chickpea lines with resistance to *H. armigera* is considered one of the best approaches against yield loss.

Tissue Culture

Traditional breeding approaches are time-consuming and laborious for the development and formal release of registered varieties. This necessitates new modern breeding approaches such as tissue culture, plant regeneration strategies, gene transfer and plant transformation. Traditional breeding approaches, such as hybridization and the introduction of artificial mutations, are based on the identification and improvement of accessible genetic variability in the trait of interest. However, most legume species are self-fertilized, which limits their genetic variability and therefore plant breeders face the problem of a narrow genetic base in crop improvement programs (Raina et al., 2016). Therefore, it is imperative to expand the genetic base through the introduction of desirable genes from wild related species into cultivated species. In plant breeding programs, in vitro culture techniques play a vital role in increasing genetic variability and accelerating the traditional breeding process. Kadiri et al. (2014) investigated suitable conditions for chickpea in vitro micropropagation using mature embryos and nodes as explants. Three chickpea genotypes (Zouaoui, ILC 483, INRA 199) were used as the source of explant cultures in Murashige and Skoog (1962) (MS) medium containing naphthyl acetic acid (NAA), Benzyl amino purine (BAP) and kinetin (KIN). The Zouaoui genotype was found to be more callogenic than INRA 199 and ILC 483. Several factors such as genotype, explant and nutrient medium affect callogenesis, cell dedifferentiation and regeneration (Yadav et al., 2012). They also reported that Zouaoui, ILC 483 and INRA 199 exhibited 52.73, 59.76 and 47.50% organogenesis capacity, respectively.

The type and concentration of exogenously applied hormones affect the behavior of the explant cultured in vitro. Hormone selection in tissue culture is based on the targeted morphogenetic response and explant type (Altaf et al., 1999). Major hormones such as auxins and cytokinins are used to achieve callogenesis or organogenesis by acting in synergy or antagonism (Zryd, 1988). Regardless of the type of explant used, MS culture media enriched with additional hormones showed different rates of organogenesis and callogenesis.

Genetic Mapping

Modern breeding approaches effectively utilize genomic resources by mapping markers associated with specific traits. The construction of genetic maps is the primary step in linkage mapping-based identification of markers associated with specific traits. Quantitative traits are governed by small additive effects of multiple genes and are also strongly influenced by environmental conditions. As a result, breeding for such polygenic traits is more complex compared to qualitative traits. The breeding behavior of quantitative traits can be studied through the construction of genetic maps because the maps focus on a single region, known as quantitative trait loci (QTL), which are responsible for the phenotypic variation of a particular trait. Due to limitations in the use of morphological markers in the development of genetic maps, various molecular markers such as diversity array technology (DArT) and single nucleotide polymorphisms (SNPs) have been found effective. In the interspecific mapping population of chickpea (ICC 4958 x PI 489777), a genetic map with more than 1200 loci covering a distance of 845 cM over 8 linkage groups was reported (Thudi et al., 2011). In chickpea, association mapping analysis using whole genome screening and candidate gene-based approach identified 312 markers associated with drought and heat response (Thudi et al., 2014). Successful genetic mapping and QTL analysis not only in chickpea but also in other legumes has been facilitated by the availability of a large number of DNA markers. Genetic maps play a vital role in the study of complex traits, especially yield and yield-dependent traits.

The advent of high-throughput genomic resources has played a critical role in genetic studies through improving yield, nutritional quality and tolerance to a wide range of biotic and abiotic stresses in chickpea. The rapid progress in the chickpea genome has also been reflected in the development of numerous molecular markers for the assessment of genetic variation. Initially SSR markers were used by several researchers for diversity assessment (Sethy et al., 2006), identification of QTLs (Aryamanesh et al., 2010) and development of genetic maps (Nayak et al., 2010). However, recently, large-scale discovery and genotyping of SNPs have been important advances in chickpea (Deokar et al., 2014).

Marker Assisted Selection

The use of marker-assisted selection (MAS) in crop improvement programs has increased in recent years. The main advantage of MAS over conventional plant breeding is the reduction in the number of generations and population size required to develop elite varieties (Yousef and Juvik, 2001; Thomas, 2003; Castro et al., 2015).

The MAS approach has not only been used to increase the speed of breeding programs, but has also led to the establishment of the gene pyramid, which allows for combining desirable QTLs from multiple parents to develop elite varieties. The successful implementation of MAS in a breeding program requires appropriate genotype selection followed by phenotypic selection of candidate genes and their associated markers. Genotypes are selected based on the presence or absence of markers, not on the trait of interest. Simon and Muehlbauer (1997) used RFLP and RAPD markers to map chickpea and reported a synthetic relationship with other legumes. Microsatellites are one of the hypervariable class of PCR-based genetic markers, as any variation in the number and/or size of microsatellite repeats at a locus between individuals results in DNA bands of different sizes (Beckmann and Soller, 1990). Microsatellite markers are very informative and have gained considerable importance over other molecular markers in the assessment of genetic diversity in field crops due to their multi-allelic structure, co-dominant inheritance, reproducibility, abundance and wide genomic distribution. SSRs are also amenable to large-scale genotyping and are therefore suitable for high-density genome mapping, gene/QTL mapping and marker-assisted selection (Winter et al., 2000; Nayak et al. 2010; Gujaria et al. 2011).

Marker Assisted Recurrent Selection

In marker-assisted recurrent selection (MARS) molecular markers are used for the identification and selection of multiple QTLs to develop elite genotypes within a single or among related populations (Ribaut et al., 2010). MARS provides a rapid method of breeding generations as it involves individual genotypic selection and crossbreeding in a selection cycle. This advanced molecular breeding approach is quite different from traditional QTL or MAS studies, as it involves a new mapping study on each fertile population and increases the desired allele frequency in populations. In short, MARS is a modern breeding approach that equips plant breeders with increased frequency of desirable alleles and the added effect of repeated crosses and small individual effects (Bernardo and Charcosset, 2006).

Multi-Parent Advanced Generation Intercrossing

The degree of genetic variability available and accessible is imperative in developing tolerance to a wide range of stresses. MAGIC, an advanced breeding approach to equip plant breeders with a high degree of genetic variability and high recombinant frequency, can also be used to understand the underlying mechanism of polygenes governing quantitative traits (Glaszmann et al., 2010). The development of MAGIC lines provides a basis for analyzing the discrimination between several QTL governing agroeconomic traits. Traditional breeding, such as hybridization, can limit productivity by creating varieties with desirable alleles in a homozygous state. Unlike conventional hybridization, MAGIC populations are very useful for accurate detection of QTLs, gene discovery, gene characterization and understanding the molecular characterization of complex traits (Buckler et al., 2009; Poland et al., 2011). In South Asia and Sub-Saharan Africa, eight chickpea parents were selected to create MAGIC lines with the aim of improving their potential to adapt to diverse agro-ecosystems. Chickpea MAGIC lines were also used to identify several QTL with high precision. The different hybridization techniques used by plant breeders have increased genetic variability for economically important traits and will also reveal rare alleles in homozygous state.

REFERENCES

Ahmad, B., Raina, A., Naikoo, M.I., Khan, S., 2019a. Role of methyl jasmonates in salt stress tolerance in crop plants. In: Khan MIR, Reddy PS, Ferrante A, Khan NA (eds) Plant signalling molecules. Woodhead Publishing, Elsevier, Duxford, pp. 371–384. https://doi.org/10.1016/B978-0-12-816451-8.00023-X.

Ahmad, B., Raina, A., Khan, S. (2019). Impact of biotic and abiotic stresses on plants, and their responses. *Disease resistance in crop plants: molecular, genetic and genomic perspectives*, 1-19.

Altaf, N., Ahmad, M. S. (1986). Plant regeneration and propagation of chickpea (Cicer arietinum L.) through tissue-culture techniques. In Nuclear techniques and in vitro culture for plant improvement.

Altaf, N., Iqbal, J., Salih, A.M., 1999. Tissue culture of microsperma lentis (*Lens culinaris* Medik) cv. Massoor-85. Pak J Bot 31(2):283–292.

Aryamanesh, N., Nelson, M. N., Yan, G., Clarke, H. J., Siddique, K. H. M., 2010. Mapping a major gene for growth habit and QTLs for ascochyta blight resistance and flowering time in a population between chickpea and Cicer reticulatum. *Euphytica*, *173*, 307-319.

Beckmann, J.S., Soller, M., 1990. Towards a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellite sites. Nat Biotechnol 8:930–932.

Bennett, J., 1994. DNA-based techniques for control of rice insects and diseases. In: Teng PS, Heong KL, Moody K (eds) Transformation, gene tagging and DNA fingerprinting. Rice pest science and management. IRRI, Los Baños, Philippines, pp 147–172.

Bernardo, R., Charcosset, A., 2006. Usefulness of gene information in marker assisted recurrent selection: a simulation appraisal. Crop Sci 46:614–621.

Buckler, E.S., Holland, J.B., Bradbury, P.J., et al 2009. The genetic architecture of maize flowering time. Sci 325(5941):714–718

Byth, D.E., Green, J.M., Hawtin, G.C., 1980. ICRISAT/ICARDA chickpea breeding strategies. In: Proceedings of the international workshop on chickpea improvement. ICRISAT, Hyderabad, pp 11–27.

Castro, P., Rubio, J., Madrid, E., Fernández-Romero, M. D., Millán, T., Gil, J., 2015. Efficiency of marker-assisted selection for ascochyta blight in chickpea. *The Journal of Agricultural Science*, *153*(1), 56-67.

Croser, J.S., 2002. Haploid and zygotic embryogenesis in chickpea (*Cicer arietinum* L.). In: PhD Thesis. University of Melbourne, Melbourne.

Croser, J. S., Lülsdorf, M. M., Davies, P. A., Clarke, H. J., Bayliss, K. L., Mallikarjuna, N., Siddique, K. H. M., 2006. Toward doubled haploid production in the Fabaceae: progress, constraints, and opportunities. *Critical Reviews in Plant Sciences*, *25*(2), 139-157.

Deokar, A. A., Ramsay, L., Sharpe, A. G., Diapari, M., Sindhu, A., Bett, K., ... Tar'an, B., 2014. Genome wide SNP identification in chickpea for use in development of a high density genetic map and improvement of chickpea reference genome assembly. *BMC genomics*, *15*, 1-19.

Gatehouse, J.A., 2008. Biotechnological prospects for engineering insect-resistant plants. Plant Physiol 146:881–887.

Gaul, H., 1964. Mutations in plant breeding. Rad Bot 4:155–232.

Glaszmann, J. C., Kilian, B., Upadhyaya, H. D., Varshney, R. K., 2010. Accessing genetic diversity for crop improvement. *Current opinion in plant biology*, *13*(2), 167-173.

Grewal, R.K., Lulsdorf, M., Croser, J., 2009. Doubled-haploid production in chickpea (*Cicer arietinum* L.): role of stress treatments. Plant Cell Rep 28(8):1289–1299.

Gujaria, N., Kumar, A., Dauthal, P., Dubey, A., Hiremath, P., Bhanu Prakash, A., ... Varshney, R. K., 2011. Development and use of genic molecular markers (GMMs) for construction of a transcript map of chickpea (Cicer arietinum L.). *Theoretical and Applied Genetics*, *122*, 1577-1589.

Huda, S., Islam, R., Bari, M.A., Asaduzzaman, M., 2001. Anther culture of chickpea. Int Chickpea Pigeonpea Newsl 8:24–26.

Joung, J.K., Sander, J.D., 2013. Innovation Talens: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol 14:49–55.

Kadiri, A., Halfaoui, Y., Bouabdallah, L., Ighilhariz, Z., 2014. Chickpea (*Cicer arietinum* L.) in vitro micropropagation. Türk Tarım ve Doğa Bilimleri Dergisi 1:1304–1309.

Ladizinsky, G., Adler, A., 1976. The origin of chickpea *Cicer arietinum* L. Euphy 25:211–217.

Lal, S., Singh, S. N., Lal, S. B., Tripathi, B. D. H., 1973. Improvement in gram through pedigree method of breeding. In *Proceedings of the 10th Workshop on Rabi Pulses* (pp. 70-80).

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Phys Plant 15:473–497.

Naikoo, M. I., Dar, M. I., Raghib, F., Jaleel, H., Ahmad, B., Raina, A., ... Naushin, F., 2019. Role and regulation of plants phenolics in abiotic stress tolerance: An overview. *Plant signaling molecules*, 157-168.

Nayak, S. N., Zhu, H., Varghese, N., Datta, S., Choi, H. K., Horres, R., ... Varshney, R. K., 2010. Integration of novel SSR and gene-based SNP marker loci in the chickpea genetic map and establishment of new anchor points with Medicago truncatula genome. *Theoretical and Applied Genetics*, *120*, 1415-1441.

Poland, J. A., Bradbury, P. J., Buckler, E. S., Nelson, R. J., 2011. Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. *Proceedings of the National Academy of Sciences*, *108*(17), 6893-6898.

Pundir, R.P.S., Mengesha, M.H., 1995. Cross compatibility between chickpea and its wild relative *Cicer echinospermum* Davis. Euphy 83:241–245.

Raina, A., Laskar, R., Khursheed, S., Amin, R., Tantray, Y., Parveen, K., Khan, S., 2016. Role of mutation breeding in crop improvement-past, present and future. *Asian Research Journal of Agriculture*, *2*(2), 1-13.

Ribaut, J.M., De Vicente, M.C., Delannay, X., 2010. Molecular breeding in developing countries: challenges and perspectives. Curr Opin Plant Biol 13(2):213–218.

Sandhu, J. S., Gupta, S. K., Singh, G., Sharma, Y. R., Bains, T. S., 2006. Inter-specific hybridization between Cicer arietinum and Cicer pinnatifidum for improvement of yield and other traits. In *4th International Food Legumes Research Conference, New Delhi, India* (Vol. 192, pp. 18-22).

Sethy, N.K., Shokeen, B., Edwards, K.J., Bhatia, S., 2006. Development of microsatellite markers and analysis of intra-specific genetic variability in chickpea (*Cicer arietinum* L.). Theor Appl Genet 112:1416–1428.

Simon, C.J., Muehlbauer, F.J., 1997. Construction of a chickpea linkage map and its comparison with maps of pea and lentil. J Hered 88:115–119.

Singh, K. B., Malhotra, R. S., Halila, M. H., Knights, E. J., Verma, M. M., 1993. Current status and future strategy in breeding chickpea for resistance to biotic and abiotic stresses. *Euphytica*, *73*, 137-149.

Singh, K.B., 1987. Chickpea breeding. In: Saxena MC, Singh KB (eds) The chickpea. CAB International, Wallingford, pp 127–162.

Singh, K.B., Ocampo, B., 1993. Interspecific hybridization in annual *Cicer* species. J Genet Breed 47:199–204.

Singh, K.B., Ocampo, B., 1997. Exploitation of wild *Cicer* species for yield improvement in chickpea. Theor Appl Genet 95:418–423.

Singh, S., Gumber, R. K., Joshi, N., Singh, K., 2005. Introgression from wild Cicer reticulatum to cultivated chickpea for productivity and disease resistance. *Plant Breeding*, *124*(5), 477-480.

Singh, M., Khan, Z., Kumar, K., Dutta, M., Pathania, A., Dahiya, O. P., Kumar, J., 2012a. Sources of resistance to Fusarium wilt and root-knot nematode in indigenous chickpea germplasm. *Plant Genetic Resources*, *10*(3), 258-260.

Singh, R. P., Singh, I., Singh, S., Sandhu, J. S., 2012b. Assessment of genetic diversity among interspecific derivatives in chickpea. *Journal of Food Legumes*, *25*(2), 150-152.

Singh, I., Singh, R. P., Singh, S., Sandhu, J. S., 2012c. Introgression of productivity genes from wild to cultivated Cicer. In *International conference on sustainable agriculture for food and livelihood security, Crop Improve* (Vol. 39, pp. 155-156).

Stalker, H. T., 1980. Utilization of wild species for crop improvement. *Advances in Agronomy*, *33*, 111-147.

Thomas, W.T.B., 2003. Prospects for molecular breeding of barley. Ann Appl Biol 142:1–12.

Thudi, M., Bohra, A., Nayak, S. N., Varghese, N., Shah, T. M., Penmetsa, R. V., ... Varshney, R. K., 2011. Novel SSR markers from BAC-end sequences, DArT arrays and a comprehensive genetic map with 1,291 marker loci for chickpea (Cicer arietinum L.). *PLoS One*, *6*(11), e27275.

Thudi, M., Upadhyaya, H. D., Rathore, A., Gaur, P. M., Krishnamurthy, L., Roorkiwal, M., ... Varshney, R. K., 2014. Genetic dissection of drought and heat tolerance in chickpea through genome-wide and candidate gene-based association mapping approaches. *Plos one*, *9*(5), e96758.

Torres, A. M., 2009. Application of molecular markers for breeding disease resistant varieties in crop plants. *Molecular Techniques in Crop Improvement: 2nd Edition*, 185-205.

Winter, P., Benko-Iseppon, A.M., Huttel, B., … Muehlbauer, F. J. 2000. A linkage map of chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* x *C. reticulatum* cross: localization of resistance genes for fusarium wilt races 4 and 5. Theor Appl Genet 101:1155–1163.

Yadav, S. K., Katikala, S., Yellisetty, V., Kannepalle, A., Narayana, J. L., Maddi, V., ... Bharadwaja, K. P., 2012. Optimization of Agrobacterium mediated genetic transformation of cotyledonary node explants of Vigna radiata. *SpringerPlus*, *1*(1), 1-8.

Yousef, G.G., Juvik, J.A. 2001. Comparison of phenotypic and marker-assisted selection for quantitative traits in sweet corn. Crop Sci 41:645–655.

Zahid, M. A., Islam, M. M., Reza, M. H., Prodhan, M. H. Z., Begum, M. R., 2008. Determination of economic injury levels of Helicoverpa armigera (Hubner) in chickpea. *Bangladesh Journal of Agricultural Research*, *33*(4), 555-563.

Zheng, Z., Wang, H. B., Chen, G. D., Yan, G. J., Liu, C. J., 2013. A procedure allowing up to eight generations of wheat and nine generations of barley per annum. *Euphytica*, *191*, 311-316.

Zryd, J.P., 1988. Cultures de cellules, tissus et organs végétaux. Fondements théoriques et utilisations pratiques. Presses Polytechniques Romandes.

Computational Analysis of the FKBP Prolyl Isomerase 5 Gene in Medaka (*Oryzias latipes*): Insights in to Molecular Mechanisms and Functional Implications

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ABSTRACT: The FKBP Prolyl Isomerase 5 gene, also known as *fkbp5*, plays a crucial role in various biological processes, including protein folding, cellular signaling, and stress response. In recent years, computational analysis has emerged as a powerful tool to unravel the molecular mechanisms and functional implications of genes. In this article, we present a comprehensive computational analysis of the *FKBP5* gene in Medaka (*Oryzias latipes*). By utilizing advanced bioinformatics techniques and computational models, we aim to gain insights into the genetic variations, protein structure predictions, gene expression profiling, molecular interactions, and functional implications of *FKBP5* in Medaka. This study provides valuable information that can enhance our understanding of the molecular mechanisms underlying *fkbp5* and its potential role in various physiological and pathological conditions.

Keywords: *Medaka*, fkbp5, in silico analysis, Bioinformatics studies

INTRODUCTION

The FKBP Prolyl Isomerase 5 (*fkbp5*) gene is an exciting area of research in the field of molecular biology (Zannas et al., 2016). It plays a crucial role in several biological processes, including protein folding and cellular signaling (Rutherford and Zuker 1994). In medaka, a small freshwater fish, the *fkbp5* gene has been studied extensively due to its potential implications in health and disease (Schartl et al., 2015).

Understanding the *fkbp5* gene in medaka can shed light on its molecular mechanisms and functional implications, providing valuable insights into the broader understanding of genetic variations and their impact on biological systems (Azetsu et al., 2019). This knowledge can have significant implications in agriculture.

Medaka is a small freshwater fish that has been extensively studied for the past 100 years (Wittbrodt et al., 2002). It is a fully-fledged model vertebrate that is deeply rooted within the life sciences (Hilgers and Schwarzer, 2019). Medaka is a useful model organism for studying various fields of research, such as developmental biology, toxicology, carcinogenesis, and behavior (Bhandari, 2016). It is also a powerful biomaterial for the studies of multiple disciplines, including molecular biology, genetics, and genomics (Lin et al., 2016). Medaka has several advantages that make it a suitable model organism for research, including: Short generation times: Medaka has a short generation time, which allows for rapid breeding and the production of large numbers of offspring Large and transparent eggs: Medaka has large and transparent eggs that allow for easy observation of development. High tolerance to inbreeding: Medaka has a high tolerance to inbreeding, which allows for the production of inbred strains. Comparably small genome size: Medaka has a comparably small genome size (~700 Mb), which makes it a suitable model organism for genomics research (Hilgers and Schwarzer, 2019).

Medaka is also a useful model organism for studying stress genes. For example, adult marine medaka was used as a model organism for the determination of environmental stress of hypoxia, and the results showed that there were significant alterations in levels of cell proliferation marker and protein in liver conditions of the species (Azra et al., 2022). Additionally, the open-field test, which is the most important behavioral paradigm for studying anxiety and stress in animals, can be used to study stress in medaka (Lucon-Xiccato et al., 2020).

In conclusion, medaka is a useful model organism for studying various fields of research, including stress genes. Its short generation times, large and transparent eggs, high tolerance to inbreeding, and comparably small genome size make it a suitable model organism for genomics research.

The main objective of this study is to explore the FKBP5 gene in Medaka using computational analysis methods. By utilizing various bioinformatics tools and techniques, we aim to unravel the genetic variations, and predict protein structures and functions.

MATERIAL AND METHODS

Overview of Computational Analysis Methods

Sequence Alignment and Homology Modeling

Sequence alignment is a fundamental technique used to compare and identify similarities between genetic sequences. In this study, we will employ sequence alignment tools (the BLOSUM62 matrix algorithm (Gromiha, 2010)) to analyze the *fkbp5/FKBP5* gene's genetic variations and identify co nserved regions.

```
Medaka Fkbp5
                           10        20        30        40        50        60
                  ....|....|....|....|....|....|....|....|....|....|....|....|
Medaka Fkbp5       1   MGRALPDAPPQDRPEHSLSAHPRRTVAAGPAGGEDVFVSAPRGNSCCLMPSVKMTTDQDL
Stickleback Fkbp5  1   -----------------------------------------------------.......
Fugu Fkbp5         1   --------------------MRI..GGHRLSSP.ESSA..AQII.----.HAD.....E.
Goldfish Fkbp5     1   ---------------------M.G.SNKREIL.TRLCM.G------------E.SSLE.I
Zebrafish Fkbp5    1   -----------------------------------------------------.SSLE.I
Common Carp Fkbp5  1   -------------MDGYMVKDT.G.SNKREIL.TR.CM.G------------E.SSLE.I
Human FKBP5        1   -----------------------------------------------------....EGA
Mouse FKBP5        1   -----------------------------------------------------....EGT

                           70        80        90       100       110       120
                  ....|....|....|....|....|....|....|....|....|....|....|....|
Medaka Fkbp5       61  LMDGPAATSVIAAKGIDLTPNKDQGVIKVVQCPGFDVDRPMIGDRVTVHYTGRLLTGKKF
Stickleback Fkbp5  8   T...QS.AA.F......V.TT..R......KHQ.LNGE..............K....E..
Fugu Fkbp5         37  PP..QST.ALFT.....V..........I.KRL.HAG.......K..........NR...
Goldfish Fkbp5     28  FSTNQCPAD.F.SQ...V...G.R..C.I.KQH.VEGEK.......C........N....
Zebrafish Fkbp5    8   FSTNQCP.A.FTSR.TAV...G.S..C.I.KQH.VEGE........F........S....
Common Carp Fkbp5  36  FSTNQCP.A.FTSQ.T.V...G.R..C.I.KQH.VEGEK.......H........N....
Human FKBP5        8   KNNEESP.ATV.EQ.E.I.SK..R..L.I.KRV.NGEET.....K.Y...K.K.SN....
Mouse FKBP5        8   SNN.ENPAATMTEQ.E.I.TK..R..L.I.KRV.TSDEA..F..K.Y...K.M.SD....

                          130       140       150       160       170       180
                  ....|....|....|....|....|....|....|....|....|....|....|....|
Medaka Fkbp5       121 DCSRERKEPFSFNAGKGQVLKSWDIGVLSMQRGEVCTLLCKPEYAYGSAGNPDKIPPNAS
Stickleback Fkbp5  68  ....D...ALC..V.......A.........M....SF.........FN...E.....SL
Fugu Fkbp5         97  ..THD........V.......A..V..S..E....AVF.........V..........SA
Goldfish Fkbp5     88  .S.LD.....V..V.....I.A...VIC...K....LM........S...S.P.V....T
Zebrafish Fkbp5    68  .S.LD.....V..V.....I.A...C.C...K....LM............S.P.V...ST
Common Carp Fkbp5  96  .S.LD...T.V..V.....I.A...V.C...K....VM............S.P.V....T
Human FKBP5        68  .S.HD.N...V.SL.....I.A.....AT.KK..I.H.............SLP...S..T
Mouse FKBP5        68  .S.HD..K..A.SL.Q...I.A.....ST.KK..I.H.............HLQ...S..T

                          190       200       210       220       230       240
                  ....|....|....|....|....|....|....|....|....|....|....|....|
Medaka Fkbp5       181 VVFEMELLSFEGESLTDDGGVVRRIKIKGEGYSQPNDGASVDV----YLEGRCDGRLFDS
Stickleback Fkbp5  128 ........M....I....S.I.....V..D..TN....SR.E.GPTVH.....G.....C
Fugu Fkbp5         157 ....I...D.HA....N...IL....V....F.N..E..K.H.----H...S.G.....C
Goldfish Fkbp5     148 L...I.....R..E..E.........V.....YN..E..T.HA----H...W.G......
Zebrafish Fkbp5    128 L...I...N.R..E..E...I.....V......N..E..T.H.----H...W.G......
Common Carp Fkbp5  156 L...I.....R..E.LE...I.....V.....NN..E.ST.N.----H.K.W.G......
Human FKBP5        128 LF..I...D.K..D.FE...II..T.R......N..E..T.EI----H.....G..M..C
Mouse FKBP5        128 LF..I...D.K..D.FE.S..I....R......N..E..T.K.----H...C.G..T..C

                          250       260       270       280       290       300
                  ....|....|....|....|....|....|....|....|....|....|....|....|
Medaka Fkbp5       237 RNVSFIVGEAEDKGVPLGVDRAMDKMQKGECCLLYLKPKYGFGSKGKAEYKIGPDRDIVY
Stickleback Fkbp5  188 .D..............................S..........RE..P.......S..I.
Fugu Fkbp5         213 .D...V...........F.......Q...........QS..A...E....F....NK.VE.
Goldfish Fkbp5     204 .D...V...S..VD.........E.I..........N....Y.KE..K..D..SNAELL.
Zebrafish Fkbp5    184 .D.T.A...S..V..........E.L..............A..KE.RK.HD..SNAELL.
Common Carp Fkbp5  212 .D.T.V...S..V..........E.I.................KE..K..D..SNAELL.
Human FKBP5        184 .D.A.T...G..HDI.I.I.K.LE...RE.Q.I...G.R....EA..PKFG.E.NAELI.
Mouse FKBP5        184 .D.V.V...G..HDI.I.I.K.LV...RE.Q.I...G.R....EA..PKFG.D.NAELM.

                          310       320       330       340       350       360
                  ....|....|....|....|....|....|....|....|....|....|....|....|
Medaka Fkbp5       297 EVTLKDFRRAKESWEMDIYEKVNLAPGVKNKGNQYFKTGQYHQAVIQYQRIISWLEMECG
Stickleback Fkbp5  248 .....S.E....T....LP..LD..A...H.......G.L.Y..................
Fugu Fkbp5         273 .......Q....C....LN..LQ..AE..I.......A.RHF.........V.......S
Goldfish Fkbp5     264 .......EK........LK..LEH.VL..Q..T....A.R.NF........VN.......
Zebrafish Fkbp5    244 .......EK...T....LK..LER.VL..Q..T....A.R.NY........VN.......
Common Carp Fkbp5  272 .....N.EK...F....LK..LER.DL..Q..T....A.R.SY......Q.VN.......
Human FKBP5        244 .....S.EK........TK..LEQ.AI..E..TV...G.K.M......GK.V......Y.
Mouse FKBP5        244 .....S.EK........TK..LTQ.AI..E..TV...G.K.T......RK.V......Y.

                          370       380       390       400       410       420
```

```
                         ....|....|....|....|....|....|....|....|....|....|....|....|
Medaka Fkbp5         357 TGIEQHQRIQEFVLTAHLNLALCFLRLKDFSHVVDNCNKVLELDENNEKAFYRRGEARLY
Stickleback Fkbp5    308 F...QK....S.L.............I....QA.......I...KS....L........L
Fugu Fkbp5           333 AKP.EQK...D.L.KS.......Y..M.EP....E.....I....H....L....K...L
Goldfish Fkbp5       324 A.K..Q.A..ALL.V............REY.QT.E.....M...PV....L........L
Zebrafish Fkbp5      304 N.K..L.A..ALL.V........Y...REY.QT.E.....M...PE....L........L
Common Carp Fkbp5    332 ..K..L.A..ALL.V............REY.QT.E.....ID..PE....L........L
Human FKBP5          304 LSEKESKASES.L.A.F....M.Y.K.REYTKA.EC.D.A.G..SA...GL......Q.L
Mouse FKBP5          304 LSEKESKASES.L.A.F....M.Y.K.REYNKA.EC.D.A.G..SA...GL......Q.L

                                   430       440       450       460       470       480
                         ....|....|....|....|....|....|....|....|....|....|....|....|
Medaka Fkbp5         417 RNEFGLAKEDFQKVLQVNPANQAARAQILICQNKIKEHHEQDKKIYANMFQKFAEQDAKT
Stickleback Fkbp5    368 ....PM.M....Q..R.....R..HG.L.L..T.......R...T..........R.T.I
Fugu Fkbp5           393 ....S..MT...Q...K.TS.R......A..............R................
Goldfish Fkbp5       384 ....SM.LM..KQ.....SF.R...S..A...R..R....R..........R...H...V
Zebrafish Fkbp5      364 ....S..LM..KQ.....SF.R...S......H..R....R..........R...H...V
Common Carp Fkbp5    392 ....SM.LV..KQ.....SF.H...S..AV.RR..R..N.R..........R...H...E
Human FKBP5          364 M...ES..G..E...E...Q.K...L..SM..K.A...N.R.RR......K........E
Mouse FKBP5          364 M.D.ES..G..E...A...Q.R...L..SM..R.A...N.R.RRV.....K....R...E

                                   490       500       510       520       530       540
                         ....|....|....|....|....|....|....|....|....|....|....|....|
Medaka Fkbp5         477 G-----KTKRRRDDIVRTTMNGELSIKRRRRSQDANVALTFKKGEEKPSCHGNRGRNEVQ
Stickleback Fkbp5    428 .-----.......ESA.SG.....GN........CP------------------------
Fugu Fkbp5           453 .-----.R.K.K-----SGVK..I.L.......ECSS-----------------------
Goldfish Fkbp5       444 .---RL.R.KEE---SGISDQNKQGL..PCL...GS------------------------
Zebrafish Fkbp5      424 C---RL.R.KEE---SVISDQNKQGL..A.L...GS------------------------
Common Carp Fkbp5    452 ANEEAAH.NEEEEEFTTVSLKE.TGVHVCYGCHERLQSHKL..IKRCSYSVLM-------
Human FKBP5          424 EAN---.AMGKKTSEGV.NEK.TD.QAMEEEKPEGH.-----------------------
Mouse FKBP5          424 EAS---.AGSKKAVEGAAG-KQHE.QAMEEGKAKGH.-----------------------
```

		Identity(%)	similarity(%)
Medaka Fkbp5	531	100	100
Stickleback Fkbp5	458	65	74
Fugu Fkbp5	479	62	75
Goldfish Fkbp5	473	55	68
Zebrafish Fkbp5	453	54	68
Common Carp Fkbp5	504	53	69
Human FKBP5	457	46	62
Mouse FKBP5	456	45	60

Figure1. Identity-Similarity Rates between Fkbp5 protein Sequences of Medaka and Other Vertebrates' Fkbp5/FKBP5 Protein Sequences. The dots and lines refer to repeating amino acids and undetectable amino acids, respectively.

We conducted a BLAST analysis using the cDNA sequences of the *fkbp5* gene from the NCBI database to as certain whether the *fkbp5* gene is indeed a functional gene. We further investigated the conservation of this gene in medaka, zebrafish (*Danio rerio*), and humans (*Homo sapiens*) by identifying the chromosome regions of the medaka *fkbp5* genes and comparing them to the gene regions in zebrafish and humans. This allowed us to detect regions of these genes in zebrafish and humans, ultimately determining the conservation of gene synteny based on the identified gene regions.

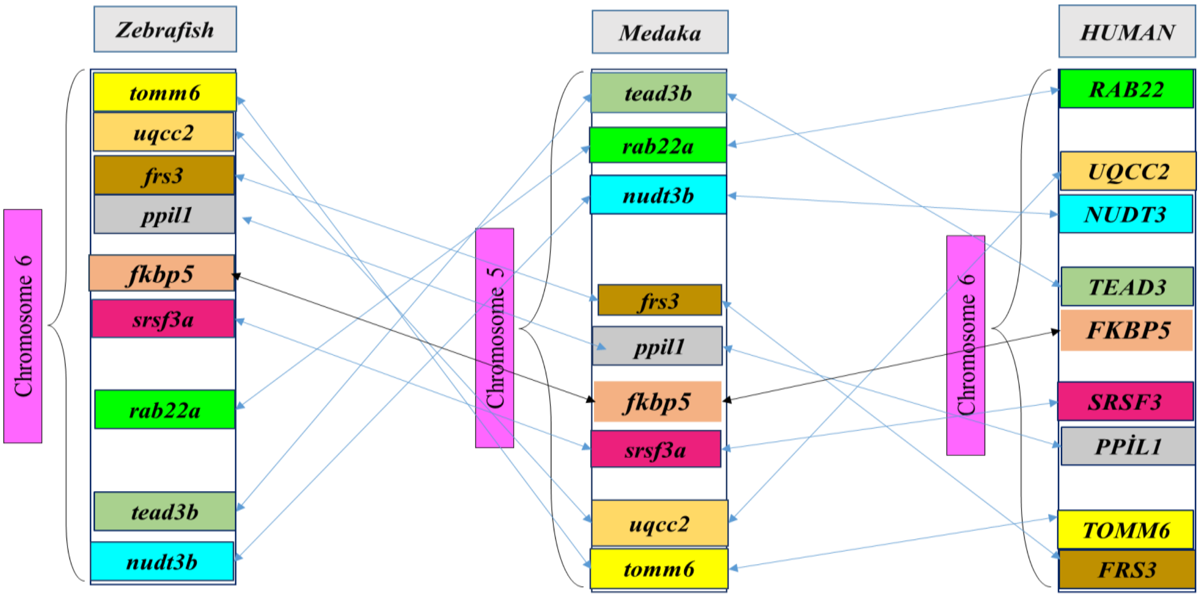

Figure 2. Conserved gene synteny in medaka

To establish the phylogenetic relationship of the medaka fkbp5 gene with other aquatic model organisms and vertebrates, we conducted a phylogenetic tree analysis using the maximum likelihood method. To accomplish this, we utilized software tools such as CLUSTALW, BioEdit (accessible at http://www.mbio.ncsu.edu/bioedit/page2.html), and MEGA6 (Tamura et al., 2013). The resulting phylogenetic tree was constructed using protein sequences from medaka (*Oryzias latipes*) and other organisms, including, zebrafish (*Danio rerio*), common carp (*Cyprinus carpio*), fugu (*Takifugu rubripes*), stickleback (*Gasterosteus aculeatus*), goldfish (*Carassius auratus*), tetraodon (*Tetraodon nigroviridis*), platyfish (*Xiphophorus maculatus*), human (*Homo sapiens*), and mouse (*Mus musculus*) *fkbp5/FKBP5genes* according to maximum likelihood method using MEGA10 program.

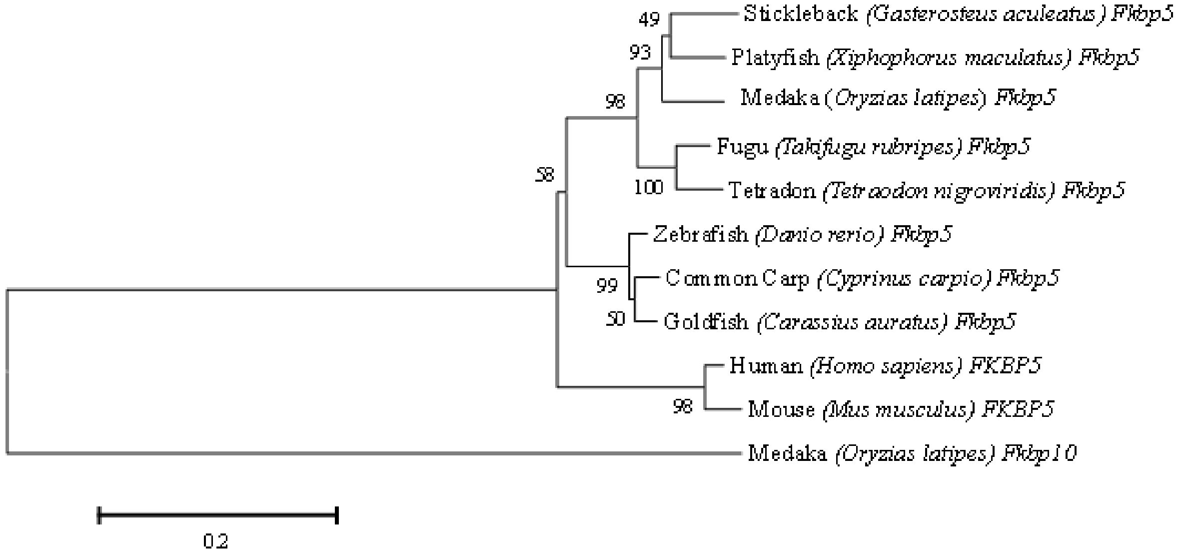

Figure 3. Phylogenetic relationship between medaka and some vertebrates

In silico analyses were performed using the longest cDNA transcripts of the medaka *fkbp5* gene. The ensembl database was utilized to determine the gene structure of *fkbp5* genes, examine the exon-intron organization of the genes, and identify the protein-encoding nucleotides as well as the 5'-3' ends. Additionally, the starting point of the detected transcription (+1), TATA box, and poly A tail were all documented and presented in Table 1.

Medaka *fkbp5* ENSORLG00000029600

```
5’cagctgagcaaagagtacagcctgctgcggaacagcctgttcccggcctggcacggaa
cgggttacaggtcagggtgtctattggaacacagtgttctcatcagcggcggtacagcta
cagtacagccggaacattgtgttactttgtggctgctttccaagacctgccctTAATttt
acactaaacgccctcccacaactctcttctcgtttctgacccccccgcgctgtctgcctc
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+1
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               -S--C--C--L--M--P--S--V--K--M--T--T--D--Q--D-
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-F--D--V--D--R--P--M--I--G--D--R--V--T--V--H--Y--T--G--R--L-
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-L--T--G--K--K--F--D--C--S--R--E--R--K--E--P--F--S--F--N--A-
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```

```
-G--K--                     G--Q--V--L--K--S--W--D--I--G--V--L--
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S--M--Q--R--G--E--V--C--T--L--L--C--K--P--E--Y--A--Y--G--S--
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--V--S--F--I--V--G--E--A--E--D--K--G--V--P--L--G--V--D--R--A
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E--S--W--E--M--D--I--Y--E--K--V--N--L--A--P--G--V--K--N--K--
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L--K--D--F--S--H--V--V--D--N--C--N--K-                -V--L--
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E--L--D--E--N--N--E--K--A--F--Y--R--R--G--E--A--R--L--Y--R--
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N--E--F--G--L--A--K--E--D--F--Q--K--V--L--Q--V--N--P--A--N--
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Q--A--A--R--A--Q--I--L--I--C--Q--N--K--I--K--E--H--H--E--Q--
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D--K--K--I--Y--A--N--M--F--Q--K--F--A--E--Q--D--A--K-
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         -T--G--K--T--K--R--R--R--D--D--I--V--R--T--T--M--N-
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```

Table 1. Gene structure of medaka *fkbp5* gene

RESULTS AND DISCUSSION

The sequence identity-similarity ratio was calculated to investigate the orthology between the medaka and the other vertebrates *fkbp5* genes. For this purpose The putative amino acid sequences coded by the medaka *fkbp5* gene was aligned with *fkbp5* sequences from various vertebrate species, including, zebrafish, medaka, fugu, human and rat using CLUSTAL W multiple alignment tool BLOSUM62 matrix algorithm was used to determine sequence identity and similarity. The percentage sequence identity varied among the examined *fkbp5* genes, but medaka *fkbp5* shared the highest sequence identity and similarity with the stickleback (65%, and 74% identity respectively) (Figure1).

Conserved gene synteny of medaka *fkbp5* gene with the *fkbp5/FKBP5* genes of zebrafish and human was identified manually using the Ensembl genome database. We detected the conserved genes and their location in medaka and then we looked for if they are take part in human and zebrafish chromosome. As a result of researching ensembl database, it was seen there are highly conserved genes which are shown in the synteny (Figure 2).

Phylogenetic relationship is given in the tree (Figure 3) which created using protein sequences of medaka (*Oryzias latipes*), zebrafish (*Danio rerio*), common carp (*Cyprinus carpio*), fugu (*Takifugu rubripes*), stickleback (*Gasterosteus aculeatus*), Goldfish (*Carassius auratus*), tetraodon (*Tetraodon nigroviridis*), Platyfish (*Xiphophorus maculatus*), Human (*Homo sapiens*), Mouse (*Mus musculus*) *fkbp5/FKBP5genes* according to maximum likelihood method using MEGA10 program. It was observed that the medaka showed clustering with other teleost fishes, and that living organisms such as humans and mouse were clustered in a different region where medaka *fkbp10* gene is chosen as the outgroup.

The longest cDNA transcript of the medaka *fkbp5* gene was used to perform in silico analyzes. The ensembl database was used to determine the gene structure of this gene. Medaka fkbp5 gene exhibits a highly conserved gene organization consisting of 13 exons separated by for 12 introns.

CONCLUSION

The use of aquatic organisms as model organisms has also increased recently. In this study, the bioinformatics of the *fkbp5* gene in the medaka, which is one of the aquatic model organisms, was studied. Identification and characterization of *fkbp5 gene* which is differentially expressed between high-stress-tolerant and non-stress-tolerant fish can be used in aquaculture selection programs to help improve stress tolerance, as well as provide important genetic markers that serve as a model for other vertebrates, including humans. Furthermore, the comparative analysis conducted in this study, in which we compared the gene FKBP Prolyl Isomerase 5 with other species, can offer valuable insights into its evolution. This analysis can help us better comprehend the conservation and divergence patterns of *fkbp5*. In summary, this research paves the way for future studies that aim to delve deeper into our understanding of the *fkbp5* gene in Medaka.

Assessing the expression of proteins and enzymes produced by stress-related genes is crucial for gauging cellular stress responses when teleost fish are exposed to stressors. This analysis is especially significant for molecular-level studies. As fish species vary in their stress tolerance and stress gene expression, it's vital to identify and characterize these genes for use in aquaculture selection programs, providing valuable data for further advancements. The utilization of in-silico analysis on the *fkbp5* gene in medaka, an aquatic model organism, yields vital genetic insights. Therefore, we conducted bioinformatics-based identification and characterization of the *fkbp5* gene in medaka.

This study sets the stage for further investigations in several directions. First, experimental validations are needed to confirm the computational predictions made regarding genetic variations, protein structures, and molecular interactions associated with *fkbp5* in medaka. Additionally, functional studies can explore the precise mechanisms through which *fkbp5* influences various physiological processes. Furthermore, comparative analysis with other species can provide valuable evolutionary insights and help us understand the conservation and divergence patterns of FKBP5. Overall, this study opens up new avenues for future research to deepen our understanding of FKBP Prolyl Isomerase 5 gene in medaka.

REFERENCES

Azetsu, Y., Chatani, M., Dodo, Y., Karakawa, A., Sakai, N., Negishi Koga, T., & Takami, M. (2019). "Treatment with synthetic glucocorticoid impairs bone metabolism, as revealed by in vivo imaging of osteoblasts and osteoclasts in medaka fish." Biomedicine and Pharmacotherapy, 118, 109101. doi:10.1016/j.biopha.2019.109101.

Azra, M. N., Noor, M. I. M., Tan, M. P., Dawood, M., Amin, M., Zekker, I., Abdullah, M. F., Latif, Z. A., & Pardi, F. (2022). "Zebrafish and Medaka as model organisms for climate change research: Global literature scientometric analysis." Frontiers in Earth Science, 10. doi:10.3389/feart.2022.988710.

Bhandari, R. K. (2016). "Medaka as a model for studying environmentally induced epigenetic transgenerational inheritance of phenotypes." Environmental Epigenetics, 2(1), 1-9. doi:10.1093/eep/dvv010.

Gromiha, M. M. (2010). "Protein Bioinformatics: From Sequence to Function." Academic Press. ISBN-13: 978-81-312-2297-3.

Hilgers, L., & Schwarzer, J. (2019). "The untapped potential of medaka and its wild relatives." Elife, 8, e46994. doi:10.7554/eLife.46994.

Lin, C. Y., Chiang, C. Y., & Tsai, H. J. (2016). "Zebrafish and Medaka: new model organisms for modern biomedical research." Journal of Biomedical Science, 23, 1-11. doi:10.1186/s12929-016-0236-5.

Lucon-Xiccato, T., Conti, F., Loosli, F., Foulkes, N. S., & Bertolucci, C. (2020). "Development of open-field behavior in the medaka, Oryzias latipes." Biology, 9(11), 389. doi:10.3390/biology9110389.

Rutherford, S. L., & Zuker, C. S. (1994). "Protein folding and the regulation of signaling pathways." Cell, 79(7), 1129-1132. doi:10.1016/0092-8674(94)90003-5.

Schartl, M., Shen, Y., Maurus, K., Walter, R., Tomlinson, C., Wilson, R. K., Postlethwait, J., & Warren, W. C. (2015). "Whole body melanoma transcriptome response in medaka." PLoS One, 10(12), e0143057. doi:10.1371/journal.pone.0143057.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). "MEGA6: Molecular Evolutionary Genetics Analysis version 6.0." Molecular Biology and Evolution, 30. doi:10.1093/molbev/mst197.

Wittbrodt, J., Shima, A., & Schartl, M. (2002). "Medaka, a model organism from the far East." Nature Reviews Genetics, 3(1), 53-64. doi:10.1038/nrg704.

Zannas, A. S., Wiechmann, T., Gassen, N. C., & Binder, E. B. (2016). "Gene stress epigenetic regulation of FKBP5: clinical and translational implications." Neuropsychopharmacology, 41(1), 261-274. doi:10.1038/npp.2015.235.

Biostimulant Application Mitigates Drought Stress in Curly Lettuce

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ABSTRACT: In recent years, drought is one of the most important environmental factors that has a negative impact on agricultural production. However, in recent years, studies have been conducted to increase drought stress tolerance of plants, and the effectiveness of various applications have been emphasized. In this study, the mitigative effect of biostimulant application against water stress in curly lettuce was investigated. For this purpose, irrigation was done at 100%, 80% and 60% of the field capacity and two different doses of biostimulant (T1: 4 kg/ha, T2: 8 kg/ha BioHealth WSG) were applied. In the result of the study, as the water stress level increased, the decrease in plant fresh weight, root fresh weight, plant dry weight and root dry weight was greater. However, biostimulant applications lowered these negative impacts on plant. In particular, the T2 application was the application that gave the best results. Also, as water stress increased, the H_2O_2, MDA, proline and sucrose content, CAT, POD and SOD activities in the plant increased. These increases with biostimulants were lower than in control plants. It was determined that biostimulant application could be significantly effective in reducing the damage caused by water stress in curly lettuce.

Keywords: *Lettuce*, Biostimulant, Water, Stress, Antioxidant, Growth

INTRODUCTION

In recent years, drought is considered to be one of the most important stress factors causing yield and quality losses in agricultural production. Various morphological, physiological and biochemical processes in plants are prevented by drought stress. In addition to reducing seed germination, leaf area, overall plant growth, and total biomass accumulation, drought stress impairs photosynthetic and gas exchange properties, chlorophyll biosynthesis, and cell membrane integrity (Hussain et al., 2019).

Alternative solutions have been sought against drought, which will clearly cause significant problems in crop production in the future with global climate change. Various applications are used for this purpose. In many studies, it has been determined that the negative effects of drought stress on plants can be alleviated with various exogenous applications such as biochar (Yildirim et al., 2021a; Güllap et al., 2022), nitric oxide (Ekinci et al., 2020), melatonin (Liu et al., 2015), methylamine (Yildirim et al., 2019), and plant growth regulators (Sahin et al.,2015; Yildirim et al., 2021b).

Biostimulants are among the most studied alternatives for reducing drought impacts in recent years. Biostimulants are defined as various substances obtained from different organic, inorganic substances and microorganisms. They are plant-growth-promoting substances that are both nutrients and soil improvers such as humic substances, amino acids, algea, inorganic compounds and beneficial microorganisms (Rouphael and Colla, 2020; Ramzan and Younis, 2022). Biostimulants can be in the form of single or multi-component formulations, can be applied to the plant from the leaves or soil, can be hydrolyzed, fermented or extracted products. Additionally, it is stated that biostimulants are not harmful to the environment and can be used in sustainable plant production (Tarafdar, 2022). Biostimulants promote seed germination, seedling development and plant growth and increase productivity (Tarafdar, 2022). While biostimulants reduce the need for fertilizer in the plant, they protect plants against abiotic stresses by promoting plant growth and increasing tolerance.

Lettuce (Lactuca sativa L.) is one of the most commonly produced leafy vegetable species. It has important functions in nutrition with the vitamins, fibres, carotenoids, polyphenols and antioxidants it contains (Llorach et al., 2008). Curly lettuce (Lactuca sativa var. crispa) has a high-water content and is also sensitive to drought stress due to its shallow root structure (Kıran, 2019). For this reason, in this study, it was investigated whether biostimulant applications had a positive effect on curly lettuce grown under drought stress.

MATERIAL AND METHOD

In the study curly lettuce (Lactuca sativa L. var. crispa) were used as plant material and two different doses of biostimulant (T1: 4 kg/ha, T2: 8 kg/ha BioHealth WSG) were applied. Seeds were planted in seedling trays filled with peat:perlite mixture prepared in a 2:1 (v:v) ratio. In the experiment, seedlings with 2-3 leaves were planted into the pods after 30 days sowing. The 1.5 liter pots used in the study were filled with soil: sand: peat (2:1:1). The applications were made as irrigation to the plant root zone 3 times at one-week intervals. Irrigation was done according to soil moisture values measurements at 100%, 80% and 60% of the field capacity. The study was terminated 45 days after planting. The seedlings were harvested for some measurements and analyses. The effects of the treatments on plant and root fresh weight and plant and root dry weight were determined. In addition, H2O2, MDA, proline and sucrose content, CAT, POD and SOD enzyme activities in plants were investigated. The study was designed with 3 replications and 10 plants in each replication. The difference among and between means were examined applying the Duncan test at the 0.05 probability level and was made statistically analyze using SPSS.

RESULTS AND DISCUSSION

As a result of the study, it was determined that there was a significant decrease in the parameters examined in lettuce with decreasing irrigation. In the study, water deficit was applied at two levels. As the water stress level increased, the decrease in plant fresh weight, root fresh weight, plant dry weight and root dry weight was greater. While plant fresh weight decreased by 41% in 80% irrigation and 62% in 60% irrigation compared to the control, root fresh weight decreased by 31% in 80% irrigation and 38% in 60% irrigation. Plant dry weight and root dry weight decreased by 30% and 24% in 80% irrigation, and by 46% and 36% in 60% irrigation (Figure 1).

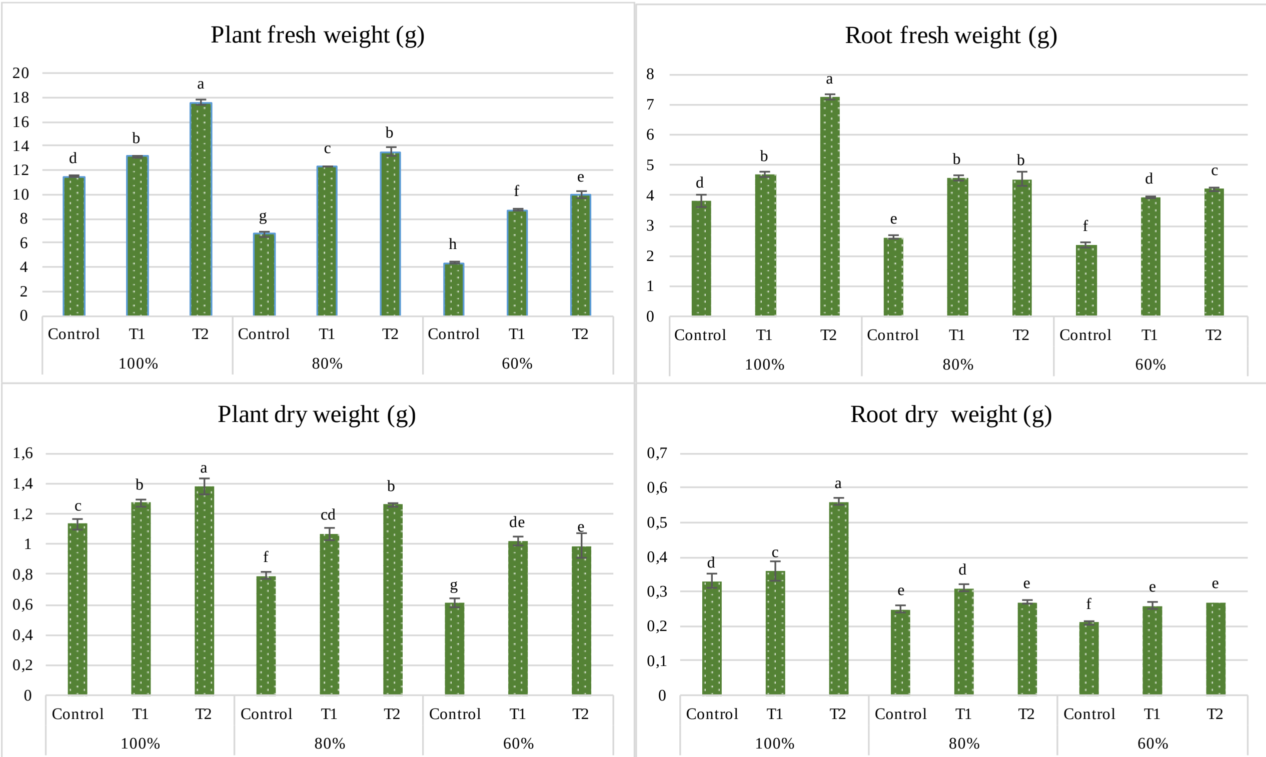

Figure 1. Effect of biostimulant application on weight of curly lettuce. There is no statistically (p<0.001) difference between the means shown with the same letter in the bar.

However, biostimulant applications lowered these negative impacts on plant fresh weight, root fresh weight, plant dry weight and root dry weight caused by water stress. In particular, the T2 application was the application that gave the best results. With T2 application at 100% irrigation, plant fresh weight, root fresh weight, plant dry weight and root dry weight increased by 53%, 90%, 22% and 70%, respectively. At 80% irrigation, while there was an increase in plant fresh weight and plant dry weight with T2 application and in root fresh weight with T1 application, there was a very low decrease in root dry weight with T1 application. At 60% irrigation, there was a decrease in plant fresh weight, root fresh weight, plant dry weight and root dry weight, and this decrease was almost half as much as in those without any application (Figure 1).

The H_2O_2, MDA, proline and sucrose content, CAT, POD and SOD activities increased in the plant under drought stress. While these increases were 38%, 65%, 46%, 48%, 55%, 43% and 42% respectively for 80% irrigation, there was a significant increase of 60%, 106%, 62%, 72%, 84%, 109% and 98% in these parameters for 60% irrigation. In full irrigation (100%), these parameters were at lower levels in both T1 and T2 applications, and these parameters were lower than the control in both water stress levels (Figure 2). Only at 60% irrigation, there was a slight increase in the content of MDA and proline, CAT, POD and SOD activities, there was an increase in the rate of 45% (T1), 7% (T2), 4% (T2), 8% (T2) and 75% (T1), respectively. These increases were lower than in control drought stressed plants (Figure 3).

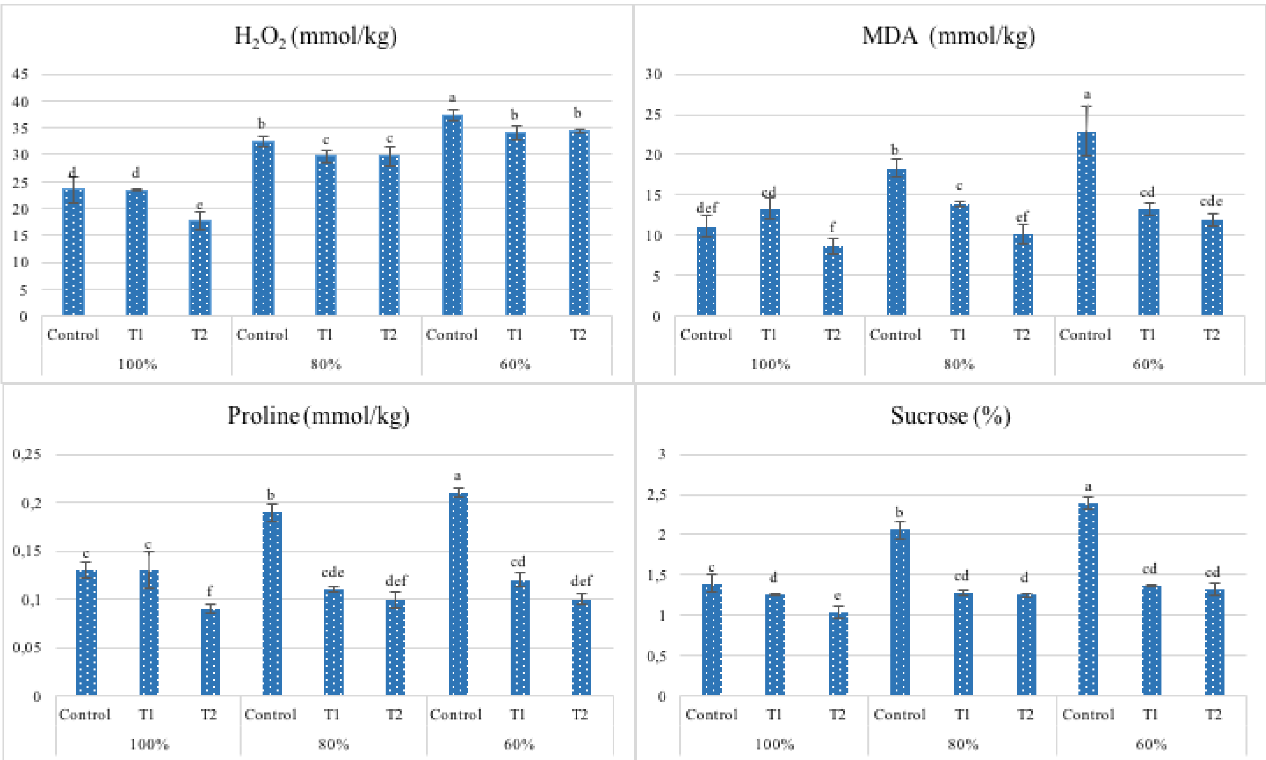

Figure 2. Effect of biostimulant application on H_2O_2, MDA, proline and sucrose content of curly lettuce. There is no statistically ($p<0.001$) difference between the means shown with the same letter in the bar.

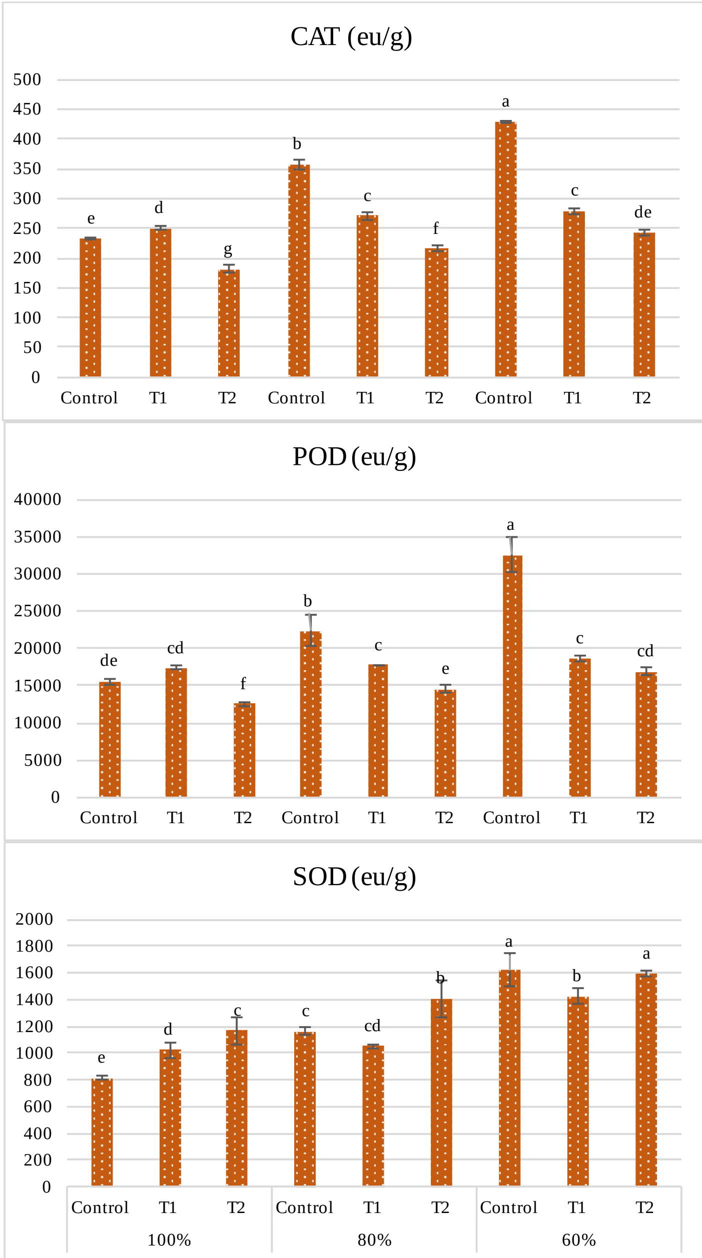

Figure 3. Effect of biostimulant application on CAT, POD and SOD enzyme activity of curly lettuce. There is no statistically (p<0.001) difference between the means shown with the same letter in the bar.

Drought disrupts plant growth and water relations, reduces leaf size, inhibits stem elongation and root development, etc. Metabolic, physiological, biochemical and molecular events occurring in the plant play a role in the drought stress reaction. The negative effects of water deficit in terms of yield and quality in agricultural production have been detected in many plant species. Lettuce, for which water is essential in its cultivation, is one of the vegetables facing this problem. In this study, plant growth decreased with increasing drought stress. However, biostimulant application had a mitigating effect on stress damage in curly lettuce in this study. In addition to facilitating nutrient uptake, many plant biostimulants also provide protective effects against environmental stress such as water deficiency (Van Oosten et al., 2017). With the humic acid and microorganisms it contains, it not only improves plant growth under stress-free conditions, but also has a positive effect on water stress for curly lettuce.

Humic substances are the main organic components of lignite, soil and peat, and humic acids are produced by the biodegradation of organic matter (Van Oosten et al., 2017). Humic acid is known to increase drought stress tolerance in plants. As a matter of fact, Chen et al., (2022) stated that humic acid application increases drought resistance and growth in corn plants, and can achieve this by improving the water content and nutrient availability of the soil, preserving photosynthetic activity, increasing osmotically active solutes and making changes in phytohormones. Humic acid application increased shoot fresh weight, shoot dry weight and leaf area in melon plants under drought stress, and also increased drought tolerance by stimulating SOD and CAT antioxidant enzyme activity (Kıran et al., 2019). In addition, it has been stated that under drought stress conditions, humic acid application increases the concentration of osmotic regulators such as soluble sugar and proline, maintains the osmotic pressure in the cells and increases the plant's resistance to drought (Beheshti and Tadayyon, 2018).

Microorganisms, which are also used as biofertilizers, are considered biostimulants, they support plant growth with various mechanisms such as increasing nutrient uptake in the plant, increasing root biomass or root area, etc (Calvı et al., 2014). Microbial biostimulants are rhizobacteria (PGPR-----) or other microorganisms that stimulate plant growth. They promote nutrient uptake, produce secondary metabolites, siderophores, hormones and organic acids, participate in nitrogen fixation and ensure stress tolerance, thus increasing product quality and yield (Kaushal et al., 2023). In a study conducted in corn, a microbial biostimulant increased the plant's resistance to drought by changing basic metabolic pathways such as redox homeostasis, plant cell wall strengthening, osmoregulation, energy production and membrane remodeling, which are involved in drought resistance mechanisms (Nephali et al., 2021). It was determined by Ouhaddou et al (2023) that microbial biostimulants in lettuce provide the ability to withstand drought stress damage, achieve this by improving various physiological and biochemical processes in the plant, and also protect lettuce plants against oxidative stress by increasing flavonoids and antioxidant activities. Due to the content of the biostimulant used in this study, it increased drought stress tolerance in curly lettuce with similar effects mentioned above.

CONCLUSION

As a result of this study, it was determined that biostimulant application could be significantly effective in reducing the damage caused by drought stress in curly lettuce.

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REFERENCES

Beheshti, S., Tadayyon, A., 2018. Effects of drought stress and humic acid on some physiological parameters of lima bean (Phaseolus lunatus L.). Journal of Plant process and Function, 6(19), 1-14.

Calvo, P., Nelson, L., Kloepper, J. W., 2014. Agricultural uses of plant biostimulants. Plant and soil, 383, 3-41.

Chen, Q., Qu, Z., Ma, G., Wang, W., Dai, J., Zhang, M., Wei, Z., Liu, Z., 2022. Humic acid modulates growth, photosynthesis, hormone and osmolytes system of maize under drought conditions. Agricultural Water Management, 263, 107447.

Ekinci, M., Ors, S., Yildirim, E., Turan, M. E. T. I. N., Sahin, U., Dursun, A., Kul, R., 2020. Determination of physiological indices and some antioxidant enzymes of chard exposed to nitric oxide under drought stress. Russian Journal of Plant Physiology, 67, 740-749.

Gullap, M. K., Severoglu, S., Karabacak, T., Yazici, A., Ekinci, M., Turan, M., Yildirim, E., 2022. Biochar derived from hazelnut shells mitigates the impact of drought stress on soybean seedlings. New Zealand Journal of Crop and Horticultural Science, 1-19.

Hussain, S., Hussain, S., Qadir, T., Khaliq, A., Ashraf, U., Parveen, A., Saqib, M., Rafiq, M., 2019. Drought stress in plants: An overview on implications, tolerance mechanisms and agronomic mitigation strategies. Plant Science Today, 6(4), 389-402.

Kaushal, P., Ali, N., Saini, S., Pati, P. K., Pati, A. M., 2023. Physiological and molecular insight of microbial biostimulants for sustainable agriculture. Frontiers in Plant Science, 14, 1041413.

Kıran, S., 2019. Effect of Vermicompost on Mineral Contents of Lettuce (Lactuca sativa var. crispa) Under Drought Stress. KSU J. Agric. Nat., 22, 133-140.

Kıran, S., Furtana, G. B., Talhouni, M., Ellialtıoğlu, Ş. Ş., 2019. Drought stress mitigation with humic acid in two Cucumis melo L. genotypes differ in their drought tolerance. Bragantia, 78, 490-497.

Liu, J., Wang, W., Wang, L., Sun, Y., 2015. Exogenous melatonin improves seedling health index and drought tolerance in tomato. Plant Growth Regulation, 77, 317-326.

Llorach, R., Martínez-Sánchez, A., Tomás-Barberán, F. A., Gil, M. I., Ferreres, F., 2008. Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chemistry, 108(3), 1028-1038.

Nephali, L., Moodley, V., Piater, L., Steenkamp, P., Buthelezi, N., Dubery, I., Burgess, K., Tugizimana, F., 2021. A metabolomic landscape of maize plants treated with a microbial biostimulant under well-watered and drought conditions. Frontiers in Plant Science, 12, 676632.

Ouhaddou, R., Ech-chatir, L., Anli, M., Ben-Laouane, R., Boutasknit, A., Meddich, A., 2023. Secondary metabolites, Osmolytes and antioxidant activity as the main attributes enhanced by biostimulants for growth and resilience of lettuce to drought stress. Gesunde Pflanzen, 1-17.

Ramzan, F., Younis, A., 2022. Use of biostimulants in tolerance of drought stress in agricultural crops. In Emerging Plant Growth Regulators in Agriculture (pp. 429-446). Academic Press.

Rouphael, Y., Colla, G., 2020. Biostimulants in agriculture. Frontiers in Plant Science, 11, 40.

Sahin, U., Ekinci, M., Kiziloglu, F. M., Yildirim, E., Turan, M., Kotan, R., Ors, S., 2015. Ameliorative effects of plant growth promoting bacteria on water-yield relationships, growth, and nutrient uptake of lettuce plants under different irrigation levels. HortScience, 50(9), 1379-1386.

Tarafdar, J. C., 2022. Biostimulants for sustainable crop production. In New and Future Developments in Microbial Biotechnology and Bioengineering (pp. 299-313). Elsevier.

Van Oosten, M. J., Pepe, O., De Pascale, S., Silletti, S., Maggio, A., 2017. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. Chemical and Biological Technologies in Agriculture, 4, 1-12.

Yildirim, E., Ekinci, M., Kul, R., Turan, M., Gur, A., 2019. Mitigation of drought stress effects on pepper seedlings by exogenous methylamine application. International Letters of Natural Sciences, (76).

Yildirim, E., Ekinci, M., Turan, M., 2021a. Impact of biochar in mitigating the negative effect of drought stress on cabbage seedlings. Journal of Soil Science and Plant Nutrition, 21(3), 2297-2309.

Yıldırım, E., Güneş, A., Turan, M., Kotan, R., Ekinci, M., 2021b. Amino Acid and Hormone Content of Plant Growth-Promoting Rhizobacteria Grown in Drought Stress Created by PEG6000. Avrupa Bilim ve Teknoloji Dergisi, (21), 95-112.

Smart Seed Technology in Sugar Beet

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ABSTRACT: The system consisting of a herbicide plus a herbicide-tolerant variety (Conviso Smart) is expected to provide a solution to the typical problems associated with weed control in sugar beet. Conviso Smart is a new system for controlling monocotyledonous and dicotyledonous weeds in ALS inhibitors. The herbicide Conviso is a combination of two active ingredients, foramsulfuron and thiencarbazone methyl. Foramsulfuron is the foliar active compound, while thiencarbazone-methyl is active in both foliar and soil. This system consists of a herbicide and a sugar beet variety that inhibits ALS. Field trials have demonstrated the broad spectrum of action and long-term persistent activity of Conviso herbicide. This system will allow for a reduction in the number of herbicide treatments and the overall intensity of weed control measures. However, if the mentioned herbicide group provides effective protection in the soil for a long time, it may disrupt agricultural systems. Herbicide residues may enter the ecosystem through surface waters and groundwater, polluting the environment and negatively affecting some plant groups (cereals, legumes, industrial plants) in crop rotation. This study was conducted to examine the Conviso Smart system.

Keywords: Sugar beet, ALS inhibitör, herbicide

INTRODUCTION

Sugar beet is an industrial crop cultivated in many countries, although it is mainly grown in temperate and Mediterranean climates (Figure 1). In most regions, sugar beet is sown in early spring. In some countries, such as Spain, winter beet production (autumn sowing) is quite common. However, in some countries, such as California, sugar beet is planted almost all year round except in July and August. In dry farming conditions (e.g. Western USA, Spain, Turkey) sugar beet is irrigated (Petersen, 2004). Therefore, due to these conditions, weed infestation of sugar beet and weed control strategies are quite different in each region. The weed flora is also influenced by crop rotation, soil type and other factors.

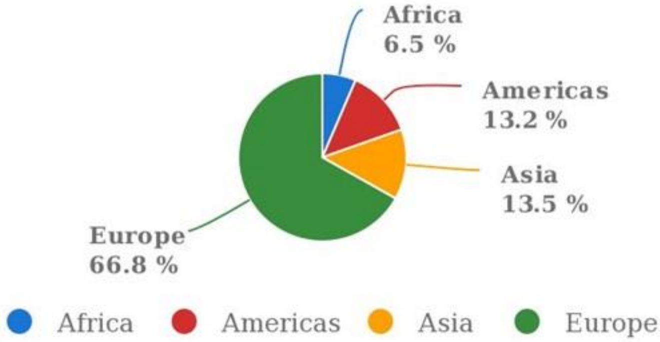

Figure 1. Sugar beet production areas (FAO, 2023)

Sugar beet is sown in wide rows, 45 to 75 cm apart (depending on the condition of machinery and irrigation system etc.). This and the slow growth of young plants are some of the reasons why weed competition is characterised as poor. Without weed control, yields will be significantly reduced by up to 95 per cent. Depending on harvesting and beet cleaning systems, weeds can interfere with beet harvesting and processing. Weeds can also be hosts for diseases and pests (GleiBl et al., 1989; Schlosser, 1989). Intensive mechanical or chemical weed control (or a combination of both methods) is common.

Sugar beet is grown in various countries under different climates, soils and conditions. The limited use of herbicides in the past has resulted in a change in the weed flora. For example, *Polygonum convolvulus* L. or *Aethusa cynapium* L. were not mentioned among the important sugar beet weeds in Germany in 1959/64 (Hanf, 1959; Rademacher, 1964). Nowadays these two species are quite common (Merkes et al., 2001). Another reason for changes in weed flora is changes in crop rotations. Crops with high economic value are grown more and more, thus reducing crop diversity on farms (Petersen, 2004).

To increase the production potential in sugar beet cultivation, it is necessary to control weeds. The increasing number of weeds resistant to herbicides has become a major problem in today's crop production. Under current conditions, they cause more global crop losses than both insect pests and pathogens.

WEEDS AND CROP INTERACTIONS

Sugar beet and weed interactions are based on competition for water, nutrients, light and allelopathic effects. Among these factors, light is of primary importance (Kropff and Spitters, 1992). As photosynthetic radiation increases, the sugar beet canopy needs to be optimally regulated because the greater the amount of photosynthetic radiation reaching the weeds, the stronger these weeds become (Schaufele, 1986; Schaufele, 1991; Mittler et al., 2002). This effect of reduced light interference by the crop is also the reason for some observations: broad-leaved weeds are more competitive than annual grasses in sugar beet (Zimdahl and Fertig, 1967). On the other hand, there are also some differences between sugar beet varieties that affect their susceptibility to weeds. In some sugar beet cultivars, leaves that are horizontal so that the canopy closes earlier can suppress weeds more easily than those that close the canopy later and grow more upright (Lotz et al., 1991). However, in some cultivars, lower leaf areas but areas with vertical leaf habit have higher yield potential. For these, higher intensity weed control may be required in cultivars.

N-fertiliser can also affect competition between sugar beet and weeds (Scott and Moisey, 1972; Brautigam and Schaufele, 1994). The leaf area of sugar beet increases at high levels of N-fertilisers, but weeds did not benefit positively from this in terms of dry matter yield or N uptake at increased N levels. On the other hand, amounts above the optimum N can harm beet quality (especially extractable sugar content).

Weeds can be sources or host plants for diseases (e.g. *Beet Necrotic Yellow Vein Virus*; Schlosser, 1989) and pests (e.g. *Heterodera schachtii*; GeiBl et al.,1989). However, the presence of weeds does not always have a negative impact. For example, aphids are vectors of virus jaundice disease in sugar beet (*Beet Yellow Virus* and *Beet Mosaic Virus*). Aphid populations are reduced by the presence of beet weeds and in this case virus jaundice infections are also reduced (Dubois et al., 1993). For ecological and phytopathological reasons the positive balance of weed effects in sugar beet is such that control is recommended rather than trying to eradicate differentiated and selective weeds (Bosch, 1987).

Dicotyledonous weeds belonging to the Chenopodiaceae, Asteraceae and Polygonaceae families are observed in many sugar beet growing areas. The most important factor in the spread of these weeds is ecological factors (soil, climate, etc.). In addition, under unfavourable conditions (e.g. cold spring) in sugar beet growing areas, some plants vernalise and flower in the first year. This process results in viable seed production. If the seed-binding plants are not uprooted and removed, seeds with long dormancy accumulate in the soil seed bank. As a result, annual weed beetroot can germinate and compete with the next sugar beet.

Many selective sugar beet herbicides are not sufficiently effective in controlling weeds belonging to the Polygonaceae and Asteraceae families. In general, monocots are less important compared with dicot weeds. However, weed control problems of the Poaceae and Cyperaceae families can cause serious diseases in some species. Perennial broad-leaved weeds can cause serious weed control problems. Most herbicides will not provide adequate control and mechanical methods are not fully effective due to regrowth from vegetative plants. Important perennial dicot weeds in the sugar beet field are: *Calystegia sepium* L. R. Br., *Cirsium arvense* L. Scop., *Convolvulus arvensis* L., *Mentha arvensis* L., *Polygonum amphibium* L., *Sonchus arvensis* L., *Rumex* sp., *Tussilago farfara* L. and *Equisetum arvense* L. on population dynamics of weeds.

HERBICIDES

Weeds were controlled by hand hoeing until the 1960s, when herbicides became available. Since then, new active ingredients such as metamitron, fenmedifam and etofumesate have been introduced. Trawling has become the main method of weed control due to its broad efficacy against mono- and dicotyledonous weeds (Wendt et al., 2017). High doses of active ingredients were common in pre-emergence and post-emergence treatments. Since the 1980s, for economic and ecological reasons, the main applications have been shifted to lower doses in post-emergence treatments. However, reduced doses require application at the cotyledon stage, the most sensitive developmental stage of weeds (Petersen, 2004).

Currently, the main herbicide strategy for weed control in sugar beet is the split application system, which combines different active ingredients at a reduced dosage. Due to its widespread use in practice, the combination of weed species commonly used in sugar beet cultivation has changed over time. *Brassica napus* L., *Chenopodium album* L., *Galium aparine* L., *Matricaria chamomilla* L., *Polygonum aviculare* L., *Aethusa cynapium* L. and other species have become increasingly difficult to control (Vasel et al., 2012). Unfavourable application conditions further limit this low flexibility.

Herbicide efficacy depends on numerous factors and their interactions, such as weather conditions before, during and after application (Blair and Martin, 1988), application technique (Prokop and Veverka, 2003; Skuterud et al., 1988), and herbicide sensitivity of target weeds (Heap, 2014). Temperature, light, rainfall and humidity affect photosynthesis, which drives the uptake, translocation and metabolism of active ingredients through stomatal regulation, transpiration and plant growth (Bakeri 1974; DiTomaso, 2002). These processes also depend on weed species, developmental stage, and leaf anatomy (cuticle, leaf wax, leaf hairiness) (Hock et al., 1995). These factors can reduce efficacy and a precise timing of application becomes necessary (May and Wilson, 2006; Cioni and Maines, 2010). The application of reduced dosages of herbicides is well recognised (Kudsk and Streibig, 2003). However, particularly unfavourable weather conditions may require higher doses for effective weed control (Lundkvist, 1997).

With the continuous use of chemical products, the risk of the establishment of weed species increases (Heap, 2014). A possible improvement is the use of herbicide-resistant crops. One approach is to introduce resistance genes into sugar beet and use the modification. Another option is to use genotypes that are not sensitive to ALS-inhibiting herbicides (Wendt et al., 2017). However, the sustainable use of soil in the use of these herbicides is a very important issue and should be emphasized.

The widespread use of herbicides to manage weeds in agricultural ecosystems has negative impacts on the environment and human health. Although the Conviso smart system offers less herbicide application, the residue left in the soil is an issue that needs to be emphasised. It is especially noteworthy that herbicides affect the ecosystem function and prevent the growth and development of non-target plants. The response of different plants to herbicide residues largely depends on the genetic make-up of these plants. Plants with high genetic similarity may show the same reaction to certain herbicides. It is an issue that needs to be investigated whether herbicide residues applied to plants that have high economic returns to the producer and are suitable for the cultivation of the ecology in which the producer is located cause a decrease in productivity in these plants.

SUGAR BEET VARIETIES TOLERANT TO ALS-INHIBITING HERBICIDES

The spectrum of herbicides used in sugar beet is rather narrow and further restrictions are expected shortly (Jursík and Holec, 2019). Most often ready mixtures of fenmedifam + desmedifam + etofumesate are used. Other herbicides were added to increase the efficacy on some problematic weeds and prolong the residual activity in the soil (Deveikyte and Seibutis, 2008). Triflusulfuron is often added to increase the activity on Brassica weeds, Amaranthus L., Matricaria L., Aethusa cynapium L. or Abutilon theophrasti Med. In some areas the most troublesome weed in sugar beet is weed beet, an annual, highly polymorphic hybrid of sugar beet and wild beet (*Beta vulgaris* subsp. *maritima* (L.) Arcang). Conventional herbicides cannot control this weed due to their selectivity to beetroot and the seeds have long-lasting longevity in the soil (Landová et al., 2010).

In the USA, farmers are using herbicide tolerant (HT) technologies for weed control in sugar beet, especially glyphosate tolerant. Currently 95% of the sugar beet growing area in the USA is glyphosate-tolerant sugar beet. The acetolactate synthase (ALS) inhibiting herbicide foramsulfuron (FSN) + thiencarbazone-methyl (TCM) tolerant sugar beet technology was developed and commercialised as Conviso® Smart, mainly for European farmers who cannot use technologies with genetically modified varieties. The herbicide containing these two active ingredients is highly selective against herbicide-tolerant sugar beet hybrids (Wendt et al., 2017) and has shown excellent efficacy on common sugar beet weeds (Gotze et al., 2018; Balgheim et al., 2018).

Sugar beet is normally sensitive to herbicides that inhibit ALS (Acetolactate synthase) (Wittenbach et al., 1994). Varieties have been developed that are not sensitive to the ALS inhibitors foramsulfuron (FSN) and thiencarbazone-methyl (TCM) of the complementary herbicide Conviso®. These varieties have been developed by classical breeding methods and do not show any phytotoxic effect for Conviso® herbicide. Conviso® is a broad-spectrum herbicide effective on difficult-to-control broad-leaved weed species, annual weeds and wild beet species. In beetroot with Conviso® herbicide-tolerant enzyme, the herbicide cannot bind to the enzyme thanks to this tolerant gene, but can still be used as a substrate. In this way, the sugar beet continues to grow. Since the enzymes of weeds do not undergo this change, the Conviso® herbicide can bind to the enzyme, which prevents the substrate from binding to the enzyme and causes the plant to die. In one study, FSN + TCM was reported to control all weeds tested very well when used in a split application with an oil adjuvant. Efficacy on C. album and A. theophrasti was found to be poor (less than 90%) when a single application of FSN + TCM was made under dry weather conditions. The main benefit of herbicide-tolerant technology is the excellent control of weed beetroot with residual activity over the whole growing season. The reproductive ability of most of the weeds tested after the split application of FSN+TCM was significantly lower compared to the reproductive ability of weeds in plots treated with conventional herbicides alone. Minimal weed infestation and absence of phytotoxicity were among the results of the study, which led to higher yields of herbicide-tolerant sugar beet (Jursik vd., 2020).

RESULTS

Acetolactate synthase (ALS)-inhibiting herbicides, used since the 1980s, are characterised by high efficacy at low total amounts of active ingredients. ALS-inhibiting herbicides may provide a new system for controlling weeds common in sugar beet cultivation. However, the amount of residues that this group of herbicides may leave in the soil is an issue that needs to be investigated. As a result of the interviews with the producers, it was concluded that there was a decrease in yield in some crop groups (such as wheat, maize, sunflower, and beans) included in the crop rotation after the use of varieties with ALS inhibitor. Further studies on this subject are needed for sustainable utilisation of soils. However, in the case of intensive use of ALS inhibitors as the sole mechanism of action, the possibility of increased development of ALS-resistant weeds should be taken into consideration.

REFERENCES

Baker, E.A. (1974). The influence of environment on leaf wax development in Brassica oleracea var. gemmifera. New Phytologist, 73(5), 955-966.

Balgheim, N., Wegener, M., & Mumme, H. (2018). CONVISO Smart-first experiences with the new sugar beet production system. Julius-Kühn-Archiv, (458), 510-515.

Blair, A.M., & Martin, T.D. (1988). A review of the activity, fate and mode of action of sulfonylurea herbicides. Pesticide Science, 22(3), 195-219.

Bosch, J. (1987). Der Einfluß einiger dominanter Ackerunkräuter auf Nutz-und Schadarthropoden in einem Zuckerrübenfeld/The influence of some dominating weeds on beneficial arthropods and pests in a sugar beet field. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection, 398-408.

Bräutigam, H., & Schäufele, W.R. (1994). Einfluss der Stickstoffdüngung auf die Verunkrautung in Zuckerrüben. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, Sonderheft, 14, 467-476.

Cioni, F., & Maines, G. (2010). Weed control in sugarbeet. Sugar Tech, 12(3-4), 243-255.

Deveikyte, I., & Seibutis, V. (2008). The influence of postemergence herbicides combinations on broad-leaved weeds in sugar beet. Zemdirbyste-Agriculture, 95(3), 43-49.

DiTomaso, J.M. (2005). Understanding Herbicides: What They are and How They Work. University of California. Division of Agriculture and Natural Resources, Davis, USA.

Dubois, D., Häni, A., Ammon, H.U., & Keller, S. (1993). Einfluss der Unkrautbekämpfung auf Ertrag und Virusbefall von Zuckerrüben. Landwirtschaft Schweiz, 6(3), 133-138.

FAO, 2023. https://www.fao.org/faostat/en/#data/QCL. Erişim Tarihi: 02.12.2023.

Gleißl, W., Bachthaler, G., & Hoffmann, G.M. (1989). Untersuchungen zur Wirtseignung verschiedener geographischer Herkünfte von Ackerunkräutern gegenüber dem Rübenzystenälchen Heterodera schachtii Schmidt. Weed Research, 29(3), 221-228.

Gleissl, W., Bachthaler, G., & Hoffmann, G.M. (1989). Investigations on the suitability of weeds of various geographic origins as hosts of the sugar-beet nematode Heterodera schachtii Schmidt. Weed Research, UK, 29 (3), 221-228.

Gotze, P., Kenter, C., Wendt, M.J., & Ladewig, E. (2018). Survey of efficacy trials for Conviso® One in sugar beet. Julius-Kühn-Archiv, (458), 498-500.

Heap, I. (2014). Global perspective of herbicide-resistant weeds. Pest management science, 70(9), 1306-1315.

Hock, B., Fedtke, C., & Schmidt, R.R. (1995). Herbizide: Entwicklung, Anwendung, Wirkungen, Nebenwirkungen. Stuttgart: Thieme.

Jursik, M., & Holec, J. (2019). Future of weed management in sugar beet in Central Europe. Listy cukrovarnicke a reparske, 135(5-6), 180-186.

Jursík, M., Soukup, J., & Kolářová, M. (2020). Sugar beet varieties tolerant to ALS-inhibiting herbicides: A novel tool in weed management. Crop Protection, 137, 105294.

Kropff, M.J., & Spitters, C.J.T. (1992). An eco-physiological model for interspecific competition, applied to the influence of Chenopodium album L. on sugar beet. I. Model description and parameterization. Weed Research, 32(6), 437-450.

Kudsk, P., & Streibig, J. C. (2003). Herbicides–a two-edged sword. Weed Research, 43(2), 90-102.

Landová, M., Hamouzová, K., Soukup, J., Jursík, M., Holec, J., & Squire, G.R. (2010). Population density and soil seed bank of weed beet as influenced by crop sequence and soil tillage. Plant Soil Environ, 56(11), 541-549.

Lotz, L.A.P., Groeneveld, R.M.W., & De Groot, N.A.M.A. (1991). Potential for reducing herbicide inputs in sugar beet by selecting early closing cultivars. In Brighton Crop Protection Conference-Weeds.

Lundkvist, A. (1997). Influence of weather on the efficacy of dichlorprop-P/MCPA and trihenuron-methyl. Weed research, 37(5), 361-371.

May, M.J., & Wilson, R.G. (2006). Weeds and weed control. Sugar beet, 359-386.

Mittler, S., Petersen, J., & Koch, H.J. (2002). Bekämpfungsschwellen bei der Unkrautbekämpfung in Zuckerrüben. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz. Sonderheft, 18, 499-509.

Petersen, J. (2004). A Review on Weed Control in Sugarbeet: From tolerance zero to period threshold. Weed biology and management, 467-483.

Petersen, J. (2004). A Review on Weed Control in Sugarbeet: From tolerance zero to period threshold. Weed biology and management, 467-483.

Prokop, M., & Veverka, K. (2003). Influence of droplet spectra on the efficiency of contact and systemic herbicides. Plant Soil and Environment, 49(2), 75-80.

Schaufele, W.R. (1986). Einfluss hochwachsenden Unkrautes (Chenopodium album) auf Entwicklung und Ertrag der Zucherrübe. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz (1970), 93(2), 125-135.

Schäufele, W.R. (1991). Einfluß niedrigwachsender Unkräuter zwischen den Reihen auf den Ertrag von Zuckerrüben. Gesunde Pflanzen, 43(6), 175-179.

Schlosser, E. (1989). Rizomania. XI: Weeds as alternate hosts for virus and vector?. Mededelingen van de Faculteit landbouwwetenschappen. Rijksuniversiteit Gent, 54(2b), 479-484.

Scott, R.K., & Moisey, F.R. (1972). effect of weeds on the sugar beet crop. In Brit Weed Contr Conf Proc.

Skuterud, R., Nordby, A., & Tyldum, A. (1988). Effects of application methods, spray volumes, pressures and herbicide rates on weed control in spring cereals. Crop protection, 7(5), 303-308.

Vasel, E.H., Ladewig, E., & Märländer, B. (2012). Weed composition and herbicide use strategies in sugar beet cultivation in Germany. Journal für Kulturpflanzen, 64(4), 112-125.

Wendt, M. J., Kenter, C., Ladewig, E., Wegener, M., & Märländer, B. (2017). Duration of soil activity of foramsulfuron plus thiencarbazone-methyl applied to weed species typical of sugar beet cultivation. Weed Technology, 31(2), 291-300.

Wittenbach, V., Koeppe, M.K., Lichtner, F.T., Zimmerman, W. T., & Reiser, R. W. (1994). Basis of selectivity of triflusulfuron methyl in sugar beets (*Beta vulgaris*). Pesticide biochemistry and physiology, 49(1), 72-81.

Zimdahl, R.L., & Fertig, S.N. (1967). Influence of weed competition on sugar beets. Weeds, 15(4), 336-339.

A Research on O-Type Plants in Sugar Beet

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ABSTRACT: CMS is an important character in agriculture because it provides ideal seed parents for large-scale hybrid seed production. Sugar beet [*Beta vulgaris* ssp. *vulgaris* (Sugar Beet Group)] is representative of crops where CMS (Cytoplasmic Male Sterility) has been used to develop commercial varieties. Sugar beet hybrid seed production uses CMS plants discovered by Owen. Since CMS plants cannot self-pollinate, their reproduction requires a specific pollen parental line that has the same nuclear genotype as the CMS line (i.e. lacks any dominant *Rf*), but the pollen parental line is male fertile due to normal mitochondria. The determination of the sustainer genotypes is one of the most important tasks in sugar beet breeding. This research was carried out to identify O-type plants in sugar beet by traditional breeding methods and marker assisted selection.

Keywords: Sugar beet, O-type, Cytoplasmic Male Sterility, Plant breeding, Hybrid

INTRODUCTION

Sugars, which are an important food source for humans and animals, are natural substances produced by plants. Sugars are composed of C, H, O elements in a 1:2:1 ratio (Kadıoğlu, 2016). Sucrose (sugar), which is economically important, is a soluble disaccharide formed by the combination of two monosaccharides known as glucose and fructose. Some plants such as sugar beet and sugar cane are plants that store large amounts of sucrose in their vacuoles (Özen and Onay, 2013). Today, two plants have a very important place in the production of crystalline sugar. Approximately 79% of the 173 million tonnes of sugar produced in the world today is obtained from sugar cane and 21% from sugar beet (Kolçak and Sarıhan, 2022). In our country, sugar beet is used as a source of crystal sugar in terms of ecological suitability.

One of the most important factors affecting yield and quality in crop production is variety selection. Almost all of the sugar beet varieties in the world are hybrid varieties. This situation is also valid for our country. Cytoplasmic male sterility (CMS) is used in hybrid sugar beet breeding. In CMS plants, when a sterile mother is crossed with a fertile siring father, all of the offspring are sterile. Plants that have normal cytoplasm and carry recessive genes that ensure sterility in the nucleus, have the ability to produce pollen and can give fertile offspring to themselves, but all of the offspring of the pollinated CMS plant are sterile are called maintainer or sustainer plants. These plants maintain sterility in CMS. Since such plants were first identified in sugar beet by Dr F.V. OWEN in 1945, they were named Owen type or O type (Owen, 1945).

SUGAR BEET FLOWER STRUCTURE AND FERTILISATION BIOLOGY

Sugar beet needs to be exposed to an average temperature of 5-10 ℃ for about 40 days for flowering (Sparkes, 2003). During the flowering stage, the plant uses the assimilates produced by photosynthesis not to develop the root but to form flowers and then seeds. The flowering period lasts an average of 28 days. The colour of the flowers varies from green to white. Sugar beet is a diploid (2n=18 chromosomes) plant that is foreign fertilised (allogom). The flower has a hermaphrodite flower structure with 5 male and 3 female organs together.

It is very important to know the flower conditions in sugar beet and to make selections accordingly. The anthers of sterile plants are white, whitish-yellow, whitish-green, small and deformed. Semi-male sterile anthers are larger than fully sterile anthers but smaller than normal anthers, the anthers are usually unopened, white or yellow in colour, usually do not carry pollen, and if pollen is present, it is attached to the anthers. Some semi-male sterile anthers are more swollen and have more pollen, their anther size is close to normal fertile anthers, they are in different shades of yellow. The anthers of fertile plants have different shades of yellow colour, the anthers are completely filled with pollen and are swollen.

O-TYPE PLANTS IN SUGAR BEET

In 1945, Owen proposed a CMS model in sugar beet after the discovery of sterile male plants in the cultivar "US1". Cytoplasmic male sterility (CMS) is one of the characteristics of sugar beet resulting from interactions between the nucleus and cytoplasm (Arakawa et al., 2018). In terms of this model, the double recessive gene xxzz and CMS-associated factor S work together to trigger CMS (Owen 1945). The *Rf*1 allele on chromosome III is thought to correspond to the Owen-type CMS gene X, while the allele *Rf*2 on chromosome IV is thought to correspond to the Owen-type CMS gene Z (Taguchi et al. 2014). A continuum line of the sterile genotype [S]xxzz, also known as the O line, represents the [N]xxzz genotype when the normal cytoplasmic factor N and the double recessive nuclear gene xxzz are both present. According to Moritani et al. (2013) and Matsuhira et al. (2012), all plants with cytoplasmic type N show male fertility, regardless of the allelic genotype of the *Rf* locus. Owen suggested that completely male sterile plants have the genotype [S]xxzz together with other combinations of genotypes ([S]XXZZ, [S]XXZz, [S]XXZz, [S]XXzz, [S]XxZZ, [S]XxZz, [S]Xxzz, [S]Xxzz, [S]xxZZ and [S]xxZz) often show varying degrees of pollen fertility (Bosemark, 2006).

Propagation of CMS plants requires an equivalent pollen-efficient maintainer with the genotype [N]xxzz. The frequency of the maintainer genotype in sugar beet is usually less than 5% (Bosemark, 1993). It is difficult to distinguish between the maintenance and repairing lineages of sugar beet by phenotypic observation because the male-fertile pollen between the two genotypes is identical. These reasons make non-repairing genotypes highly valuable. In conventional breeding, sustainer pollinated lines are crossed with sterile lines and this requires several years for the identification of sustainer lines (Schnable and Wise 1998). Since the traditional method of testing crosses with CMS has the disadvantages of being both time-consuming and expensive (Norouzi and Rajabi 2018), molecular marker-assisted selection (MAS) methods can be used to precisely identify associated cytoplasmic and nuclear factors.

DNA marker-assisted selection (MAS) can significantly accelerate the identification of non-repaired genotypes of beetroot. The identification of male sterile cytoplasm (S) and normal cytoplasm (N) using the locus that is the most polymorphic among a variable number of successively repeated mitochondrial loci is well established (Cheng et al. 2010; Nishizawa et al. 2000). The composition of the *Rf*1 and *Rf*2 fertility genes is analysed using two different (*Rf*1: s17; *Rf*2; o7) molecular markers and their amplification bands help to distinguish sterile and sustainer lineages of sugar beet (Taguchi et al., 2014). Elucidation of the genotypic composition of the maintainer lineages and the roles of *Rf*1 and *Rf*2 genes during the fertility restoration period may facilitate the validation of maintainer and sterile lineages for future breeding studies.

CONCLUSIONS

The selection of infertile and pollinator lines is particularly important in sugar beet breeding because sugar beet is an ever-flowering crop and almost all sugar beet species are hybrids. The establishment of sugar beet hybrid seed is highly dependent on this single source of CMS. The choice of CMS lines and pollinator lines therefore has a significant impact on the production process. The genotypes of the maintenance lines typically constitute only 3-5% of the pollinator line population, which poses greater challenges in the selection of maintenance lines. All studies in biotechnological methods and classical plant breeding techniques are of great importance for sugar beet hybrid seed studies.

REFERENCES

Arakawa, T., Uchiyama, D., Ohgami, T., Ohgami, R., Murata, T., Honma, Y., ... & Kubo, T. (2018). A fertility-restoring genotype of beet (*Beta vulgaris* L.) is composed of a weak restorer-of-fertility gene and a modifier gene tightly linked to the *Rf*1 locus. PLoS One, 13(6), e0198409.

Bosemark, N. O. (2006). Genetics and breeding, Chapter 4.

Bosemark, N.O. (1993). Genetics and breeding. In The sugar beet crop (pp. 67-119). Dordrecht: Springer Netherlands.

Cheng, D., Yoshida, Y., Kitazaki, K., Negoro, S., Takahashi, H., Xu, D., ... & Kubo, T. (2011). Mitochondrial genome diversity in *Beta vulgaris* L. ssp. *vulgaris* (Leaf and Garden Beet Groups) and its implications concerning the dissemination of the crop. Genetic resources and crop evolution, 58, 553-560.

Kadıoğlu, A. (2016). Bitki Fizyolojisi (6.Baskı), Gündüz Ofset Matbaacılık ve Yayıncılık, Trabzon.

Kolçak, M., Sarıhan, E.O. (2022). Kıyı Pancarı (*Beta vulgaris* subsp. *maritima* (L.) Arcang) Türünün In-vitro Şartlarda Mikro Çoğaltımı. Uşak Üniversitesi Fen ve Doğa Bilimleri Dergisi, 6(2), 94-112.

Matsuhira, H., Kagami, H., Kurata, M., Kitazaki, K., Matsunaga, M., Hamaguchi, Y., ... & Kubo, T. (2012). Unusual and typical features of a novel restorer-of-fertility gene of sugar beet (*Beta vulgaris* L.). Genetics, 192(4), 1347-1358.

Moritani, M., Taguchi, K., Kitazaki, K., Matsuhira, H., Katsuyama, T., Mikami, T., Kubo, T. (2013). Identification of the predominant nonrestoring allele for Owen-type cytoplasmic male sterility in sugar beet (*Beta vulgaris* L.): development of molecular markers for the maintainer genotype. Molecular Breeding, 32, 91-100.

Nishizawa, S., Kubo, T., Mikami, T. (2000). Variable number of tandem repeat loci in the mitochondrial genomes of beets. Current Genetics, 37, 34-38.

Norouzi, P., Rajabi, A. (2018). Identification and confirmation of new molecular markers linked to the fertility restorer genes in sugar beet. Journal of Crop Science and Biotechnology, 21, 335-341.

Owen, F.V. (1945). Cytoplasmically inherited male-sterility in sugar beets. Jour. Agr. Res., 71, 423-440.

Özen, H.Ç., Onay, A. (2013). Bitki Fizyolojisi (2. Baskı), Hazar Reklam Matbaacılık ve Yayıncılık, Ankara.

Pillen, K., Sleinrücken, G., Herrmann, R. G., Jung, C. (1993). An extended linkage map of sugar beet (Beta vulgaris L.) including nine putative lethal genes and the restorer gene X. Plant breeding, 111(4), 265-272.

Schnable, P.S., Wise, R.P. (1998). The molecular basis of cytoplasmic male sterility and fertility restoration. Trends in plant science, 3(5), 175-180.

Sparkes, D. (2003) Growth and development, field crops. In Encyclopedia of Applied Plant Sciences (pp 595–600). Elsevier Academic Press, Amsterdam.

Taguchi, K., Hiyama, H., Yui-Kurino, R., Muramatsu, A., Mikami, T., Kubo, T. (2014). Hybrid breeding skewed the allelic frequencies of molecular variants derived from the restorer of fertility 1 locus for cytoplasmic male sterility in sugar beet (*Beta vulgaris* L.). Crop Science, 54(4), 1407-1412.

Taguchi, K., Nakatsuka, K., Takahashi, H., Okazaki, K., Yoshida, T. (2006). Relationship between the coefficient of parentage and sugar yield in sugar beet [*Beta vulgaris*] f1 hybrid. Breeding Research (Japan), 8(4).

Investigation of the Use of Probiotics as Heavy Metal Regulators in Aquaculture

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ABSTRACT: The decreasing habitability of the natural life population is one of the most serious problems of today. Many factors such as global warming, industrialization, use of chemicals in agriculture and anthropogenic activities disproportionately affect the functioning of the ecosystem. The release of hazardous heavy metals into nature as a result of industrial developments poses a major threat to environmental safety due to their high persistence in nature and widespread exposure. The disruption of global balance causes serious concerns about the health of living things and causes mass deaths in fish populations. These mass deaths cause food pollution, economic losses, and a decrease in food resources. Microorganisms have become increasingly important as a powerful alternative tool for the removal of heavy metals from aquaculture media. The use of probiotics among microorganisms is an efficient, cost-effective and environmentally friendly strategy that has attracted great attention in combating heavy metal pollution. The fact that they do not pose a threat to the ecosystem, reduce the damage caused by heavy metals, play a role in rebalancing the microbiota and improve the immune system makes the use of probiotic bacteria in heavy metal biosorption in aquaculture media important. In this review, some studies on the regulation of heavy metals in the aquatic ecosystem using probiotic bacteria were discussed and evaluated.

Keywords: Heavy metal, Probiotic bacteria, Aqua culture, Fish, Biosorption

INTRODUCTION

Aquaculture, the cultivation of aquatic organisms such as fish, shellfish, and algae, is an important sector of the global economy. However, human activities including mining, global warming, industrialization, the discharge of industrial waste, the use of pesticides, fertilizers, and herbicides, as well as heavy metal contamination, pose many problems for aquatic environments. (Petitjean et al., 2019). Disruption of ecological balance can lead to mass mortality. Ecological imbalances, public health hazards, economic losses, nutrient contamination, and a decrease in food availability are all caused by this mass mortality (Naylor et al., 2021). For example, the Food and Agriculture Organization of the United Nations (FAO) has documented that over 30% of the world's fish stocks are declining and another 60% are completely depleted (Coates et al., 2023).

Heavy metal-contaminated wastewater is a major contributing factor to environmental pollution and the disruption of the natural equilibrium. These are the top industrial companies in this regard. These industries process a variety of heavy metals, and the metal ions in their wastewater include mercury, copper, cobalt, iron, lead, silver, zinc, arsenic, and chromium

(Sağlam and Cihangir 1995). Heavy metals such as lead, mercury, and cadmium can harm aquatic life, including damage to the nervous system, impaired growth and development, and reduced survival rates (Emenike et al., 2021).

The use of probiotics as an efficient, cost-effective, and environmentally friendly strategy, which is a powerful alternative to mitigate the adverse consequences of massive accumulation of heavy metals, has recently gained much attention (Bhatnagar and Kumari, 2013; Arya et al., 2015). The use of probiotics in aquaculture is of great interest due to their potential to increase the health and productivity of aquatic organisms without posing a threat to the ecosystem. Probiotics are microorganisms that are beneficial to the health of the host organism when administered in sufficient amounts. They are known to improve gut health, boost immunity, modulate gut microbiota, aid nutrition, and enhance the quality of water (Hasan and Banerjee, 2020).

The term "gut microbiota" refers to microbes or microorganisms found in different areas of the gastrointestinal system; this group has been linked to the brain-gut axis (Butt and Volkoff, 2019). Under normal conditions, the populations of gut bacteria are crucial for preserving homeostasis as well as growth, digestion, reproduction, and defense (Butt and Volkoff, 2019). Exposure to heavy metals causes intestinal dysbiosis. Dysbiosis is a decrease in beneficial microbiota or alteration of all gut bacteria. Supplements such as prebiotics, probiotics, and postbiotics in aquatic ecosystems are used to reorganize and improve the altered gut microbiota. Among these, probiotics and prebiotics are among the most important agents modulating the gut flora (Kakahel et al., 2023a). Probiotics used in aquaculture can be broadly categorized into two groups: those that grow in the aquatic environment, consuming all available nutrients and eradicating pathogenic bacteria, and those that are taken orally with food to enhance the beneficial microbial flora associated with the gut (Hasan and Banerjee, 2020; El-Saadony et. al. 2021).

The literature on the effects of newly discovered heavy metal contamination on the bacterial composition and structure of fish guts is presented in this study from a variety of angles. The potential of various probiotics to regulate heavy metal concentrations in aquaculture systems and the potential of using probiotics in heavy metal detoxification are investigated and discussed.

Heavy Metal Pollution in Water Ecosystems

The term 'heavy metal' describes metalloids or metallic elements that have toxic effects on humans and other living organisms (Singh et al., 2011). Heavy metal pollution is one of the most serious environmental and human health risks linked to industrial progress (Ammen et. al. 2020). It is known that a number of industrial processes, such as those in the leather tanning, chrome plating, battery manufacturing, glass, agricultural, home waste, and pharmaceutical industries, are significant sources of heavy metals that discharge hazardous metal ions into the atmosphere (Wuana et al., 2011; Singh et al., 2021). The release of hazardous heavy metals poses a devastating threat even at low concentrations, resulting in serious concerns worldwide (Monachese et al. 2012; Kinoshita et al., 2013). Heavy metals are the major source of toxicity in aquatic ecosystems, both from natural sources and anthropogenic sources (Evariste et al., 2019). Aquatic ecosystems contain metals in water, sediments, or aquatic organisms in dissolved, particulate, or chelated/combined forms (Al Naggar et al., 2018; Amankwaa et al., 2021).

Research has shown that heavy metals like Zn, Pb, Mn, Fe, Ni, and Cr are present in the Pacific and Atlantic Oceans in both dissolved and particulate form (Benson et al., 2017; Sujitha et al., 2020). Paul (2017) detected high concentrations of heavy metals (such as Cr, Cd, Cu, Zn and Pb) in freshwater bodies such as rivers and lakes in their study. Some studies produced in Turkey have also reported the abundance of heavy metals in water and sediments (Topaldemir et al., 2023 ; Yüksel et al., 2022). Through atmospheric deposition, erosion, runoff, and precipitation, these metals are introduced into water bodies (Yang et al., 2020). Long-term accumulation of heavy metals in sediments is caused by the steady state of water, longer retention times, and deeper water columns (Kakade et al., 2023). Each trophic level may have a different heavy metal accumulation rate. Benthic fish species accumulated higher concentrations of Pb, Cd, and Hg than pelagic (herbivorous) fish and shrimp, according to a Densu River study (Amankwaa et al., 2021). Fish in the Red Sea accumulated less zinc, copper, lead, mercury, and zinc ions than crustaceans and squid (Younis et al., 2015).

Heavy Metal Exposure and Microbiota

According to Rajeshkumar et al. (2017), toxicity happens when heavy metals enter the body through permeable membranes (like gills) and are absorbed through food, water, and ion exchange in addition to skin adsorption. Once within the body, various organs experience adsorption and absorption, which leads to bioaccumulation (Rajeshkumar and Li, 2018). Toxic effects from heavy metal accumulation include genetic mutations, oxidative stress, inflammation, and histological damage. Furthermore, by altering the makeup and functionality of the gut microbiota, this harmful effect causes microbial alterations (Walther and Siebe, 2011; Amoatey and Baawain, 2019; Qian et al., 2020).

Microbiota refers to the diverse community of microorganisms, including bacteria, fungi and viruses, living in the gastrointestinal tract and on other surfaces of aquatic organisms (Bist et al., 2022). Disruption of the microbiota in aquaculture causes environmental impacts such as reduced growth and productivity, impaired immune system and disruption of the balance in the aquatic ecosystem (Emenike et al., 2021). Changes in the composition and function of the gut microbiota can have effects on overall gut health and host metabolism. Heavy metal exposure can lead to dysbiosis, an imbalance in the gut microbiota (Assefa and Köhler 2020). Dysbiosis can disrupt the normal functioning of the gut, including nutrient absorption, immune response and gut barrier integrity. This disruption causes a variety of health problems, including inflammation, metabolic disorders and even cognitive dysfunction (Chiu et al., 2020).

Various mitigation strategies can be used to minimize heavy metal exposure's negative effects on aquaculture microbiota. Various technologies are available for the regulation of heavy metals in water. For the treatment of heavy metals, techniques like filtration, reverse osmosis, ion exchange, precipitation, and physical adsorption are commonly employed (Khulbe and Matsuura, 2018). These techniques are expensive and produce secondary chemicals (Anirudhan and Sreekumari, 1998). Considering the disadvantages of these methods, probiotics have been proposed as an alternative and effective practice to replace existing conventional physical and chemical processes for successfully removing heavy metals from water (Wołowiec et al., 2019; Duan et al., 2020).

Probiotics in Aquaculture and Probiotic Use in Heavy Metal Regulation

There is a bidirectional relationship between heavy metals and the gut microbiota, which serves as the first line of defense against the toxic effects of heavy metals. The gut microbiota's composition and metabolic profile can change as a result of exposure to heavy metals. According to Duan et al. (2020), the gut microbiota functions as a physical barrier to prevent the absorption of heavy metals and influences their uptake and metabolism by modifying the pH, oxidative balance, and concentrations of detoxification enzymes and proteins involved in heavy metal metabolism. (Duan and others, 2020).

Probiotics are live microorganisms that provide health benefits to the host and play an essential role in aquaculture (El-Saadony et al., 2021). Modifying the microbiome through the administration of probiotics for animals is a fairly new concept and these probiotics are of high importance for aquatic organisms (Ehsanniar et al., 2022). The removal of heavy metals from an organism using probiotics has been successfully applied in recent years and has recently been termed "gut healing" (Liu et al., 2019). Probiotics positively affect fish health by reducing the side effects of pollutants in fish (Kirillova et al., 2017). Probiotics, beneficial microorganisms, can be applied to aquaculture species to restore and maintain a healthy microbiota. Probiotics have many beneficial properties that may be useful for heavy metal detoxification and bioremediation, including strong adhesion to the intestinal mucosa, high tolerance to gastrointestinal fluid, robust suppression of pathogen growth, and potent antioxidant and immunoregulatory capacities (Zhai et al., 2013; Giri et al., 2018; Wang et al., 2020).

According to Munoz-Atienza et al. (2013), microorganisms meant for use as probiotics in aquaculture should be safe for humans, the environment, and aquatic hosts. According to Ibrahem (2015), the different probiotic groups utilized in aquaculture can be divided into living bacteria that react both Gram-positive and Gram-negative, unicellular algae, bacteriophages, and yeasts. Probiotic strains that are most frequently used are those that are bacterial, such as *Lactobacillus* sp., *Lactococcus* sp., *Bifidobacterium* sp., *Micrococcus* sp., *Microbacterium* sp., *Bacillus* sp., *Pseudomonas* sp., *Streptococcus* sp., *Arthrobacters* sp., *Phaeobacter* sp., *Streptomyces* sp., *Enterococcus* sp., etc., and yeast probiotics. Tetrasehnis suecica, *Spirulinaplatensis*, microalgae probiotics, *Saccharomyces cerevisiae*, *Debrayomyces hansenii*, yeast cell wall, etc. (Rohani et al., 2022).

The most commonly used probiotics are *Lactobacillus* and *Bifidobacterium* species. In addition, the yeast Saccharomyces cerevisiae and some strains of *E. coli* and *Bacillus* species are also used (Guarner et al., 2008). Specifically, probiotics have been reported to reduce the absorption of heavy metals in the intestinal tract by increasing heavy metal uptake in the intestine, detoxifying heavy metals in the intestine, altering the expression of metal transporter proteins and maintaining intestinal barrier function (Giri et al., 2018; Duan et al., 2020).

In one study, *Lactobacillus rhamnosus* GR-1 was found to immobilize cadmium and lead, reducing their translocation across the intestinal epithelium. This suggests that some probiotic strains may have the ability to bind and remove heavy metals from the gut, potentially reducing their harmful effects (Bist and Choudhardy 2022). Studies have reported that the use of *Lactobacillus plantarum* CCFM8610 (Zhai et al., 2016) against Cd-exposed Nile tilapia (*Oreochromis niloticus*), *Lactobacillus acidophilus* (Abu-Baraka et al., 2017) against Cd-exposed Nile tilapia, and *Lactobacillus reuteri* P16 (Giri et al., 2018) strains against Pb-exposed fish reversed heavy metal-induced changes. It was also reported to significantly improve the growth performance of fish and prevent mortality of fish exposed to heavy metals, reduce heavy metal accumulation, alleviate oxidative stress in tissues, and reverse changes in hemato-biochemical parameters in the blood of fish (Zhai et al., 2016; Giri et al., 2018). Heavy metal biosorption by microorganisms such as *Lactobacillus* strain has been reported to involve ion exchange, complexation, precipitation, reduction and chelation, which will be greatly influenced by pH and other metal ions (Lin et al., 2019; Duan et al., 2020).

Yu et al. (2021) investigated the protection of *L. plantarum* CCFM639 against waterborne Al exposure in tilapia. It was reported that *L. plantarum* CCFM639 strain effectively reduced mortality and Al accumulation in tissues and improved growth performance in Al-exposed fish. It was also reported that Al-induced changes in hemato-biochemical parameters and hepatic oxidative stress and histopathology were alleviated by probiotic bacteria (Yu et al., 2017). *L. plantarum* CCFM8610 was reported to effectively bind dissociated aqueous Cd ions in vitro and sequester food-derived Cd in vivo, thus preventing the absorption of this toxic metal through the gills and intestines of fish (Zhai et al., 2013). In another study, it was reported that the use of *Lactococcus lactis* as a dietary additive to Hg-exposed black sea bass reduced Hg toxicity and can be used as an immune stimulant (El-Bouhy et al., 2021).

The effects of feeding probiotic Bacillus cereus, which is frequently used in aquaculture, to Carassius auratus gibelio after exposure to Cd were assessed in terms of bioaccumulation, oxidative stress, and gut microbiota. It has been observed that dietary supplementation with B. cereus can undo changes in the gut microbiota's composition brought on by exposure to mercury. (Wang et al., 2020). The probiotic *Bacillus subtilis* was reported to be able to adsorb lead in the intestines and gills, thereby reducing the concentration of lead in the organ (Yin et al., 2018). Because of their high peptidoglycan and teichoic acid content in their cell walls, *Bacillus* species have been shown to have high adsorption capacities, making them useful for the adsorption of heavy metals. This demonstrates that the use of B. cereus to adsorb heavy metal decreased the amounts of Cd in *C. gibelio's* organs exposed to cadmium. It is further corroborated by the observation that in this investigation, the interaction between *B. cereus* and cadmium significantly affected the accumulation of cadmium in *Carassius gibelio* (Wang et al., 2020).

MATERIAL AND METHOD

Methodology and data collection

We employed a methodical search approach using resources like ScienceDirect, ResearchGate, Google Scholar, and Web of Science to locate pertinent research. Numerous subjects were covered by the search terms, such as probiotics, aquatic organisms, aquaculture, bacteria, microbiota, dysbiosis, heavy metals, and biosorbent. Once the initial searches were completed, research articles were reviewed to confirm which ones were relevant to the study. The regulation of heavy metals by probiotics in aquaculture and the studies conducted were reviewed and selected for this study.

RESULTS AND CONCLUSION

Probiotics are acknowledged as new functional agents that have the ability to affect any aquatic organism's gut microbiota. Probiotics have the potential to improve aquatic environments, safeguard biodiversity, increase the overall sustainability of our water resources, and be in line with green technology principles when incorporated into environmental management practices. Probiotic use provides an all-encompassing strategy that not only addresses the problem of heavy metal toxicity but also enhances the general health of aquatic environments.

In conclusion, probiotics are frequently used in aquaculture to remove heavy metals from the environment, but more study is required in this field.

Statement of Conflict of Interest

The authors declare no competing interests.

Authors' Contributions

MŞK and FİA: Investigation, resources, writing – review & editing

REFERENCES

Abdel-Megeed, R. M. (2021). Probiotics: a promising generation of heavy metal detoxification. *Biological trace element research*, *199*(6), 2406-2413.

Abu-Braka, A. Z., Zaki, M. S., Abbas, H. H., Ismail, N. E. D. A., Khalil, R., Tanekhy, M., & Saad, T. (2017). Filed studies on some probiotics to minimize hazard effects of prevailing heavy metals contamination for improving immunity and growth performance of Oreochromis niloticus. *Electronic Physician*, *9*(4), 4138.

Al Naggar, Y., Khalil, M. S., & Ghorab, M. A. (2018). Environmental pollution by heavy metals in the aquatic ecosystems of Egypt. *Open Acc. J. Toxicol*, *3*, 555603.

Amankwaa, G., Lu, Y., Liu, T., Wang, N., Luan, Y., Cao, Y., ... & Gyimah, E. (2021). Heavy metals concentration profile of an aquatic environment and health implications of human exposure to fish and prawn species from an urban river (Densu). *Iranian Journal of Fisheries Sciences*, *20*(2), 529-546.

Ameen, F. A., Hamdan, A. M., & El-Naggar, M. Y. (2020). Assessment of the heavy metal bioremediation efficiency of the novel marine lactic acid bacterium, Lactobacillus plantarum MF042018. Scientific reports, 10(1), 314.

Amoatey, P., & Baawain, M. S. (2019). Effects of pollution on freshwater aquatic organisms. *Water Environment Research*, *91*(10), 1272-1287.

Anirudhan T.S., Sreekumari S.S. Adsorptive removal of heavy metal ions from industrial effluents usingactivated carbon derived from waste coconut buttons. J. Environ. Sci. 2011;23:1989–1998. doi: 10.1016/S1001-0742(10)60515-3.

Aryal, M. & Liakopoulou-Kyriakides, M. Ağır metallerin bakteriyel biyokütle ile biyolojik olarak uzaklaştırılması. *Çevre. Monit. Değer biçmek.* **187** , 4173 (2015).

Assefa, S., & Köhler, G. (2020). Intestinal microbiome and metal toxicity. *Current opinion in toxicology*, *19*, 21-27.

Bhatnagar, S. & Kumari, R. Bioremediation: A sustainable tool for environmental management – A review. *Ann. Rev. Res. Biol.* **3**, 974–993 (2013).

Bist, P., & Choudhary, S. (2022). Impact of heavy metal toxicity on the gut microbiota and its relationship with metabolites and future probiotics strategy: a review. *Biological trace element research*, *200*(12), 5328-5350.

Butt, R. L., & Volkoff, H. (2019). Gut microbiota and energy homeostasis in fish. *Frontiers in endocrinology*, *10*, 9.

Chiu, K., Warner, G., Nowak, R. A., Flaws, J. A., & Mei, W. (2020). The impact of environmental chemicals on the gut microbiome. *Toxicological Sciences*, *176*(2), 253-284.

Diwan, A. D., Harke, S. N., & Panche, A. N. (2023). Host-microbiome interaction in fish and shellfish: An overview. *Fish and Shellfish Immunology Reports*, 100091.

Duan, H., Yu, L., Tian, F., Zhai, Q., Fan, L., & Chen, W. (2020). Gut microbiota: A target for heavy metal toxicity and a probiotic protective strategy. Science of the Total Environment, 742, 140429.

Egerton, S., Culloty, S., Whooley, J., Stanton, C., & Ross, R. P. (2018). The gut microbiota of marine fish. *Frontiers in microbiology*, *9*, 873.

Ehsannia, S., Ahari, H., Kakoolaki, S., Anvar, S. A., & Yousefi, S. (2022). Effects of probiotics on Zebrafish model infected with Aeromonas hydrophila: spatial distribution, antimicrobial, and histopathological investigation. BMC microbiology, 22(1), 167.

El-Bouhy, Z. M., Reda, R. M., Mahboub, H. H., & Gomaa, F. N. (2021). Chelation of mercury intoxication and testing different protective aspects of Lactococcus lactis probiotic in African catfish. Aquaculture Research, 52(8), 3815-3828.

El-Saadony, M. T., Alagawany, M., Patra, A. K., Kar, I., Tiwari, R., Dawood, M. A., ... & Abdel-Latif, H. M. (2021). The functionality of probiotics in aquaculture: An overview. Fish & shellfish immunology, 117, 36-52.

Elsanhoty, R., Al-Turki, I. ve Ramazan, M. Ağır metallerin ve aflatoksin B1'in kirli sudan uzaklaştırılmasında laktik asit bakterilerinin uygulanması. *Su Bilimi. Teknoloji.* **74** , 625–638 (2016).

Emenike, E. C., Iwuozor, K. O., & Anidiobi, S. U. (2021). Heavy metal pollution in aquaculture: sources, impacts and mitigation techniques. *Biological Trace Element Research*, 1-17.

Enan, G., Mamdouh, M., Negm, S., Ismaiel, A. & Abdel-Shafi, S. Laktik asit bakterilerinin sınıflandırılması, antimikrobiyal potansiyeli, endüstriyel uygulamaları ve probiyotik kapasitesi: Bir inceleme makalesi. *Res. J. Başvuru. Bilim.* **13** , 742–757 (2018).

Evariste, L., Barret, M., Mottier, A., Mouchet, F., Gauthier, L., & Pinelli, E. (2019). Gut microbiota of aquatic organisms: a key endpoint for ecotoxicological studies. *Environmental pollution*, *248*, 989-999.

Fargašová, A. (1994). Toxicity determination of plant growth hormones on aquatic Alga—Scenedesmus quadricauda. *Bulletin of environmental contamination and toxicology*, *52*, 706-711.Garrabou et al., 2019

Giri, S. S., Yun, S., Jun, J. W., Kim, H. J., Kim, S. G., Kang, J. W., ... & Park, S. C. (2018). Therapeutic effect of intestinal autochthonous Lactobacillus reuteri P16 against waterborne lead toxicity in Cyprinus carpio. Frontiers in immunology, 9, 1824.

Guarner, F., Khan, A. G., Garisch, J., Eliakim, R., Gangl, A., Thomson, A., ... & Szajewska, H. (2008). World Gastroenterology Organisation Practice Guideline: Probiotics and Prebiotics-May 2008: guideline. South African Gastroenterology Review, 6(2), 14-25.

Hasan, K. N., & Banerjee, G. (2020). Recent studies on probiotics as beneficial mediator in aquaculture: a review. *The Journal of Basic and Applied Zoology*, *81*(1), 1-16.

Ibrahem, M. D. (2015). Evolution of probiotics in aquatic world: Potential effects, the current status in Egypt and recent prospectives. Journal of advanced research, 6(6), 765-791.

Jia, P. P., Junaid, M., Wen, P. P., Yang, Y. F., Li, W. G., Yang, X. G., & Pei, D. S. (2021). Role of germ-free animal models in understanding interactions of gut microbiota to host and environmental health: A special reference to zebrafish. *Environmental Pollution*, *279*, 116925.

Kakade, A., Sharma, M., Salama, E. S., Zhang, P., Zhang, L., Xing, X., ... & Li, X. (2022). Heavy metals (HMs) pollution in the aquatic environment: Role of probiotics and gut microbiota in AĞIR METALs remediation. *Environmental research*, 115186.

Kakakhel, M. A., Narwal, N., Kataria, N., Johari, S. A., Din, S. Z. U., Jiang, Z., ... & Xiaotao, S. (2023a). Deciphering the dysbiosis caused in the fish microbiota by emerging contaminants and its mitigation strategies-A review. *Environmental Research*, 117002.

Kakakhel, M. A., Bibi, N., Mahboub, H. H., Wu, F., Sajjad, W., Din, S. Z. U., ... & Wang, W. (2023b). Influence of biosynthesized nanoparticles exposure on mortality, residual deposition, and intestinal bacterial dysbiosis in Cyprinus carpio. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *263*, 109473.

Khulbe K.C., Matsuura T. Removal of heavy metals and pollutants by membrane adsorption techniques. Appl. Water Sci. 2018;8:19. doi: 10.1007/s13201-018-0661-6.

Kinoshita, H., Soağır metala, Y., Ohtake, F., Ishida, M., Kawai, Y., Kitazawa, H., ... & Kimura, K. (2013). Biosorption of heavy metals by lactic acid bacteria and identification of mercury binding protein. *Research in microbiology*, *164*(7), 701-709.

Kirillova, A. V., Danilushkina, A. A., Irisov, D. S., Bruslik, N. L., Fakhrullin, R. F., Zakharov, Y. A., ... & Yarullina, D. R. (2017). Assessment of resistance and bioremediation ability of Lactobacillus strains to lead and cadmium. International journal of microbiology, 2017.

Kumar, R., Qureshi, M., Vishwakarma, D. K., Al-Ansari, N., Kuriqi, A., Elbeltagi, A., & Saraswat, A. (2022). A review on emerging water contaminants and the application of sustainable removal technologies. *Case Studies in Chemical and Environmental Engineering*, *6*, 100219.

Lin, D., Ji, R., Wang, D., Xiao, M., Zhao, J., Zou, J., ... & Li, S. (2019). The research progress in mechanism and influence of biosorption between lactic acid bacteria and Pb (II): A review. Critical reviews in food science and nutrition, 59(3), 395-410.

Liu, M., Kakade, A., Liu, P., Wang, P., Tang, Y., & Li, X. (2019). Hg2+-binding peptide decreases mercury ion accumulation in fish through a cell surface display system. Science of The Total Environment, 659, 540-547.

Liu, W., Feng, H., Zheng, S., Xu, S., Massey, I. Y., Zhang, C., ... & Yang, F. (2021). Pb toxicity on gut physiology and microbiota. *Frontiers in Physiology*, *12*, 574913.

Medina-Félix, D., Garibay-Valdez, E., Vargas-Albores, F., & Martínez-Porchas, M. (2023). Fish disease and intestinal microbiota: A close and indivisible relationship. *Reviews in Aquaculture*, *15*(2), 820-839.

Monachese, M., Burton, J. & Reid, G. Mikrobiyal süreçler yoluyla insanların ağır metallere karşı biyolojik iyileştirme ve toleransı: probiyotikler için potansiyel bir rol? *Başvuru Çevre. Mikrobiyol.* **78** , 6397–6404 (2012).

Morshed, S. M., & Lee, T. H. (2023). The role of the microbiome on fish mucosal immunity under changing environments. *Fish & Shellfish Immunology*, 108877.

Mrvčić, J., Stanzer, D., Šolić, E. ve Stehlik-Tomas, V. Laktik asit bakterilerinin metal iyonlarıyla etkileşimi: gıda güvenliğini ve kalitesini iyileştirme fırsatları. *Dünya J. Microbiol. Biyoteknoloji.* **28** , 2771–2782 (2012).

Muñoz-Atienza, E., Gómez-Sala, B., Araújo, C., Campanero, C., Del Campo, R., Hernández, P. E., ... & Cintas, L. M. (2013). Antimicrobial activity, antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic origin intended for use as probiotics in aquaculture. BMC microbiology, 13, 1-22.

Nayak, S. K. (2010). Role of gastrointestinal microbiota in fish. *Aquaculture research*, *41*(11), 1553-1573.

Naylor, R. L., Hardy, R. W., Buscağır metalann, A. H., Bush, S. R., Cao, L., Klinger, D. H., ... & Troell, M. (2021). A 20-year retrospective review of global aquaculture. *Nature*, *591*(7851), 551-563.

Paul, D. (2017). Research on heavy metal pollution of river Ganga: A review. *Annals of Agrarian Science*, *15*(2), 278-286.

Qian, D., Xu, C., Chen, C., Qin, J. G., Chen, L., & Li, E. (2020). Toxic effect of chronic waterborne copper exposure on growth, immunity, anti-oxidative capacity and gut microbiota of Pacific white shrimp Litopenaeus vannamei. *Fish & shellfish immunology*, *100*, 445-455.

Rajeshkumar, S., & Li, X. (2018). Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China. *Toxicology reports*, *5*, 288-295.

Rajeshkumar, S., Liu, Y., Ma, J., Duan, H. Y., & Li, X. (2017). Effects of exposure to multiple heavy metals on biochemical and histopathological alterations in common carp, Cyprinus carpio L. *Fish & shellfish immunology*, *70*, 461-472.

Rohani, M. F., Islam, S. M., Hossain, M. K., Ferdous, Z., Siddik, M. A., Nuruzzaman, M., ... & Shahjahan, M. (2022). Probiotics, prebiotics and synbiotics improved the functionality of aquafeed: Upgrading growth, reproduction, immunity and disease resistance in fish. Fish & shellfish immunology, 120, 569-589.

Rosenbaum, M., Knight, R., & Leibel, R. L. (2015). The gut microbiota in human energy homeostasis and obesity. *Trends in Endocrinology & Metabolism*, *26*(9), 493-501.

Sağlam, N., & Cihangir, N. Ağır metallerin biyolojik süreçlerle biyosorbsiyonu çalışmaları.

Singh R., Gautam N., Mishra A., Gupta R. Heavy metals and living systems: An overview. Indian J. Pharmacol. 2011;43:246–253. doi: 10.4103/0253-7613.81505.

Singh V., Mishra V. Microbial Removal of Cr (VI) by a New Bacterial Strain Isolated from the Site Contaminated with Coal Mine Effluents. *J. Environ. Chem. Eng.* 2021;9:106279. doi: 10.1016/j.jece.2021.106279.

Soon, T. K., & Ransangan, J. (2019). Extrinsic factors and marine bivalve mass mortalities: an overview. *Journal of Shellfish Research*, *38*(2), 223-232.

Stressmann, F. A., Bernal-Bayard, J., Perez-Pascual, D., Audrain, B., Rendueles, O., Briolat, V., ... & Ghigo, J. M. (2021). Mining zebrafish microbiota reveals key community-level resistance against fish pathogen infection. *The ISME journal*, *15*(3), 702-719.

Sujitha, S. B., Jonathan, M. P., Villegas, L. E. C., & Hernández-Camacho, C. J. (2020). Occurrences and ecotoxicological risks of trace metals in the San Benito Archipelago, Eastern Pacific Ocean, Mexico. *Ocean & Coastal Management*, *184*, 105003.

Topaldemir, H., Taş, B., Yüksel, B., & Ustaoğlu, F. (2023). Potentially hazardous elements in sediments and Ceratophyllum demersum: An ecotoxicological risk assessment in Miliç

Wetland, Samsun, Türkiye. *Environmental Science and Pollution Research*, *30*(10), 26397-26416.

Tye, S. P., Siepielski, A. M., Bray, A., Rypel, A. L., Phelps, N. B., & Fey, S. B. (2022). Climate warming amplifies the frequency of fish mass mortality events across north temperate lakes. *Limnology and Oceanography Letters*, *7*(6), 510-519.

Walther B, Sieber R (2011) Gıdalarda biyoaktif proteinler ve peptitler. Int J Vitam Nutr Res 81:181–192.

Wang, N., Jiang, M., Zhang, P., Shu, H., Li, Y., Guo, Z., & Li, Y. (2020). Amelioration of Cd-induced bioaccumulation, oxidative stress and intestinal microbiota by Bacillus cereus in Carassius auratus gibelio. Chemosphere, 245, 125613.

Wołowiec M., Komorowska-Kaufman M., Pruss A., Rzepa G., Bajda T. Removal of Heavy Metals and Metalloids from Water Using Drinking Water Treatment Residuals as Adsorbents: A Review. Minerals. 2019;9:487. doi: 10.3390/min9080487.

Wu, G., Xiao, X., Feng, P., Xie, F., Yu, Z., Yuan, W., ... & Li, X. (2017). Gut remediation: a potential approach to reducing chromium accumulation using Lactobacillus plantarum TW1-1. Scientific Reports, 7(1), 15000.

Wuana R.A., Okieimen F.E. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *Int. Sch. Reseach Not.* 2011;2011:402647. doi: 10.5402/2011/402647.

Xie, H., Yang, X., Xu, J., & Zhong, D. (2023). Heavy metals pollution and potential ecological health risk assessment in the Yangtze River reaches. *Journal of Environmental Chemical Engineering*, *11*(2), 109489.

Xiong, J. B., Nie, L., & Chen, J. (2019). Current understanding on the roles of gut microbiota in fish disease and immunity. *Zoological research*, *40*(2), 70.

Yang, J., Holbach, A., Wilhelms, A., Krieg, J., Qin, Y., Zheng, B., ... & Norra, S. (2020). Identifying spatio-temporal dynamics of trace metals in shallow eutrophic lakes on the basis of a case study in Lake Taihu, China. *Environmental Pollution*, *264*, 114802.

Yin, Y., Zhang, P., Yue, X., Du, X., Li, W., Yin, Y., ... & Li, Y. (2018). Effect of sub-chronic exposure to lead (Pb) and Bacillus subtilis on Carassius auratus gibelio: bioaccumulation, antioxidant responses and immune responses. *Ecotoxicology and environmental safety*, *161*, 755-762.

Yoshida, M. A., Tanabe, T., Akiyoshi, H., & Kawamukai, M. (2022). Gut microbiota analysis of Blenniidae fishes including an algae-eating fish and clear boundary formation among isolated Vibrio strains. *Scientific reports*, *12*(1), 4642.

Younis, A. M., Amin, H. F., Alkaladi, A., & Mosleh, Y. Y. (2015). Bioaccumulation of heavy metals in fish, squids and crustaceans from the Red Sea, Jeddah Coast, Saudi Arabia. *Open Journal of Marine Science*, *5*(04), 369.

Yu, L., Zhai, Q., Zhu, J., Zhang, C., Li, T., Liu, X., ... & Chen, W. (2017). Dietary Lactobacillus plantarum supplementation enhances growth performance and alleviates aluminum toxicity in tilapia. Ecotoxicology and Environmental Safety, 143, 307-314.

Yukgehnaish, K., Kumar, P., Sivachandran, P., Marimuthu, K., Arshad, A., Paray, B. A., & Arockiaraj, J. (2020). Gut microbiota metagenomics in aquaculture: factors influencing gut microbiome and its physiological role in fish. *Reviews in Aquaculture*, *12*(3), 1903-1927.

Yüksel, B., Ustaoğlu, F., Tokatli, C., & Islam, M. S. (2022). Ecotoxicological risk assessment for sediments of Çavuşlu stream in Giresun, Turkey: association between garbage disposal facility and metallic accumulation. *Environmental Science and Pollution Research*, *29*(12), 17223-17240.

Zhai, Q., Wang, G., Zhao, J., Liu, X., Tian, F., Zhang, H., & Chen, W. (2013). Protective effects of Lactobacillus plantarum CCFM8610 against acute cadmium toxicity in mice. *Applied and environmental microbiology*, *79*(5), 1508-1515.

Zhai, Q., Yu, L., Li, T., Zhu, J., Zhang, C., Zhao, J., ... & Chen, W. (2017). Effect of dietary probiotic supplementation on intestinal microbiota and physiological conditions of Nile tilapia (Oreochromis niloticus) under waterborne cadmium exposure. Antonie Van Leeuwenhoek, 110, 501-513.

Zhang, X., Wen, K., Ding, D., Liu, J., Lei, Z., Chen, X., ... & Lin, Y. (2021). Size-dependent adverse effects of microplastics on intestinal microbiota and metabolic homeostasis in the marine medaka (Oryzias melastigma). *Environment International*, *151*, 106452.

Wheat Yellow Rust (*Puccinia striiformis* f.sp. *tritici*) Disease and Resistance Genes

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ABSTRACT: Wheat (*Triticum aestivum* L.), belonging to the Gramineae family, has high nutritional value and yield, and accounts for 35% of the world's total cereal production. It ranks third in the world in terms of production and second after rice and maize. Most of wheat production is used for human consumption and some uses include beverage, starch and straw production. It is also occasionally used for animal feed and biofuel production. During its growth and development, wheat is affected by a variety of biotic and abiotic stresses such as high temperature, drought, salinity, pests and diseases that severely affect the quality and yield of wheat. Fungal diseases are the most important biotic factors that are problematic in wheat fields. Among the biotic stresses is wheat yellow rust caused by *Puccinia striiformis* f. sp. *tritici* (Pst). They cause chlorophyll loss by invading the leaf surface of wheat. Direct yield losses are inevitable, especially when the flag leaf just below the spike is infested by fungi. In wheat, Pst causes yield losses of up to 50% due to shriveling of grains and weakened plant growth. Although chemical control and wheat cropping measures can reduce the loss due to yellow rust to some extent, the most economical and environmentally friendly way to control the disease is through the spread of genetic resistance. In this study, we present the biology of Pst and breeding for resistant cultivars and resistance genes as an alternative, preventive, effective and sustainable control method for the future in wheat crops important in human nutrition.

Keywords: *Triticum aestivum*, *Puccinia striiformis* f. sp. *tritici*, Resistant, Yr, Marker

INTRODUCTION

Triticum aestivum L., or wheat, is a member of the Gramineae family and is very nutritious (Li et al., 2023). Its yield is third in the world in terms of production, behind only rice and maize, and makes up 35% of all cereal produced worldwide (Nhemachena and Kirste, 2017; Khan et al., 2017). One of the three principal cereal crops farmed worldwide is wheat. With a global per capita consumption of 67.4 kg/year^{-1}, it is the most consumed food grain (Djanaguiraman et al., 2019). Even though the world produces more than 700 million tons of wheat annually, food scarcity-related malnutrition remains a major issue that is linked to rapid population growth (FAO, 2017). Turkey is a major producer and consumer of wheat worldwide.

Turkey has one of the highest per capita annual wheat consumption rates in the world, at 170 kg (FAO, 2022a). Additionally, Turkey is the world's top exporter of flour and ranks second in terms of pasta (FAO, 2022b). Turkey grows more than 6.6 million hectares of wheat (*Triticum* sp.) each year, with an estimated 20 million tons of wheat produced there (TurkStat, 2022).

The majority of wheat production is directed to human consumption, some uses include beverage, starch and straw production, it is also occasionally used for animal feed and biofuel production (Tadesse et al., 2019). Wheat production has declined steadily on a hectare basis over the past three decades and has continued to fall ever since (Nhemachena and Kirste, 2017; Breitenbach and Fényes, 2000). Among many other factors, environmental stressors such as poor soil fertility and high pH, climate change, poor variety selection and disease outbreaks contribute to the observed losses in production rates, quality and yield (Dube et al., 2020). In addition, wheat is affected by various biotic and abiotic stresses during its growth and development, such as high temperature, drought, salinity, pests and diseases, which severely affect the quality and yield of wheat (Jiang et al., 2020). Fungal diseases are the most important biotic agents that are problematic in wheat fields. They cause chlorophyll loss by invading the leaf surface of wheat. Especially when the flag leaf just below the spike is infested by fungi, direct yield losses are inevitable (He et al., 2019). Biotic stresses include wheat yellow rust caused by *Puccinia striiformis* f. sp. *tritici* (Pst). When favorable weather conditions for Pst occur, it can cause devastating outbreaks leading to extreme crop losses in susceptible wheat varieties. Fluctuations in the annual production of wheat, which is a strategic product for our country, cause serious economic and sociological problems. Yellow rust disease causes serious decreases and fluctuations in production, especially in epidemic years. The yellow rust epidemic that occurred in our country in 2010 caused approximately 1 million tons of yield loss and led to an economic loss of 1.2 billion TL. The frequency, severity and prevalence of wheat stripe rust varies depending on many factors such as weather, phenology and genetic background of the host.

Pst is a member of the large family of rust fungi and causes wheat stripe rust, the most widespread, devastating disease, resulting in significant yield loss in wheat production (Chen et al., 2014; Line, 2002). Pst always severely impairs the photosynthetic function of the leaf and affects the supply and movement of assimilation products, leading to reduced thousand grain weight (TKW) and yield loss (He et al., 2011; Li et al., 2005). Pst is one of the most widespread destructive wheat diseases in the world, especially in regions with cool and humid environments. Pst is an obligate biotroph and can therefore infect the host plant at different growth stages and feed on live wheat plants for nutrient uptake (Carmona et al., 2020). The fungus is hemicyclic, no sexual cycle has been identified, and host species other than wheat (and perhaps a few very closely related species in areas where these occur) are not important for epidemiology and survival (Stele et al., 2001). The wind dispersal of its spores over hundreds or thousands of kilometers has resulted in its widespread spread on a continental or global scale, allowing the disease to reappear regularly in areas where the climate is seasonally unfavorable (Brown and Hovmoller, 2002). In wheat and barley, yellow rust has caused yield losses of up to 50% due to shriveling of grains and weakened plant growth (Line, 2002). Although chemical control and wheat intercropping measures can reduce losses from stripe rust to some extent, the most economical and environmentally friendly way to control the disease is through the spread of genetic resistance (Line and Chen, 1995; Uauy et al., 2005).

Pst, which causes yellow rust disease in wheat, is one of the most destructive pathogens and frequently mutates in major wheat cultivars, defeating resistance genes (Chen and Penman, 2005; Lu et al., 2011; Zhan et al., 2022). Large yield losses brought on by wheat pathogens make this situation worse (Pardey et al. 2013; Moscou and van Esse, 2017). It is crucial to maximize wheat yields through efficient control of wheat diseases in order to ensure the growth of global wheat production required to feed a growing population (Pardey et al., 2013). Reproductive resistant varieties are the most affordable, environmentally friendly, and successful way to control biotic and abiotic stresses on wheat, as demonstrated by theory and practice (Yin et al., 2018). In order to prepare gene resources for wheat resistance breeding, it is imperative that additional genes with stress resistance roles be discovered (Yin et al., 2023). The emergence of new and more aggressive strains of Pst has greatly increased the concerns of the wheat industry. In recent years, efforts have been made to control the progression of Pst, for example, the application of foliar fungicides has long been an important component of Pst control mechanisms and is still used to this day (Carmona et al., 2020). However, agrochemicals have been criticized for their negative impact on the environment (Bernardes et al., 2015; Gill and Garg, 2014; Mahmood et al., 2016). Alternatively, breeding for Pst-resistant varieties offers a preventive, effective and sustainable control method for the future and should be the focus of breeders (Figlan et al., 2020). The introduction of host plant-resistant wheat varieties has been a mostly reliable, environmentally friendly and cost-effective strategy for Pst control (Figlan et al., 2020). Breeding resistant varieties is the most economical and environmentally friendly way to control the disease. There are many resistance genes to yellow rust on wheat chromosome 2B (Luo et al., 2009). Alternatively, breeding for Pst-resistant varieties offers a preventive, effective and sustainable control method for the future and should be the focus of breeders (Figlan et al., 2020). The introduction of host plant-resistant wheat varieties has been a mostly reliable, environmentally friendly and cost-effective strategy for Pst control (Figlan et al., 2020). The dissemination of resistance genes is the most economical and sustainable technique to control yellow rust (Bai et al., 2014; Lan et al., 2014). More than 80 resistance genes (Yr1 - Yr83) have been discovered so far (Zhao and Kang, 2023; McIntosh et al., 2017; Li et al., 200), not all of which are widely used in wheat programs globally. Furthermore, widely bred resistant varieties often lose their resistance due to the emergence of new races (Xian et al., 2023). However, climate change and fungal evolution are always expected to create newer pathotypes/races of the pathogen. In light of this, new genes need to be genetically edited and characterized for ready mobilization when needed (Mohammad et al., 2023). The aim of this study is to present resistance genes and breeding for Pst-resistant varieties as an alternative, preventive, effective and sustainable control method for the future in wheat crop, which is important in human nutrition.

The Disease

Taxonomy and biology

Different formae speciales of *P. striiformis*, a fungus in the order Uredinales of Basidiomycetes, are responsible for stripe rusts on grasses and cereal crops. Before its current name was revived in 1953 (Hylander et al. 1953; Stubbs, 1985), the pathogen's species name had undergone several changes, including *Uredo glumarum* (Schmidt 1827), *Puccinia striaeformis* (Westendorp 1854), *Puccinia straminis* (Fuckel 1860), and *Puccinia glumarum* (Eriksson and Henning 1894). It has dikaryotic uredial and telial stages in its life cycle. Teliospores can germinate to form haploid basidiospores, however, the pathogen responsible for stripe rust lacks known alternate hosts for the basidiospores to infect, as well as pycnial and aecial stages, in contrast to the pathogens causing stem rust and leaf rust.

The Pst fungus has a complicated life cycle and is an obligatory biotroph. This fungus can produce spermatium, aeciospores, urediniospores, teliospores, and basidiospores, but it needs two separate hosts to complete its life cycle. Particularly urediniospores are dikaryotic and are used extensively in etiological and evolutionary biology studies (Aime and McTaggart, 2021). Pst infects host plants (grasses and cereal crops) when urediniospores accumulate on the leaf surface due to wind or raindrops. It then infects the host mesophyll cell and forms haustorium mother cells, which give rise to the haustorium, a balloon-shaped feeding structure (Hovmoller et al., 2011). In addition to serving as the pathogen's primary route for nutrition absorption, the pas haustorium is also the site of intense secretory protein (effector) expression and secretion (Garnica et al., 2013). But Pst cannot be grown in vitro, and its agricultural host, wheat, is not very genetically manipulable because of the difficulty of genetic transformation and the creation of mutants (Yin and Hulbert, 2011). Taken together, these host- and pathogen-related variables impede the advancement of research aimed at elucidating the intricate biology of Pst infection.

Historical impact and distribution

Cultivating cereals has been plagued by rust since the beginning of recorded history. The Old Testament's Mosaic books and the writings of early scholars both make mention of rust epidemics (Large, 1940). Stripe rust may have been the cause of epidemics documented in Sweden in 1794 and England in 1725 (Chester, 1946). Major wheat-growing nations frequently experience severe disease outbreaks (Welings, 2011). The United States (Chen, 2005), Mexico, Chile, and other Andean nations are among the Americas' most significant stripe rust producers (CPC, 2005). Comparably, stripe rust poses a significant threat in China (Chen et al., 2009), Central Asia (Morgounov et al., 2005), the Middle East (Stubbs, 1985; Wellings, 2011), Europe, and the northern Indian subcontinent (Singh, 2004). Wheat cultivated in the highlands of Ethiopia, Kenya, and Uganda is susceptible to the disease in Africa (CPC, 2005, Stubbs, 1988). Wheat stripe rust has been more prevalent recently and is now significant in Australia, New Zealand, and South Africa (Wellings, 2011).

In Turkey, Pst is the most prevalent wheat disease. In the Thrace region of northwest Turkey, winter wheat growing areas experienced an unusual outbreak of stripe rust infection in 2014. In Turkey, it was discovered that certain commercial varieties (Enola, Izgi 2001, etc.) that were previously identified as resistant to P. striiformis f. sp. tritici races were actually fully susceptible. The Enola variety was the target of the first significant infection, which was discovered in a farmer's field in Edirne (41°12′6.34° N, 26°24′44.04° E). The disease later spread to the provinces of Tekirdağ and Kırklareli. Later, trace stripe rust infections were found in several previously identified resistant Yr genes, including Yr1, Yr3, and Yr4, in a yellow rust trap nursery in Sakarya, northern Turkey (Mert et al., 2016).

Symptoms, disease development, and signs of the pathogen

The green tissues of grasses and cereal crop plants are infected by the pathogen that causes stripe rust. As long as the plants are still green, infection can occur at any time from the one-leaf stage to plant maturity. In ideal temperature conditions, sporulation begins about two weeks after infection, and symptoms start to show up around one week after infection. The rust-colored, yellow-to orange-colored pustules, known as uredia, are caused by the fungus. There are thousands of urediniospores in each uredium. The urediniospore is too small to be seen with the human eye, but in bulk, the spores have a powdery, yellow to orange color. Necrosis or uredia stripes do not appear on seedling leaves, instead, they develop later in plant life, usually following stem elongation. Different degrees of chlorosis or necrosis (hypersensitive response) with or without sporulation appear based on temperature and plant resistance. Necrotrophic pathogen-caused spots can be differentiated from necrotic stripes or elongated spots that appear on adult plant leaves. Stripe rust is a pathogen that damages host plants by taking up their nutrients and water.

This fungus can produce spermatia, aeciospores, urediniospores, teliospores, and basidiospores, and it requires two distinct hosts to complete its life cycle. Particularly, urediniospores are dikaryotic and are frequently employed in etiological and evolutionary biology research (Aime and MCTaggert, 2021). Pst infects its hosts, which are grasses and cereal crops, by depositing urediniospores onto their leaf surfaces through wind or raindrops. From there, it infects the host mesophyll cell and forms haustorial mother cells, which eventually give rise to the haustorium, a balloon-shaped feeding structure (Hovmoller et al., 2011). In addition to serving as the pathogen's primary site of nutrition absorption, the rust haustorium is also the site of massive secretory protein (effector) expression and secretion (Garnica et al., 2013). However, Pst cannot be cultivated in vitro, and wheat, the agronomic host of Pst, is not particularly accessible to genetic manipulation due to the relatively complex genetic transformation and mutant creation. (Yin and Hulbert, 2011). All of these pathogen- and host-related variables work against research efforts to fully understand the biology of Pst infection. New Pst strains have been appearing frequently in recent years, and our understanding of the virulence variation of this fungus may aid in the development of new resistant wheat cultivars.

Wheat resistance genes and molecular markers against *Pst*

Pst is one of the most important foliar diseases of wheat. It occurs in all wheat cultivated areas worldwide and can cause serious yield losses when not controlled (Wellings, 2011). Reports of yield losses ranging from 10 to 100% have been made because the pathogen disrupts the plant's primary metabolism by depleting the energy stores required for the host's growth. Chlorosis and necrosis are the results of further colonization and reproduction interfering with the plant's ability to photosynthesize (Carmona et al., 2020; Figlan et al., 2020). According to Carmona et al. (2020), high yield losses in susceptible cultivars are caused by Pst, and the extent of yield losses due to Pst is primarily determined by the plant's growth stage at the beginning of the disease and the cultivars' level of resistance to Pst. The most cost-effective way to control stripe rust is to breed resistant varieties, which also reduces environmental impact by using fewer fungicides (Chen, 2005; Line, 2002). But after cultivars with race-specific resistance genes are introduced, new virulent races (pathotypes) of the rust pathogen frequently appear a few years later (Line and Qayoum, 1922; Singh et al., 2004; Stubbs, 1988). Consequently, previously resistant varieties become susceptible when newly introduced races infect them (Chen, 2005; Stubbs, 1985). The emergence of new races that lead to the failure of resistance genes is a common cause of severe Pst outbreaks (Chen, 2007). For instance, a new race virulent to Yr9 was reported in 1985 as a result of more than 80% of the varieties released in the late 1980s having Yr9. This race caused outbreaks in China that resulted in yield losses of 2.65 million tons in 1990 (Chen et al., 2009; Wan et al., 2004). Similar to this, Yr27 was extensively employed in wheat cultivars cultivated in Pakistan and India, and between 2002 and 2004, new races virulent to Yr27 were reported to have emerged in South Asia (Singh et al., 2004). Races virulent to Yr27 were identified as the cause of severe yellow rust outbreaks in 2009 in South and Central Asian countries (BGRI, 2010). New races with more complex combinations of virulence that arise through mutation, recombination, or long-distance migration exacerbate these outbreaks (Singh et al., 2004; Stubbs, 1988; Wellings, 2011). When such outbreaks occur, a lot of fungicides are applied to control a disease that would otherwise result in a large loss of yield (Chen et al., 2009; Chen, 2007). Rust urediniospores can travel great distances by wind, so monitoring Pst races should be a top priority in all outbreak areas worldwide (Singh et al., 2004). In order to develop effective disease management strategies through resistance breeding and future race prediction, it is necessary to regularly monitor the virulence spectrum of Pst populations and identify new races (McIntosh and Brown, 1977). Characterizing non-race-specific resistance requires the identification of virulent races (Chen, 2005). When screening cultivars or breeding lines to assess resistance levels and the application of novel resistance genes, information on virulence is also helpful. Furthermore, tracking the pathogen population's virulence in various areas aids in comprehending the genetic variability of host-pathogen interactions at the moment.

According to Flor's (1971) gene-for-gene concept, host defense responses are triggered when a pathogen's Avr protein is recognized by a cognate host R protein (Petit-Houdenot and Fudal, 2017). The yellow rust (Yr) genes in rust resistance act as R genes in accordance with the traditional gene-for-gene theory. Certain Yr genes show resistance to the disease that is considered durable and non-race-specific, meaning it remains effective even after being used extensively and for an extended period of time in an environment that is conducive to the disease (Chen and Kang, 2017). This type of resistance is non-durable and race-specific because it is only effective against a small subset of Pst races and can be overcome once a new, virulent strain emerges (Chen, 2013). Other Yr genes may also provide resistance against other subsets of Pst races. Only a small number of Yr genes directly linked to resistance have been isolated to date, despite the identification of over 300 quantitative trait loci (QTL) and more than 150 temporarily or permanently designated Yr genes (Chen and Kang, 2017; Saeed et al., 2022; Farzand et al., 2022). These genes include Yr18 (Krattinger et al., 2009), Yr36 (Fu et al., 2009), Yr46 (Herrera et al., 2011; Chhetri et al., 2016), Yr5/YrSP (Marchal et al., 2018), Yr7 (Marchal et al., 2018), Yr15 (Klymiuk et al., 2018), Yr27 (Athiyannan et al., 2022), Yr28 (Zheng et al., 2020), and YrU1 (Wang et al., 2020). The mapping of yellow rust resistance genes and the development of molecular markers for marker-assisted selection has been one of the most active areas of research on stripe rust. Yr9 (Shi et al, 2001), Yr5 (Sun et al., 2002; Yan et al., 2003; Chen et al., 2003), Yr15 (Chagué et al., 1999; Peng et al., 2000), Yr24 (Zakari et al., 2003), Yr10 (Frick et al., 1998; Shao et al., 2001, Bariana et al., 2002; Smith et al., 2002, Wang et al., 2002), Yr17 (Robert et al., 1999; Seah et al., 2001), Yr26 (Ma et al., 2001), Yr28 (Singh et al., 2000), Yr18 (Suenaga et al., 2003), Yr32 (Eriksen et al., 2004), Yr33 (McIntosh et al., 2004), Yr36 (J. Dubcovsky, 2004), Yr34 (McIntosh et al., 2004), YrH52 (Peng et al., 2000) and Yrns-B1 (Börner et al., 2000) (Table 1). Given that markers are close to genes, marker-assisted selection should benefit from these markers. While markers for Yr17 were found by cloning a gene-like sequence for disease resistance, the majority of markers were found using RAPD, SSR, and AFLP techniques (Seah et al., 2001). Nucleotide-binding site and leucine-rich repeat (NBS-LRR) proteins with a non-canonical N-terminal zinc finger BED domain are encoded by the Yr5/YrSP and Yr7 genes. An intracellular immune receptor is encoded by the Yr27 gene, and a typical NBS-LRR protein is encoded by the Yr28 gene. Simultaneously, the YrU1 gene produces an NBS-LRR protein with a C-terminal WRKY domain and an N-terminal ANK domain. It has been shown that these genes are resistant to racial differences. Durable resistance in non-race-specific protection is thus desperately needed. The NBS-LRR domain-lacking Yr15, Yr18, Yr36, and Yr46 genes belong to the class of non-NBS-LRR R genes that seem to provide persistent, non-race-specific resistance to Pst (Chen and Kang, 2017). Transcript analysis between the Yr39 gene (durable resistance) and the Yr5 gene (non-durable resistance) revealed 14 transcripts that are expressed and shared by both resistance types, potentially indicating a connection to host cell death. This information provided insights into the molecular mechanisms enabling this resistance. R genes are present in some of the up-regulated genes linked to Yr39-mediated resistance but not Yr5-mediated resistance. According to Chen et al. (2013), the Yr39 gene is therefore expected to serve as a master regulator of other R genes and defense-related extra genes, thereby increasing its efficiency. Nowadays, resistance-related genes, which are probably downstream of the R gene-induced defense signaling, account for the majority of wheat genes that react to Pst infection. It is therefore necessary to locate and clone additional major R genes, like the Yr39 gene. These two genes are currently being combined using molecular markers for Yr5 and Yr15, each of which confers resistance to all races of P. striiformis f. sp. tritici in the United States. Codominant RGAP markers are crucial for cloning Yr5 using a gene insertion strategy based on resistance gene analogs since they co-localize with Yr5 and share a great deal of homology with numerous gene clones for plant resistance. For resistance to tritici races PST-41 and PST-45, P. striiformis f. sp. possesses one dominant gene and one recessive gene, designated RpstS1 and rpstS2, respectively. He generated a linkage group with 11 RGAP markers for RpsLem and Yr21, the linked genes in 'Lemhi,' and a linkage group with 12 RGAP markers for RpstS1, the dominant gene in 'Steptoe,' using the RGAP technique. The linkage group for RpsLem and Yr21 was mapped to wheat chromosome 1B using an RGAP marker repulsively linked to the resistant allele and a set of nulli-tetrasomic 'Chinese Spring' lines, confirming the chromosomal location of Yr21 (Chen et al., 1995b). A chromosome-specific microsatellite marker called Hvm68 was used to map RpstS1 to barley chromosome 4H. They demonstrate that one or more genes can control the quality of resistance to both wheat and barley stripe rust in barley. These genes may offer efficient defense against stripe rust of wheat and barley, respectively, if they are transferred from wheat to barley and from barley to wheat. It should be investigated whether P. striiformis f. sp. tritici and P. striiformis f. sp. hordei can combine virulence genes to produce new isolates that cause diseases in both wheat and barley crops before implementing this strategy. The advancement of genomic sequencing in recent times has facilitated the continuous improvement of wheat genome assembly (Adhikari et al., 2022). This development is expected to expedite the identification of Yr genes.

Usage Under field conditions, Islam et al. (2023) discovered that 53 out of 92 genotypes were resistant and 29 showed moderate resistance, while the remaining genotypes were all either susceptible or moderately susceptible. Out of 109 genotypes, only 12 were found to be resistant to all 6 virulent/pathogenic pathotypes under controlled conditions. Furthermore, after field screening identified 97 genotypes as resistant, these genotypes were confirmed molecularly using markers associated with the major R genes, Yr5, Yr10, Yr15, and Yr17. The Yr5 gene was present in 9 genotypes, the Yr10 gene in 12, the Yr15 gene in 14, and the Yr17 gene in 32. The resistance genes that this study looked at are good at preventing yellow rust disease. Yellow rust resistance breeding programs can benefit from the utilization of genotypes that have demonstrated resistance in both controlled and field settings.

Table 1. Molecular markers and resistance genes to other diseases linked to genes for resistance to stripe rust in wheat.

Yr gene	Linked gene or marker	Reference
Yr5	Xwgp-17-2B, Xwgp19-2B, Yr5STS7/8	Chen et al., 2003b
Yr8	Sr34	McIntosh et al., 1998
Yr9	Lr26, Sr31, Xwgp4, Xwgp7, Xwgp8, Xwgp9	Shi et al., 2001
Yr10	RgaYr10a, S26-M47, S13-M63	Smith et al., 2002
Yr15	Nor1, UBC212a, Xgwm413	Peng et al., 2000
Yr17	Lr37, Sr38, Vrga1	Seah et al., 2001
Yr18	Lr34, Xgwm295.1, Xgwm44	Suenaga et al., 2003

Yr24	Xgwm11-1B	Zakari et al., 2003
Yr26	Xgwm11, Xgwm18	Ma et al., 2001
Yr27	Lr13, Lr23	McDonald et al., 2004
Yr28	Xmwg634-4DS	Singh et al., 2000
Yr29	Lr46	McIntosh et al., 1998
Yr30	Sr2, Lr27	McIntosh et al., 1998
Yr31	Yr27, Yr23, Lr23	McIntosh et al., 1998
Yr32	Xwmc198, M62/P19-156, M59/P37-375	Eriksen et al., 2004
Yr33	A 7DL marker	McIntosh et al., 2004
Yr34	Xgwm6-5A, B1	McIntosh et al., 2004
Yr36	Xucw74-6B, Xucw77-6B	J. Dubcovsky, 2004
YrH52	Xgmw273a, UBC212a	Peng et al., 2000
Yrns-B1	Xgwm493	Börner et al., 2000
YrA7	Xgwm88.2, Xucw71	Santra et al., 2005

REFERENCES

Adhikari, L., Raupp, J., Wu, S. Y., Wilson, D., Evers, B., Koo, D. H., Singh, N., Friebe, B., Poland, J. (2022). Genetic characterization and curation of diploid a-genome wheat species. Plant Physiol., 188, 2101-2114.

Agenbag, G. M., Pretorius, Z. A., Boyd, L. A., Bender, C. M., Prins, R. (2012). Identification of adult plant resistance to stripe rust in the wheat cultivar Cappelle-Desprez. Theor Appl Genetics., 125:109-20.

Aime, M. C., McTaggart, A. R. (2021). A higher-rank classification for rust fungi, with notes on genera. Fungal. Syst. Evol., 7, 21-47.

Athiyannan, N., Abrouk, M., Boshoff, W. H. P., Cauet, S., Rodde, N., Kudrna, D., Mohammed, N., Bettgenhaeuser, J., Botha, K. S., Derman, S. S., et al. (2022). Long-read genome sequencing of bread wheat facilitates disease resistance gene cloning. Nat. Genet., 54, 227.

Bariana, H. S., Brown, G. N., Ahmed, N. U., Khatkar, S., Conner, R. L., Wellings, C. R., Haley, S., Sharp, P. J., Laroche, A. (2002). Characterization of *Triticum* vavilovii-derived stripe rust resistance using genetic, cytogenetic and molecular analyses and its marker-assisted selection. Theor. Appl. Genet. 104: 315-320.

Bernardes, M. F. F., Pazin, M., Dorta, L. C. P. D. J. (2015). Impact of pesticides on environmental and human health (chp 8). In: Andreazza AC, Scola G, editors. Toxicology Studies - cells, drugs and environment. London, UK: IntechOpen, pp. 195-233.

Börner, A., Röder, M. S., Unger, O., Meinel, A. (2000). The detection and molecular mapping of a major gene for non specific adult plant disease resistance against stripe rust (*Puccinia striiformis*) in wheat. Theor. Appl. Genet. 100: 1095-1099.

Breitenbach, M. C., Fényes, T. I. (2000). Maize and wheat production trends in SOUTH AFRICA in a deregulated environment. Agrekon., 39:292-312.

Brown, J. K. M., Hovmoller, M. S. (2002). Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science, 297, 537-541.

Carmona, M., Sautau, F., Perez-Hernandez, O., Reis, E. M. (2020). Role of fungicide applications on the integrated management of wheat stripe rust. Front Plant Scie., 11:733.

Chagué, V., Fahima, T., Dahan, A., Sun, G.L., Korol, A. B., Ronin, Y. I., Grama, A., Röder, M. S., Nevo, E. (1999). Isolation of microsatellite and RAPD markers flanking the Yr15 gene of wheat using NILs and bulked segregant analysis. Genome, 42: 1050-1056.

Chen, X. M. (2005). Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat. Can. J. Plant Pathol. 27:314-337.

Chen, X. M. (2007). Challenges and solutions for stripe rust control in the United States. Aust. J. Agric. Res., 58:648-655.

Chen, X. (2013). Review article: High-temperature adult-plant resistance, key for sustainable control of stripe rust. Am. J. Plant Sci., 4, 608-627.

Chen, X. M., Line, R. F. (1995a). Gene action in wheat cultivars for durable high-temperature adult-plant resistance and interactions with race-specific, seedling resistance to stripe rust caused by *Puccinia striiformis*. Phytopathology, 85: 567-572.

Chen, X. M., Line, R. F. (1995b). Gene number and heritability of wheat cultivars with durable, high-temperature, adult-plant resistance and race-specific resistance to *Puccinia striiformis*. Phytopathology, 85: 573-578.

Chen, X. M., Line, R. F. (2000). Developing molecular markers for quantitative trait loci conferring durable, high-temperature, adult-plant resistance in wheat to stripe rust with resistance gene analog polymorphism [online]. In VIIIth International Plant and Animal Genome Conference. 9-12 January 2000, Town and Country Hotel, San Diego, Calif. Available from http://www.intl-pag.org/pag/8/abstracts/pag8874.html.

Chen, X. M., Ling, P. (2004). Towards cloning wheat genes for resistance to stripe rust and functional genomics of *Puccinia striiformis* f. sp. *tritici* [online]. In Proceedings of the 11th International Cereal Rusts and Powdery Mildews Conference. 22-27 August 2004, John Innes Centre, Norwich, UK. European and Mediterranean Cereal Rust Foundation, Wageningen, Netherlands. Cereal Rusts and Powdery Mildews Bulletin, Abstr. A2.10. Available from http://www.crpmb.org/icrpmc11/ abstracts.htm.

Chen, X., Penman, L. (2005). Stripe rust epidemic and races of *Puccinia striiformis* in the United States in 2004. Phytopathology, 95, S19.

Chen, X., Kang, Z. (2017). Introduction: History of research, symptoms, taxonomy of the pathogen, host range, distribution, and impact of stripe rust. In Stripe Rust, Chen, X., Kang, Z., Eds., Springer: Dordrecht, The Netherlands, pp. 1-33.

Chen, X. M., Soria, M. A., Yan, G. P., Sun, J., Dubcovsky, J. (2003). Development of sequence tagged site and cleaved amplified polymorphic sequence markers for wheat stripe rust resistance gene Yr5. Crop Sci. 43: 2058-2064.

Chen, W. Q., Wu, L. R., Liu, T. G., Xu, S. C., Jin, S. L., Peng, Y. L., Wang, B. T. (2009). Race dynamics, diversity, virulence evolution in *Puccinia striiformis* f. sp. *tritici*, the causal agent of wheat stripe rust in China from 2003 to 2007. Plant Dis. 93:1093-1101.

Chen, W., Wellings, C., Chen, X., Kang, Z., Liu, T. (2014). Wheat stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici*. Mol. Plant. Pathol., 15, 433-446.

Chen, X. M., Coram, T., Huang, X. L., Wang, M. N., Dolezal, A. (2013). Understanding molecular mechanisms of durable and non-durable resistance to stripe rust in wheat using a transcriptomics approach. Curr. Genom., 14, 111-126.

Chester, K. S. (1946) The nature and prevention of the cereal rusts as exemplified in the leaf rust of wheat. Chronica Botanica, 269 pp.

Chhetri, M., Bansal, U., Toor, A., Lagudah, E., Bariana, H. (2016). Genomic regions conferring resistance to rust diseases of wheat in a w195/btss mapping population. Euphytica, 209, 637-649.

CPC, (2005). Crop Protection Compendium. CAB International, Wallingford, UK.

Djanaguiraman, M. et al. (2019). Alien chromosome segment from *Aegilops speltoides* and *Dasypyrum villosum* increases drought tolerance in wheat via profuse and deep root system. BMC Plant Biol., 19, 1-15.

Dube, E., Tsilo, T. J., Sosibo, N. Z., Fanadzo, M. (2020). Irrigation wheat production constraints and opportunities in South Africa. Sou Afri Journ Scie., 116:6342.

Eriksen, L., Afshari, F., Christiansen, M. J., McIntosh, R. A., Jahoor, A., Wellings, C. R. (2004). Yr32 for resistance to stripe (yellow) rust present in the wheat cultivar Carstens V. Theor. Appl. Genet. 108: 567-575.

Eriksson, J., Henning, E. (1894). Die Hauptresultate einer neuen Untersuchung über die Getreiderostpilze. Z. Pflanzenkr. 4: 197-203.

FAO (Food and Agricultural Organization), (2017). FAO, The State of Food Security and Nutrition in the World Food and Agricultural Organization, Rome.

Farzand, M., Dhariwal, R., Hiebert, C. W., Spaner, D., Randhawa, H. S. (2022). QTL mapping for adult plant field resistance to stripe rust in the aac cameron/p2711 spring wheat population. Crop Sci., 62, 1088-1106.

Figlan, S., Ntushelo, K., Mwadzingeni, L., Terefe, T., Tsilo, T. J., Shimelis, H. (2020). Breeding wheat for durable leaf rust resistance in southern africa: variability, distribution, current control strategies, challenges and future prospects. Front Plant Scie., 11:549.

Flor, H. H. (1971). Current status of the gene-for-gene concept. Annu. Rev. Phytopathol., 9, 275-296.

Frick, M. M., Huel, R., Nykiforuk, C. L., Conner, R. L., Kusyk, A., Laroche, A. (1998). Molecular characterisation of a wheat stripe rust resistance gene in Moro wheat. In Proceedings of the 9th International Wheat Genetics Symposium. 2-7 August 1998, University of Saskatchewan, Saskatoon, Sask. Edited by A.E. Slinkard. University Extension Press, University of Saskatchewan, Saskatoon, Sask. Vol. 3. pp. 181-182.

Fu, D., Uauy, C., Distelfeld, A., Blechl, A., Epstein, L., Chen, X., Sela, H., Fahima, T., Dubcovsky, J. (2009). A kinase-start gene confers temperature-dependent resistance to wheat stripe rust. Science, 323, 1357-1360.

Fuckel, L. (1860). Enumeratio fungorum Nassovia. Jahrb. Ver. Naturk. Herzogth. Nassau, 15: 9.

Garnica, D. P., Upadhyaya, N. M., Dodds, P. N., Rathjen, J. P. (2013). Strategies for wheat stripe rust pathogenicity identified by transcriptome sequencing. PLoS ONE, 8, e67150.

Gill, H. K., Garg, H. (2014). Pesticides: environmental impacts and management strategies (chp 8). In: Larramendy ML, Soloneski S, editors. Pesticides - toxic aspects. London, UK: IntechOpen, pp. 187-230.

He, C. Y., Zhang, L. J., Du, J. Y., Zhou, X. C., Zhou, J., Bai, B., et al. (2011). Study on Different Typical-resistant Responses of winter wheat to stripe rust at adult stage and their influences on yield. Journal of Triticeae Crops, 31 (4): 757-761.

Herrera-Foessel, S. A., Lagudah, E. S., Huerta-Espino, J., Hayden, M. J., Bariana, H. S., Singh, D., Singh, R. P. (2011). New slow-rusting leaf rust and stripe rust resistance genes lr67 and yr46 in wheat are pleiotropic or closely linked. Theor. Appl. Genet., 122, 239-249.

Hovmoller, M. S., Sorensen, C. K., Walter, S., Justesen, A. F. (2011). Diversity of Puccinia striiformis on cereals and grasses. Annu. Rev. Phytopathol., 49, 197-217.

Hylander, N., Jørstad, I., Nanfeldt, J. A. (1953). Enumeratio uredionearum Scandinavicarum. Opera Bot. 1: 1-102.

Jiang, W., Geng, Y., Liu, Y., Chen, S., Cao, S., Li, W., Chen, H., Ma, D., Yin, J. (2020). Genome-wide identification and characterization of SRO gene family in wheat: Molecular evolution and expression profiles during different stresses. Plant Physiol. Biochem., 154, 590-611.

Khan, M. S., Rizvi, A., Saif, S., Zaidi, A. (2017). Phosphate-solubilizing microorganisms in sustainable production of wheat: Current perspective. Probiot. Agroecosyst., 51-81.

Klymiuk, V., Yaniv, E., Huang, L., Raats, D., Fatiukha, A., Chen, S. S., Feng, L. H., Frenkel, Z., Krugman, T., Lidzbarsky, G., et al. (2018). Cloning of the wheat yr15 resistance gene sheds light on the plant tandem kinase-pseudokinase family. Nat. Commun., 9, 3735.

Krattinger, S. G., Lagudah, E. S., Spielmeyer, W., Singh, R. P., Huerta-Espino, J., McFadden, H., Bossolini, E., Selter, L.L., Keller, B. (2009). A putative abc transporter confers durable resistance to multiple fungal pathogens in wheat. Science, 323, 1360-1363.

Large, E. C. (1940). The Advance of the Fungi. Jonathan Cape, London, p 488.

Li, Q., Jing, J. X., Du, J. Y. (2005). Analysis on Durable Resistance toWheat Stripe Rust of Wheat Cul tivarsL ibel lula and Xiannong. Journal of Triticeae Crops, 25 (6): 112-115.

Li, Y., Xi, K., Liu, X., Han, S., Han, X., Li, G., Yang, L., Ma, D., Fang, Z., Gong, S., et al. (2023). Silica nanoparticles promote wheat growth by mediating hormones and sugar metabolism. J. Nanobiotechnol., 21, 2.

Line, R. F. (2002). Stripe rust of wheat and barley in North America: a retro-spective historical review. Annu. Rev. Phytopathol. 2002. 40:75-118.

Line, R. F., Qayoum, A. (1992). Virulence, aggressiveness, evolution, and distribution of races of *Puccinia striiformis* (the cause of stripe rust of wheat) in North America, 1968-87. U.S. Dep. Agric. Agric. Res. Serv. Tech. Bull. No. 1788.

Line, R. F., Chen, X. M. (1995). Success in breeding for and managing durable resistance to wheat rusts. Plant. Dis., 79 pp. 1254-1255.

Lu, N., Wang, J., Chen, X., Zhan, G., Chen, C., Huang, L., Kang, Z. (2011). Spatial genetic diversity and interregional spread of *Puccinia striiformis* f. sp. *tritici* in Northwest China. Eur. J. Plant Pathol., 131, 685-693.

Ma, J., Zhou, R., Dong, Y., Wang, L., Wang, X., Jia, J. (2001). Molecular mapping and detection of the yellow rust resistance gene Yr26 in wheat transferred from *Triticum turgidum* L. using microsatellite markers. Euphytica, 120: 219-226.

Mahmood, S., Daur, I., Al-Solaimani, S. G., Ahmad, S., Madkour, M. H., Yasir, M., et al. (2016). Plant growth promoting rhizobacteria and silicon synergistically enhance salinity tolerance of mung bean. Front Plant Scie., 7:876.

Marchal, C., Zhang, J. P., Zhang, P., Fenwick, P., Steuernagel, B., Adamski, N. M., Boyd, L., McIntosh, R., Wulff, B. B. H., Berry, S., et al. (2018). Bed-domain-containing immune receptors confer diverse resistance spectra to yellow rust. Nat. Plants, 4, 662-668.

McDonald, D. B., McIntosh, R. A., Wellings, C. R., Singh, R. P., Nelson, J. C. (2004). Cytogenetical studies in wheat. XIX. Location and linkage studies on gene Yr27 for resistance to stripe (yellow) rust. Euphytica, 136: 239-248.

McIntosh, R. A. (1988). Catalogue of gene symbols for wheat. In Proceedings of the 7th International Wheat Genetics Symposium. 14-19 July 1988, Cambridge, UK. Edited by T.E. Miller and R.M.D. Koebner. Institute of Plant Science Research, Cambridge, UK. Vol. 2. pp. 1225-1323.

McIntosh, R. A., Brown, G. N. (1997). Anticipatory breeding for re-sistance to rust diseases in wheat. Annu. Rev. Phytopathol. 35:311-326.

McIntosh, R. A., Devos, K. M., Dubcovsky, J., Rogers, W. J. (2004). Catalogue of gene symbols for wheat: 2004 supplement [online]. Available from http://grain.jouy.inra.fr/ggpages/wgc/ 2004upd.html

Mert, Z., Nazari, K., Karagöz, E., Akan, K., Öztürk, İ., Tülek, A. (2016). First incursion of the warrior race of wheat stripe rust (*Puccinia striiformis* f. sp. *tritici*) to Turkey in 2014. Plant Diseases, 100(2), 528.

Mohammad, W. H., Jaspal K., Ritu, B., Sandeep, S., Puja S., Achla, S., Rohtas, S., Jyoti K. (2023). Stripe rust resistance gene(s) postulation in wheat germplasm with the help of diferentials and tagged molecular markers. Scientifc Reports, 13:9007

Morgounov, A., Braun, H. J., Ketata, H., Paroda, R. (2005). International cooperation for winter wheat improvement in central Asia: results and per-spectives. Turk. J. Agric. For. 29:137-142.

Moscou, M. J., van Esse (2017). The quest for durable resistance. Science, 358, pp. 1541-1542

Nhemachena, C. R., Kirsten J. A. (2017). Historical assessment of sources and uses of wheat varietal innovations in South Africa. Sou Afri Journ Scie., 113:1-8.

Pardey, P. G., Beddow, J. M., Kriticos, D. J., Hurley, T. M., Park, R. F., Duveiller, E., Sutherst, R. W., Burdon, J. J., Hodson, D. (2013). Right-sizing stem-rust research Science, 340, pp. 147-148.

Peng, J. H., Fahima, T., Roder, M. S., Huang, Q. Y., Dahan, A., Li, Y. C., Grama, A., Nevo, E. (2000). High-density molecular map of chromosome region harboring stripe-rust resistance genes YrH52 and Yr15 derived from wild emmer wheat, *Triticum dicoccoides*. Genetica (The Hague), 109: 199-210.

Petit-Houdenot, Y., Fudal, I. (2017). Complex interactions between fungal avirulence genes and their corresponding plant resistance genes and consequences for disease resistance management. Front. Plant Sci., 8, 1072.

Robert, O., Abelard, C., Dedryver, F. (1999). Identification of molecular markers for the detection of the yellow rust resistance gene Yr17 in wheat. Mol. Breed. 5: 167-175.

Saeed, M., Ullah, F., Shah, L., Ahmad, W., Ali, M., Munsif, F., Zubair, A., Ibrahim, M., Shah, S. M. A., Uddin, H., et al. (2022). Identification of three novel qtls associated with yellow rust resistance in wheat (*Triticum aestivum* l.) anong-179/khaista-17 f2 population. Sustainability, 14, 7454.

Santra, D. P., Watt, C., Uauy, C., Kidwell, K. K., Chen, X. M., Dubcovsky, J., Campbell, K. G. (2005). Mapping QTL for high temperature adult-plant resistance to stripe rust in wheat (Triticum aestivum L.) [online]. In XIII Plant and Animal Genome Conference. 15-19 January 2005, Town and Country Convention Centre, San Diego, Calif. Available from http:// www.intl-pag.org/13/abstracts/pag13_p331.html.

Schmidt, J. K. (1827). Allgemeine ökonomisch-technische Flora oder Abbildungen und Beschreibungen aller in bezug auf Ökonomie und Technologie, merkwürdigen Gewächse. Jena, Germany. Vol. I. p. 27.

Seah, S., Bariana, H., Jahier, J., Sivasithamparam, K., Lagudah, E. S. (2001). The introgressed segment carrying rust resistance genes Yr17, Lr37 and Sr38 in wheat can be assayed by a cloned disease resistance gene-like sequence. Theor. Appl. Genet. 102: 600-605.

Shao, Y. T., Niu, Y. C., Zhu, L. H., Zhai, W. X., Xu, S. C., Wu, L. R. (2001). Identification of an AFLP marker linked to the stripe rust resistance gene Yr10 in wheat. Chin. Sci. Bull. 46: 1466-1469.

Shi, Z. X., Chen, X. M., Line, R. F., Leung, H., Wellings, C. R. (2001). Development of resistance gene analog polymorphism markers for the Yr9 gene resistance to wheat stripe rust. Genome, 44: 509-516.

Singh, R. P., William, H. M., Huerta-Espino, J., Rosewarne, G. (2004). Wheat rust in Asia: meeting the challenges with old and new technologies. In: New Directions for a Diverse Planet: Proc. 4th Int. Crop Sci. Congr. Brisbane, Australia. http://www.pngg.org/pp590_790/Singh-4thIntCSCong2004.pdf

Singh, R. P., Nelson, J. C., Sorrells, M. E. (2000). Mapping Yr28 and other genes for resistance to stripe rust in wheat. Crop Sci. 40: 1148-1155.

Smith, P. H., Koebner, R. M. D., Boyd, L. A. (2002). The development of a STS marker linked to a yellow rust resistance derived from the wheat cultivar Moro. Theor. Appl. Genet. 104: 1278- 1282.

Steele, K. A., Humphreys E., Wellings C. R., et al. (2001). Support for a stepwise mutation model for pathogen evolution in Australasian *Puccinia striiformis* f. sp. tritici by use of molecular markers. Plant Pathol, 50, pp. 174-180.

Stubbs, R. W. (1985). Stripe rust. Page 61-101 in: The Cereal Rusts II. Dis-eases, Distribution, Epidemiology and Control. A. P. Roelfs and W. R. Bushnell, eds. Academic Press, New York.

Stubbs, R. W. (1988). Pathogenicity analysis of yellow (stripe) rust of wheat and its significance in a global context. Pages 23-38 in: Breeding Strategies for Resistance to the Rusts of Wheat. N. W. Simmonds and S. Rajaram, eds. CIMMYT, Mexico DF, Mexico.

Suenaga, K., Singh, R. P., Huerta-Espino, J., William, H. M. (2003). Microsatellite markers for genes Lr34/Yr18 and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology, 93: 881-890.

Sun, Q., Wei, Y., Ni, C., Xie, C., Yang, T. (2002). Microsatellite marker for yellow rust resistance gene Yr5 introgressed from spelt wheat. Plant Breed. 121: 539-541.

Tadesse, W., Bishaw, Z., Assefa, S. (2019). Wheat production and breeding in Sub-Saharan Africa. Int Journ Clim Chan Strat Manag., 11:696-715.

Uauy, C., Brevis, J. C., Chen, X. M. et al. (2005). High-temperature adult-plant (HTAP) stripe rust resistance gene Yr36 form *Triticum turgidum* ssp. *dicoccoides* is closely linked to the rain protein content locus Gpc-B1. Theor Appl Genet, 112, 97-105.

Ul Islam, B., Mir, S., Dar, M.S., Khan, G.H., Shikari, A.B., Sofi, N.u.R., Mohiddin, F., Ahangar, M.A., Jehangir, I.A., Kumar, S., et al. (2023). Characterization of Pre-Breeding Wheat (*Triticum aestivum* L.) Germplasm for Stripe Rust Resistance Using Field Phenotyping and Genotyping. Plants, 12, 3239. https://doi.org/10.3390/plants12183239.

Wan, A., Zhao, Z., Chen, X. M., He, Z., Jin, S., Jia, Q., Yao, G., Yang, J., Wang, B., Li, G., Bi, Y., and Yuan, Z. (2004). Wheat stripe rust epidemic and virulence of *Puccinia striiformis* f. sp. *tritici* in China in 2002. Plant Dis., 88:896-904.

Wang, L. F., Ma, J. X., Zhou, R. H., Wang, X. M, Jia, J. Z. (2002). Molecular tagging of the yellow rust resistance gene Yr10 in common wheat, P.I. 178383 (*Triticum aestivum* L.). Euphytica, 124: 71-73.

Wang, H., Zou, S. H., Li, Y. W., Lin, F. Y., Tang, D. Z. (2020). An ankyrin-repeat and wrky-domain-containing immune receptor confers stripe rust resistance in wheat. Nat. Commun., 11, 1353.

Wellings, C. R. (2011). Global status of stripe rust: a review of historical and current threats. Euphytica 179:129-141.

Westendorp, G. D. (1854). Quatrième notice sur quelques Cryptogames récemment découvertes en Belgique. Bull. Acad. R. Sci. Belg. 21: 229-246.

Xian-xin, W. U., Chao-qun, Zang, Ya-zhao, Zhang, Yi-wei X. U., Shu, Wang, Tian-ya L. I., Li, G. A. O. (2023). Characterization of wheat monogenic lines with known Sr genes and wheat cultivars for resistance to three new races of *Puccinia graminis* f. sp. *tritici* in China. Journal of Integrative Agriculture, 22 (6), 1740-1749.

Yan, G. P., Chen, X. M., Line, R. F., Wellings, C. R. (2003). Resistance gene analog polymorphism markers co-segregating with the Yr5 gene for resistance to wheat stripe rust. Theor. Appl. Genet. 106: 636-643.

Yin, C. T., Hulbert, S. (2011). Prospects for functional analysis of effectors from cereal rust fungi. Euphytica, 179, 57-67.

Yin, J. L., Fang, Z. W., Sun, C., Zhang, P., Zhang, X., Lu, C., Wang, S. P., Ma, D. F., Zhu, Y. X. (2018). Rapid identification of a stripe rust resistant gene in a space-induced wheat mutant using specific locus amplified fragment (SLAF) sequencing. Sci. Rep., 8, 3086.

Yin, X., Yuan, Y., Han, X., Han, S., Li, Y., Ma, D., Fang, Z., Gong, S., Yin, J. (2023). Genome-Wide Identification, Characterization, and Expression Profiling of TaDUF668 Gene Family in *Triticum aestivum*. Agronomy, 13, 2178.

Zakari, A, McIntosh, R. A., Hovmoller, M. S., Wellings, C. R., Shariflou, M. R., Hayden, M., Bariana, H. S. (2003). Recombination of Yr15 and Yr24 in chromosome 1BS. In Proceedings of 10th International Wheat Genetics Symposium. 1-5 September 2003, Rome, Italy. Edited by N.E. Pogna, N. Romano, E.A. Pogna, and G. Galterio. Instituto Sperimentale per la Cerealcoltura, Rome, Italy. Vol. 1. pp. 417-420.

Zhan, G., Ji, F., Chen, X., Wang, J., Zhang, D., Zhao, J., Zeng, Q., Yang, L., Huang, L., Kang, Z. (2022). Populations of *Puccinia striiformis* f. sp. *tritici* in Winter Spore Production Regions Spread from Southwestern Oversummering Areas in China. Plant Dis., 106, 2856-2865.

Zheng, S.G., Wu, Y., Zhou, M., Zeng, L., Liu, R., Li, Y.F., Liu, Z.H., Zhang, C.H., Lu, L., Zhang, L. (2020). Characterization and diagnostic marker development for yr28-rga1 conferring stripe rust resistance in wheat. Eur. J. Plant. Pathol., 156, 623-634.

Wheat Powdery Mildew (*Blumeria graminis* f. sp. *tritici*) Disease and Resistance Genes

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ABSTRACT: Wheat (*Triticum aestivum* L.), belonging to the Gramineae family, has high nutritional value and yield. It constitutes 35% of the world's total cereal production, providing food for about 30% of the world's population and accounting for more than 20% of the calories consumed by humans. It ranks third in the world in terms of production and second only to rice and maize. During its growth and development, wheat is affected by various biotic and abiotic stresses such as high temperature, drought, salinity, pests and diseases that severely affect the quality and yield of wheat. Fungal diseases are the most important biotic factors that are problematic in wheat fields. Among the biotic stresses, the obligate biotrophic fungal pathogen *Blumeria graminis* f. sp. *tritici* [Bgt] (syn. *Erysiphe graminis* (DC) f. sp. *tritici* EJ MarchalBgt) is one of the most damaging wheat diseases. It causes serious economic losses in wheat production and causes approximately 10-40% yield loss. Favorable conditions can cause the disease to cover the upper surface of the leaf and thus wither and weaken the host, inhibit the development of wheat roots and reduce tiller formation. In addition, infections at the spike and flowering stages can reduce the number of grains in the spike, grain filling and weight. The most economical and environmentally friendly way to control the disease is to spread the transmission of genetic resistance. In this study, we present the biology of Pgt and breeding for resistant varieties as an alternative, preventive, effective and sustainable control method for the future and resistance genes in wheat plant, which is important in human nutrition.

Keywords: Wheat (*Triticum aestivum* L.), Fungia, Disease, *Blumeria graminis* f. sp. *tritici*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important commercial crop, providing food for about 30% of the world's population and accounting for more than 20% of the calories consumed by humans (Arzani and Ashraf, 2017). Wheat, an important staple dietary crop, is widely grown in many parts of the world. The world population is expected to grow to 9 billion by 2050. Current wheat yield increases are estimated to be around 0.5% to 1% per year, below the 2.4% needed to meet global demand for this commodity (Ray et al., 2013; Crespo-Herrera et al., 2018). As a result, wheat production needs to increase by up to 70% to meet projected global demand for wheat products by 2050 (Hunter et al., 2017). The average yield of wheat has remained stable in recent years, by up to 40%. This suggests that current production and productivity rates are not sufficient to ensure future food security. Wheat yields are crucial for ensuring global food security (Alam et al., 2014). Arable agricultural land scarcity, water resources pollution and pressure, and climate change limit the potential to increase production by expanding production areas. Furthermore, there are a number of reasons that reduce wheat yield potential, including biotic and abiotic factors that cause low productivity of wheat (Mondal et al., 2016; Singh et al., 2016). Therefore, to increase yield potential, it is necessary to develop a new generation of wheat varieties with improved tolerance/resistance to multiple stresses such as resistance to diseases, pests, soil alkalinity and salinity, and nitrogen use efficiency.

Powdery mildew (*Blumeria graminis* f. sp. *tritici*), septoria leaf spot (*Septoria tritici*) and rust diseases (*Puccinia striiformis* f. sp. *tritici*, *P. recondita* f. sp. *tritici*, *P. graminis* f. sp. *tritici*) are the main biotic factors limiting wheat production in the world and Turkey (Cowger, 2018; Schwessinger, 2017). Among these, Bgt disease (*Blumeria graminis* (DC) E.O. Speer f. sp. tritici Em. Marchal (synm. *Erysiphe graminis* DC. f. sp. tritici Em. Marchal)) is one of the most economically important diseases of wheat. It can cause crop losses of 34% or more (Alam et al., 2013; Cao et al., 2013; Shen et al., 2015). Bgt is a common cereal disease in temperate and rainy areas. Bgt forms dotted white gray pustules on plant leaves. Under favorable development conditions, the pustules merge and can cover the entire leaf and even reach the stem and spike. Depending on the disease race, the severity of development due to environmental conditions and the development period in which the plant is exposed to infection, the photosynthesis rate and assimilation index of the leaves decrease. As a result, it causes a decrease in grain yield and quality (Samobor et al., 2006). Wheat powdery mildew caused by the obligate biotrophic fungal pathogen Bgt is one of the most damaging wheat diseases (Basandrai and Basandrai, 2017; Mazzucotelli et al., 2021; Zhao et al., 2020). It causes serious economic losses in wheat production, resulting in yield losses of about 10-40% (Mapuranga et al., 2022). The genus *Blumeria* is monophyletic, meaning that it includes a single species, "*Blumeria graminis*", which is divided into eight forma-specific subdivisions that infect grasses and cereal crops, including wheat, barley, oats and rye (Menardo et al., 2017). Wheat can be infected by *Bg triticale* as well as *Bg dicocci* (tetraploid durum wheat), a hybrid between wheat and rye mildew with an extended host range that can infect triticale and wheat (Menardo et al., 2016). This impact is large because breeding for PM resistance in wheat makes no distinction between these specialized forms and their prevalence across different varieties and in different regions is largely unknown. Small and marginal farmers in developing countries cannot afford expensive spraying programs with high input prices due to resource constraints. Fungicides, which are frequently used in developed countries, have bad effects on human health and soil, causing water and environmental pollution. Due to these impacts, there is increasing awareness of the rapid development of fungal resistance strains (Mehta et al., 2022). Under these circumstances, the development and dissemination of Bgt-resistant wheat varieties is an environment and farmer-friendly, practically feasible and practicable, stable and economically viable alternative to manage and reduce losses due to the disease. In order to reduce losses caused by Bgt, much effort has been made to study the genetic molecular mechanisms of wheat powdery mildew so that effective control measures can be developed (Fu et al., 2016; Liu et al., 2016; Chen et al., 2018; Hu et al., 2018; Zou et al., 2018; Zheng et al., 2020a; Sánchez et al., 2021; Li et al., 2022a). In particular, resistance to PM in general follows the gene-for-gene hypothesis (Flor, 1971), eliciting boom and bust cycles that require a constant search for new genes. It is governed by race-specific master genes (Li et al., 2019) that confer resistance to Bgt isolates with corresponding avirulent genes. Furthermore, Bgt is a destructive pathogen because it is highly adaptable with the potential for mutability (Mc Donald and Linde, 2002; Laloševi´c et al., 2022). In temperate regions, the presence of the teleomorph (Sharma et al., 1990; Basandrai and Basandrai, 2017) and rapid and massive conidial production can result in a high mutation rate. The evolution of new and matching virulences results in their rapid spread. It can sensitize resistant varieties within a short period of their large-scale cultivation. To ensure a successful wheat breeding program aimed at developing PM-resistant varieties, continuous Bgt virulence monitoring is necessary. Knowledge of virulence dynamics and changes in Bgt populations is highly desirable for large-scale epiphytotic predictions as well as for the selection of effective race-specific major Pm genes and the timely and spatial replacement of ineffective progenitors (Babayants et al., 2015). Consequently, the development of Bgt resistant cultivars based on a better understanding of mildew populations and how adapted and non-adapted specific forms interact with each other should lead to better methods for discovering new and novel sources of genetic resistance to PM. The aim of this study was to demonstrate that breeding for PM-resistant cultivars is a preventive, effective and sustainable control method for the future and to present resistance genes as an alternative in wheat crops of human nutrition importance.

The Disease

Taxonomy and biology

Table 1. *Blumeria graminis* f. sp. *tritici* Marchal (syn. *Erysiphe graminis* (DC) f. sp. *tritici* Marchal) taxonomy (Basandrai et al., 2023)

Kingdom	**Fungi**
Class	Leotiomycetes
Order	Erysiphales
Family	Erysiphaceae
Genera	*Blumeria*
Species	*Graminis*
Sub species	*tritici*

Powdery mildew (*Blumeria graminis* f. sp. *tritici*), septoria leaf spot (*Septoria tritici*) and rust diseases (*Puccinia striiformis* f. sp. *tritici*, *P. recondita* f. sp. tritici, *P. graminis* f. sp. tritici) are the main biotic factors limiting wheat production in the world and Turkey (Cowger, 2018; Schwessinger, 2017). Among these, Bgt disease (*Blumeria graminis* (DC) E.O. Speer f. sp. *tritici* Em. Marchal (synm. *Erysiphe graminis* DC. f. sp. *tritici* Em. Marchal)) is one of the most economically important diseases of wheat. It can cause crop losses of 34% or more (Alam et al., 2013; Cao et al., 2013; Shen et al., 2015). Bgt is a common cereal disease in temperate and rainy areas. Bgt forms dotted white gray pustules on plant leaves. Under favorable development conditions, the pustules merge and can cover the entire leaf and even reach the stem and spike. Depending on the disease race, the severity of development due to environmental conditions and the development period in which the plant is exposed to infection, the photosynthesis rate and assimilation index of the leaves decrease. As a result, it causes a decrease in grain yield and quality (Samobor et al., 2006). Bgt powdery mildew can reproduce both asexually and sexually. This leads to the production of asexual conidial and sexual ascospores, respectively (Jankovics et al., 2015). Grain powdery mildew [*Blumeria graminis* (DC.) Wint.] forms a superficial mycelial cover on the host. Mycelia send their suckers to the epidermis cells. Mycelia developing in the epidermis form conidiophores. At the end of these, conidiospores are formed, arranged like rosary beads. The matured conidiospores separate and cause new infections. When the host begins to turn yellow, mycelial masses form cleistothecia. Each cleistothecium contains an average of 15-20 ascus (Tob, 2023). The most important destructive infections are caused by the release of sexual ascospores from fruiting bodies called chasmothecia, which infect cultivated plants in the fall and spring. Sexual ascospores usually appear in the asci within 3 to 5 days after exposure to high humidity (Sánchez-Martín et al., 2018). After the resting period in the ascus, 4-8 ascospores are formed under specific conditions. They infect the grain and cause the illness (Tob, 2023). Secondary infections are caused by the appearance of germ tubes that elongate and differentiate into a structure called appressorium. The hyphae differentiate after an average of 6 days to form a conidial structure called a conidiophore. This conidial structure matures between 8 and 10 days (Sánchez-Martín et al., 2018). Typical mold colonies start as small whitish round spots that can be surrounded by chlorosis. They then turn tan or brown. As the lesion ages, the mycelium expands in diameter and becomes denser. Then, over time, the mycelium turns gray, especially on the leaves and heads.

High relative humidity (50-100%) and low temperatures between 15 and 25 °C are optimal conditions for powdery mildew development. Temperatures above 25 °C delay the development of Pgt disease (Weise et al., 1977; Draz et al., 2019). PM outbreaks occur alternately during the winter, spring and summer seasons when there is some wind to ensure the effective spread of conidia throughout the growing season. Wheat PMs are host-specific and can only grow on one host species, with the only exception being Bg triticale. Bgt fungus survival is usually in the form of immobile mycelium or protective structures (chasmothecia) on wheat hosts. Primary infections involve chasmothecia (135 to 224 µm in diameter). Chasmothecia are produced in the mycelium in late spring or summer and are resistant to extreme weather conditions and moisture loss, thus serving as an important survival mechanism and source of inoculum for the following season (Shi et al., 2016). Rising temperatures (3-31 °C) with ~100% relative humidity and an optimum of 15 °C occurring in spring allow mycelium in winter dormancy to start growing and rapidly form conidia (Sánchez-Martín et al., 2018). While in temperate areas the agent overwinters as mycelia on plants, in other areas it overwinters as cleistothecium. When plants turn yellow, the cleistothecenium spends the winter on dry plant leaves. They form ascus and ascospores in the spring as the weather warms up and cause the main infections. Primary infections, however, are not significant in disease development. Primary infections are mostly caused by mycelia on infected cereal leaves during the fall. Conidiospores, which have been formed from these mycelia, are dispersed by the wind in the spring, leading to secondary infections. The ideal temperature for stretching of conidiospores is 10°C (0-35°C). For infections, 15-20°C is the optimum temperature (Tob, 2023). Overwintering of the Chasmothecia fungus and overexposure of mycelium and conidia in summer allow the pathogen to survive unfavorable periods (Sánchez-Martín et al., 2018). The disease can have a devastating impact on grain yield and quality (Walczak et al., 2019). Severe infections can cause yield and quality deterioration that can result in leaf death (Pietrusińska and Tratwal, 2020).

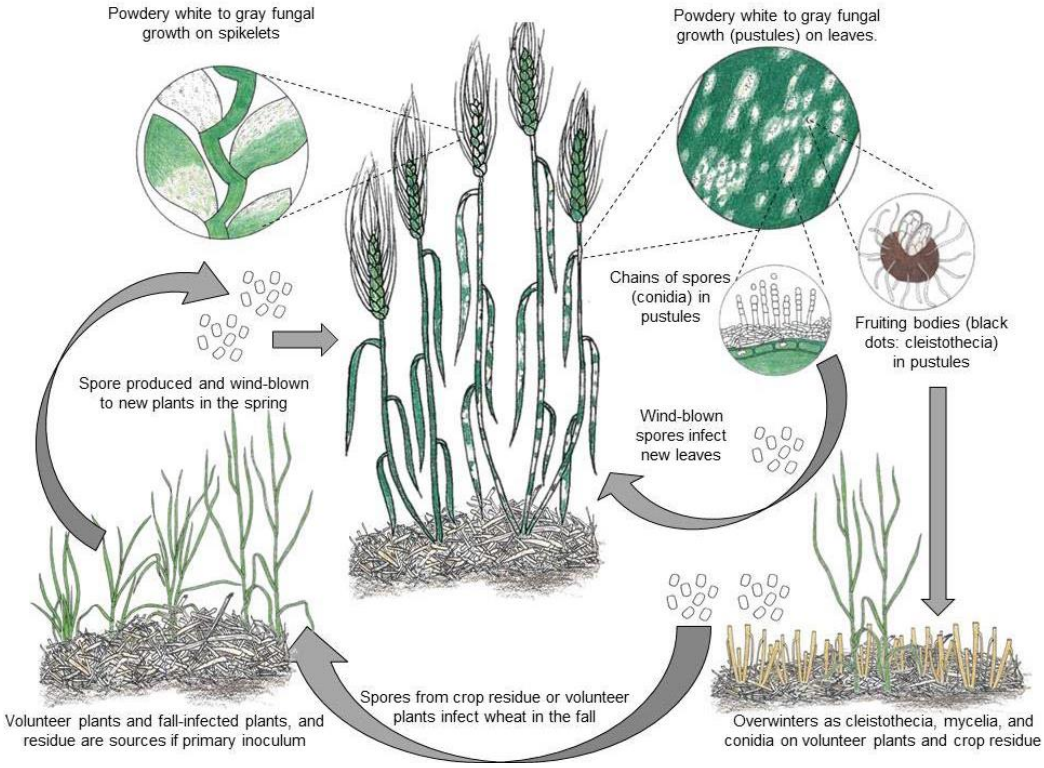

Figure 1. Powdery mildew disease cyle (Salgado, 2016)

Host, Symptoms, disease development, and signs of the pathogen

Wheat powdery mildew (Bgt), which has spread to all grain-growing regions of the world, is specialized according to plant species. Therefore, *B. graminis* f.sp. *tritici* (synm. *Erysiphe graminis*) in wheat is specific to wheat and does not infect other grassy species. Fungus (*B. graminis*) is divided into six forms. Additionally, there are many physiological races under each form. *B. graminis* f. sp. *tritici* in wheat, *B. graminis* f. sp. *hordei* in barley, *B. graminis* f. sp. *secalis* in rye, *B. graminis* f. sp. *avenae* in oats, *B. graminis* f. sp. bromi in *Bromus* spp., *B. graminis* f. sp. *poae* in Poa spp. causes disease (Tob, 2023). The disease can occur at all times after germination of the plant and the conspicuous symptoms can appear on the leaves, but can develop on all leaves such as glumes, leaf sheath, spikelets and awns (Piarulli et al., 2012; Basandrai and Basandrai, 2017). The disease is characterized by cottony white mycelium and conidiospore colonies, which turn dull gray-brown later in the season (Figure 2). Plants with severe infections are stunted (Agrios, 2005). Ascospores are sexual spores produced from cleistothecia. Conidia serve as the primary source of inoculum in early spring and are dispersed by wind. High relative humidity (RH) is required for germination of conidia and ascospores (not free water) (Abdullah, 2015). The pathogen produces large conidiospores throughout the season of production activity. The cycle is usually completed every 7-10 days (Lipps, 2010). Bgt, genetic recombination (Sharma et al., 1990; Perugini, 2007; Li et al., 2012) or teleomorphs are present. If functional, it can show great variability that continues to occur due to other mechanisms such as mutation, parasexuality, etc. Temperature < 25 °C and relative humidity > 45% have a significant impact on PM epiphytotics (TeBeest et al., 2008; Mehta et al., 2018).

Figure 2. Bgt symptom and spore (Abdullah, 2015; Basandrai et al., 2023)

Damage of the pathogen

Turkey, which is one of the homelands of durum wheat, is one of the countries that can produce high quality durum wheat in the world due to its suitable ecology. However, in order to produce the desired quality and yield, existing varieties need to be improved in terms of both disease resistance and quality (Yıldırım et al., 2019). For this purpose, local varieties (Allard and Bradshaw, 1964), which are adapted to the regions where they grow and have genetic variation even within themselves, are of great importance in terms of re-establishing the shrinking genetic variation in wheat. In addition, it has been determined that local cultivars have resistance genes against many abiotic and biotic stress factors (Hart, 2001). The majority of our current durum varieties grown in Turkey are susceptible to Bgt. The most important reason for this is that priority and weight is given to diseases such as rust diseases which are economically more important than powdery mildew in breeding studies. However, in suitable ecologies, powdery mildew can cause yield loss in wheat ranging from 13% to 34% (Griffey and Das, 1994; Yıldırım et al., 2004). In addition to yield loss, the importance of the disease increases even more when the quality loss in wheat grain and straw is taken into consideration.

At first, the upper surface of the lower leaves has dotted white-gray pustules, but gradually they turn brown. When conditions are favorable, pustules may merge and completely cover the leaf or infect the stems and spikes. Diseased leaves önce bölgesel yeşillik gösterdikten sonra necrosis gösterirler. Typical is the appearance of green areas on the leaf (green island formation). Wind, rain, and friction can disperse the mycelial cover, which forms the plant's surface layer. Infected plants are more prone to lodging and also lose yield because necrosis reduces the assimilation surface (Tob, 2023). PM is an obligate biotroph that is highly dependent on the live host plant to complete its life cycle and cause damage, unlike nectotrophs that kill host cells (Dean et al., 2012). Suitable conditions (Temperature < 25 °C and relative humidity > 45%) cause Bgt to cover the upper surface of the leaf, thus wilting and weakening the host plant and deteriorating yield and quality. PM symptoms usually appear first on the oldest lower leaves. Bgt fungus progresses with plant growth and damages the upper leaves, heads and awns of susceptible varieties (Cunfer, 2002). Shaded low areas that trap moist air due to dense planting, high plant density and poor air circulation facilitate the development of this disease. PM infections occurring at the seedling stage prevent the growth and development of wheat. In severe infections, photosynthesis is blocked and plants may die. In addition, infections occurring during the tillering stage can inhibit the development of wheat roots. It may reduce tillering. Additionally, infections occurring during the heading and flowering stages may affect yield and quality by reducing the number of grains in the spike, grain filling and weight (Feng et al., 2014). In general, PM outbreaks result in reduced grain yield and yield-related traits (number of pods, number of grains per spike, grain weight, number of grains, grain filling) and losses in grain quality. It can also affect quality parameters (e.g. wheat processing, milling, cooking quality) (Cunfer, 2002; Asad et al., 2014; Gao et al., 2014a). The conversion of sugar to starch in wheat grains is suppressed by PM at the early infection stage. At the later infection stage, a sufficient substrate for starch synthesis is available in susceptible cultivars (Gao et al., 2014b).

Geographical distribution, economic importance and control

Directional selection increases the frequency of virulent pathotypes. These then spread to border regions through natural or human selection, i.e. mediated gene flow (Gao et al., 2014a). Bgt disease is widespread in regions with temperate, cool and humid climatic zones, such as Africa, Asia, the United States, Europe, Africa and Oceania (Yang et al., 2017; Zeybek et al., 2017; Shahin et al., 2019). In epidemics occurring worldwide, Bgt reduces photosynthesis in leaves. Consequently, it delays the leaf assimilation index and causes a decrease in grain yield due to its negative effects on grain components (Bowen et al., 1991). In wheat, Bgt disease greatly affects grain yield by reducing spike number, spike fullness, grain size and weight. It also affects the number of fertile spikes and tillers. The highest and most important economic impact of Bgt disease occurs when the crop is infected at tillering, stem elongation and booting. During the infection process, diseased plants tend to increase metabolism, resulting in lower storage compounds in the grain, smaller grains and ultimately yield losses (Bowen et al., 1991; Draz et al., 2019). Bgt can cause grain yield losses of up to 13-100 percent. The disease agent occurring before and/or during the flowering stage can cause more intense and severe losses (Alam et al., 2011; Mwale et al., 2014; Laloševi´c et al., 2022). Yield losses due to Pgt reached 62% in Brazil, 50% in Denmark, 40% in China, 35% in Russia and 23% in Egypt (Shahin et al., 2019; Nordestgaard et al., 2021). The highest yield losses were reported in Western and Southern Europe (93%) and Central and Eastern Europe (72%) (Morgounov et al., 2012). Tang et al. (2017) reported that the percentage of wheat production area affected by Bgt in China increased by 8.5% per decade from 1970 to 2012. In the last decade, about 8 million hectares in China have become infected with Bgt (Meyer et al., 2021). These situations indicate the re-emergence of PM as a global food security threat. It shows that food security is threatened when Bgt causes epidemics. Therefore, global wheat breeding programs need to prioritize preventive, effective and environmentally friendly methods to control and combat the disease. To successfully control the fungal agent of Bgt, the etiology, pathogen and virulence mechanisms/spectrum, host and resistance mechanisms of the fungal agent should be well known. In addition, PM can be effectively controlled if environmental factors favoring pathogenesis and factors affecting the proliferation of Bgt resistance are understood. Cultural and chemical methods are used as control methods. Within the scope of cultural control, especially dense planting should be avoided and balanced fertilization should be made according to the results of soil analysis, especially excessive nitrogen fertilization should be avoided. In chemical control, spraying time, dose, technique, time and plant protection products and tools and machines to be used are important. Symptoms of powdery mildew disease can be seen every year under favorable conditions. For this reason, the course of the disease should be monitored by considering the climatic conditions as the time of spraying, and in cases where the disease progresses towards the upper part of the plant, green parts spraying should be started in order to prevent the upper leaves, especially the flag leaf, from being infected with the disease. In the spraying technique, sprays should be applied at the recommended doses to ensure that the upper and lower surfaces of the leaves are covered. Plant protection products and doses recommended in the books published by the Ministry of the relevant countries are used. Spraying should be done with ground tools (Tob, 2023). Especially for large cultivation areas, hydraulic field sprayers with high work width should be used for spraying. The survivorship of the disease is determined by walking along the diagonals of the sprayed barley or wheat fields 15-20 days after the spraying and determining the severity of the disease on the 3rd leaf from the top of 100 plants if the disease shows a homogeneous spread in the field, otherwise on the 3rd leaf from the top of 200 plants according to the following 0-5 scale (Aktaş, 2001). If there is no increase in disease level, the application is considered successful (Tob, 2023).

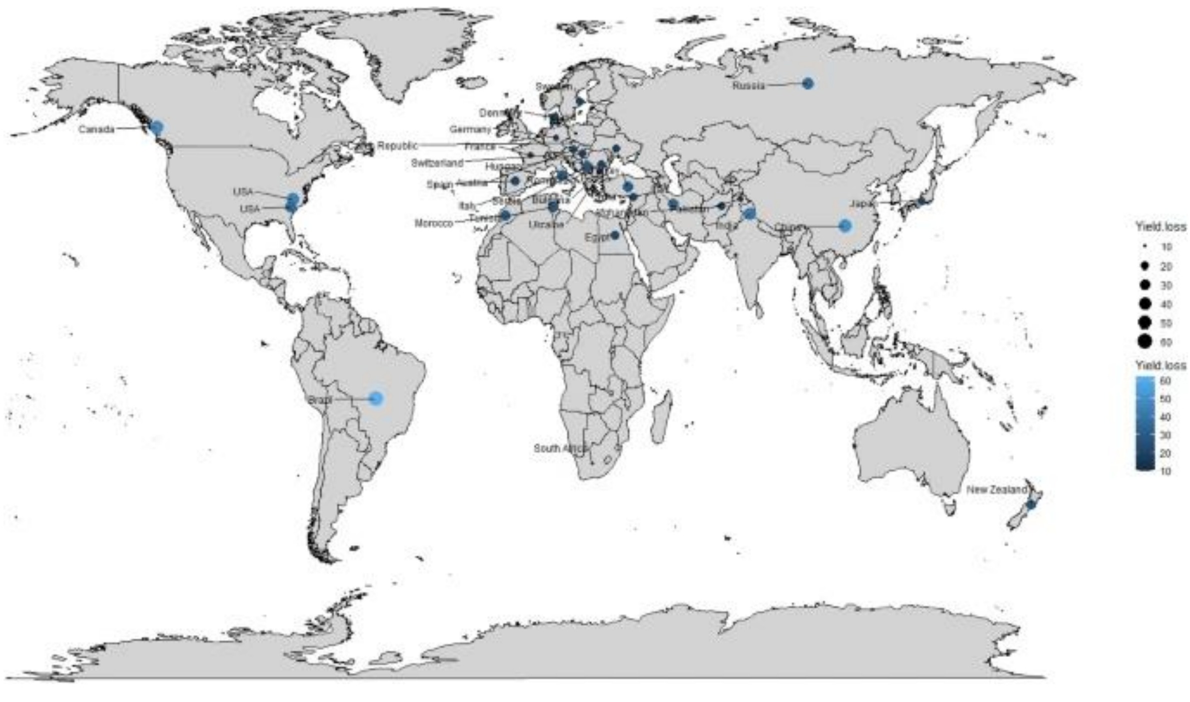

Figure 3. World distribution of wheat Bgt and yield losses (Basandrai et al., 2023)

In addition to cultural measures, chemical control is widely used in the fight against the disease. Chemical control may not be economical in small production farm areas. Fungicides used in chemical control are applied as seed and green parts spraying. However, seed application is usually not sufficient to control the disease throughout the season. On the other hand, green parts applications are not preferred because they are not economical. Again, the negative effects of chemical control on the environment and human health and the increase in social sensitivity against it have brought the use of resistant varieties to the forefront in the fight against this pathogen (Xiao et al., 2013). Specific major gene resistance to pathogen races and many resistance genes (pm genes) (such as pm1a- pm47) have been identified and used in breeding programs (Xiao et al., 2013). However, the pathogen can break these major gene resistance genes by developing new races, causing severe infections and increasing crop losses (McDonald and Linde, 2002a). Therefore, the development of new sources of resistance or new resistant varieties is of great importance in the control of the agent.

There are 78 resistance genes (Pm1- Pm53, Pm18 = Pm1c, Pm22 = Pm1e, Pm23 = Pm4c, Pm31 = Pm21) identified and mapped at 50 loci in wheat so far, 27 of which were transferred from closely or distantly related species of wheat (Sönmezoğlu et al., 2010; Elkot et al., 2015). Of the 50 loci identified, 11 were mapped to the A genome, 26 to the B genome and 13 to the D genome of wheat (Elkot et al., 2015). The majority of Bgt resistance genes studied in wheat show major (single) gene characteristics. Increasing the number of genes used in wheat breeding is very important in terms of ensuring economic and long-term resistance. In Turkey, Bezostaja-1 is one of the varieties known to be resistant to Bgt and widely produced. This variety, which has been in production for more than thirty years, has the Pm8 gene located on the 1RS/1BL translocation chromosome (rye/wheat) (Wricke et al., 1996). Bgt is one of the most important diseases that negatively affect the yield and quality of wheat. Bgt is common in regions with the highest humidity in spring. Economically, it is a disease that cannot be controlled chemically. Sönmezoğlu et al. (2019), aimed to determine the resistance of local durum wheat varieties of Turkey, which is one of the important gene sources of durum wheat, against powdery mildew. In this context, 49 local durum wheat varieties collected from different regions of Turkey and Bezostaja, Variety 1252, Tahirova-2000, Gediz 75 and Sofu varieties were used as control. Local cultivars collected from different locations and inoculated with Bgt spores representing the possible races in Turkey were analyzed for their reaction types against the disease. As a result, one local cultivar (TR 31729) was found to have single gene resistance against Bgt.

Wheat resistance genes against *Pst*

The use of variety mixtures and the resistance gene pyramid are two well-documented approaches to genetically control wheat powdery mildew (Terefe et al., 2019; Pietrusińska et al., 2011; Koller et al., 2018). The gene pyramid in particular remains the most feasible, environmentally and economically easiest way to control Bgt and ensure durable resistance (Pietrusińska et al., 2017, 2018; Zheng et al., 2020b). The majority of Bgt resistance genes studied in wheat show major (single) gene characteristics. This is especially important as the use of a single gene is transient due to evolving PM virulent races. For example, for Western Australian isolates, Pm2, Pm3a, Pm3e, Pm4a, Pm13 and Pm27 (Golzar et al., 2016), Pm3 alleles (Pm3a, Pm3b, Pm3c, Pm3d and Pm3f) (Li et al., 2016), Pm6 (Purnhauser et al., 2011) and only a limited number of PM resistance genes are still active, including Pm2, Pm4b and Pm8 (Mwale et al., 2017) and Pm1a in SA (Kloppe et al., 2022). There are 78 resistance genes (Pm1- Pm53, Pm18 = Pm1c, Pm22 = Pm1e, Pm23 = Pm4c, Pm31 = Pm21) identified and mapped at 50 loci in wheat so far, 27 of which were transferred from closely or distantly related species of wheat (Sönmezoğlu et al., 2010; Elkot et al., 2015). Of the 50 loci identified, 11 were mapped to the A genome, 26 to the B genome and 13 to the D genome of wheat (Elkot et al., 2015). In a recent study, 45% of 15,944 bread wheat genotypes screened in India were found resistant to PM isolate (Vikas et al., 2020). In the West Siberian region, adult plant resistance (APR) was observed in only 6% (six out of 97) of the cultivars (Leonova et al., 2019), which represents a small fraction of effective PM resistance genes (Leonova et al., 2019). Furthermore, only 5% (59 out of 1297) of landraces in the USA showed resistance to PM (Li et al., 2016). Plants use a variety of mechanisms to resist pathogens, including race-specific, non-race-specific, qualitative and quantitative resistance. The genetic status of both (host plant and pathogen race) determines and influences the outcome of this host-pathogen interaction. Table 2 presents the reported race-specific and non-race-specific genes for Bgt. In recent years, experts have turned their attention to major Pm resistance genes that are assumed to be race-specific or qualitative. For example, the Pm3 resistance gene (and its alleles) has been widely studied because it is simply inherited, transient and easy to manipulate. It expresses complete resistance, usually associated with a hypersensitive response that is effective against several pathogen races and can be easily overcome by new virulent pathogen races (Koller et al., 2018; Simeone et al., 2020). Currently, studies have shifted focus to adult plant resistance, non-race-specific resistance, as well as durable resistance, which involves the interaction of multiple genes that delay and reduce infection, growth and multiplication of the fungus during the adult plant period (Jakobson et al., 2012). However, more than 240 Bgt resistance genes have now been identified on all 21 wheat chromosomes. However, they are not evenly distributed on each chromosome (Table 2). In Turkey, Bezostaja-1 is one of the varieties known to be resistant to Bgt and widely produced. This variety, which has been in production for more than thirty years, has the Pm8 gene located on the 1RS/1BL translocation chromosome (rye/wheat) (Wricke et al., 1996).

Table 2. Race-specific and non-specific resistance germplasm source, associated genes and references of the Bgt disease

Reported Genes	**Germplasm Source**	**References**	**Reported Genes**	**Germplasm Source**	**References**
	Race-specific resistance			**Race-specific resistance**	
PM2	*Aegilops squarosa*	(Hsam et al., 1995)	*PM32*	*A. spelltoides*	(Hsam et al., 2003)
PM8	*Secale cereale*		*PM33*	*T. turgidum*	(Zhu et al., 2005)
PM17	*S. cereale*		*PM34*	*A. tauschii*	(Miranda et al., 2006)
PM19	*A. squarosa*		*PM35*	*A. tauschii*	(Miranda et al., 2007)
PM3a-pm3j	*Triticum aestivum* L.	(Yahiaoui et al., 2004)	*PM36*	*T. turgidum*	(Blanco et al., 2008)
PM4	*T. aestivum* L.	(Sánchez-Martín et al., 2021)	*PM37*	*T. timopheevii*	(Perugini et al., 2008)
PM4b, 4c	*T. aestivum* L. (RE714)	(Keller et al., 1999)	*PM40*	*Elytrigia intermedium*	(Luo et al., 2009)
MIRE	*T. aestivum* L.		*PM41*	*T. turgidum*	(Li et al., 2009)
PM5	*Taestivum* L.	(Robe et al., 1996)	*PM42*	*T. turgidum*	(Hua et al., 2009)
PM5a	*T. aestivum* L.		*PM43*	*Thinopyrum intermedium*	(He et al., 2009)
Pm5b	*T. aestuvum* L.	(Hsam et al., 2001)	*PM45*	*T. aestivum* L.	(Ma et al., 2011)
PM5c	*T. sphaerococcum*		*PM47*	*T. aestivum* L.	(Xiao et al., 2013)
PM5d	*T. aestivum* L.		*PM48*	*A. tauschii*	(Fu et al., 2017a)
PM5e	*T. aestivum*	(Huang et al., 2003)	*PM51*	*Thinopyrum ponticum*	(Zhan et al., 2014)
PM9	*T. aestivum* L.	(Schneider et al., 1991	*PM52*	*T. aestivum* L.	(Wu et al., 2019)
PM10	*T. aestivum* L.	(Tosa et al., 1995)	*PM54*	*T. aestivum* L.	(Hao et al., 2015)
PM11	*T. aestivum* L.		*PM57*	*A. searsii*	(Liu et al., 2017)
PM14	*T. aestivum* L.		*PM59*	*T. aestivum* L.	(Tan et al., 2018)
PM18	*T. aestivum* L.		*PM60*	*T. urartu*	(Zhao et al., 2020)
PM13	*A. longissima*	(Cenci et al., 1999)	*PM61*	*T. aestivum* L.	(Sun et al., 2018)
PM15	*T. aestivum* L.	(Tosa et al., 1990)	*PM63*	*T. aestivum* L.	(Tan et al., 2019)
PM16	*T. aestivum* L.	(Wu et al., 2021a)	*PM65*	*T. aestivum* L.	(Li et al., 2019)
PM20	*S. cereale*	(Friebe et al., 1994)	*PM66*	*A. longissima*	(Li et al., 2020a)

PM21	*Haynaldia villosa*	(Lili et al., 1995; Qi et al., 1996)	*PM68*	*T. turgidum*	(He et al., 2021)
PM22	*T. aestivum* L.	(Peusha et al., 1996; Singrün et al., 2003)	*PM69*	*T. turgidum*	(Li et al., 2022b)
PM23/PM4c	*T. aestivum* L.	(Hao et al., 2008)	*PMCH1357*	*T. aestivum* L	(Chen et al., 2019)
PM24/24b	*T. aestivum* L.	(Huang et al., 2000; Xue et al., 2012)	*PMCG15-009*	*T. aestivum* L.	(Zhang et al., 2023)
PM25	*T. monokokum*	(Shi et al., 1998)	*MIHLT*	*T. aestivum* L.	(Wang et al., 2015)
PM26	*T. turgidum*	(Piarulli et al., 2012)	*PMG3M*	*T. turgidum*	(Xie et al., 2021)
MG5323	*T. turgidum*		*MlXBD*	*T. aestivum* L.	(Jin et al., 2020)
PM27	*T. timopheevii*	(Järve et al., 2000)	*pmHYM*	*T. aestivum* L.	(Fu et al., 2017b)
PM28	*T. aestivum* L.	(Peusha et al., 2000)	*pmDGM*	*T. aestivum* L.	(Wu et al., 2021b)
PM29	*T. aestivum* L.	(Zeller et al., 2002)	*pmQ*	*T. aestivum* L.	(Li et al., 2020b)
PM30	*T. turgidum*	(Liu et al., 2002)	*PmZ155*	*T. aestivum* L.	(Sun et al., 2015)
PM31	*T. turgidum*	(Xie et al., 2004)	*MlX99*	*T. aestivum* L.	(Zhao et al., 2013)

Non-race-specific

PM6	*T. aestivum* L.	(Purnhauser et al., 2011)
PM7	*S. cereale*	(An et al., 2022)
PM12	*A. speltoides*	(Jia et al., 1996; Zhang et al., 2021)
PM38	*T. aestivum* L.	(Spielmeyer et al., 2008; Lagudah et al., 2009)
PM39	*T. aestivum* L.	(Lillemo et al., 2008; Yang et al., 2014)
PM46	*T. aestivum* L.	(Gao et al., 2012)
PM53	*A. speltoides*	(Petersen et al., 2015)
PM55	*Dasypyrum villosum*	(Zhang et al., 2016)
PM56	*S. cereale*	(Hao et a., 2018)
PM58	*A. tauschii*	(Wiersma et al., 2017)
PM62	*D. villosum*	(Zhang et al., 2018)
PM64	*T. turgidum*	(Zhang et al., 2019)
PM67	*D. villosum*	(Zhang et al., 2021)
pmX	*T. aestivum* L.	(Fu et al., 2013)
PMWFJ	*T. aestivum* L.	(Ma et al., 2015)

CONCLUSION

Powdery mildew Bgt is one of the most important diseases in wheat agriculture, affecting production economically and food security. Chemical control methods for Bgt are expensive and uneconomical for small marginal farms. In addition, chemicals pose a danger to human and environmental health. In addition to cultural measures, chemical control is widely used in the fight against the disease. Chemical control may not be economical in small production farm areas. Fungicides used in chemical control are applied as seed and green parts spraying. However, seed application is usually not sufficient to control the disease throughout the season. On the other hand, green parts applications are not preferred because they are not economical. Again, the negative effects of chemical control on the environment and human health and the increase in social sensitivity against it have brought the use of resistant varieties to the forefront in the fight against this pathogen. Therefore, integrating host plant resistance, which is more economical and environmentally friendly methods, has been recognized as sustainable for disease control. In order to develop tolerant/resistant varieties against Bgt, it is necessary to identify suitable resistance gene sources. It also depends on their effective implementation in breeding programs. More than 240 genes, including alleles, have been reported for resistance to Bgt in wheat. However, most of these genes are derived from wild relatives of wheat (Aegilops sp., Dasypyrum sp., etc.). This limits their commercial use due to linkage and linkage drift with pest genes. The use of traditional classical breeding methods for resistance to Bgt, lack of sensitization and low selection efficiency have led to limited success. Environmental condition variability, nonpersistent PM resistance and the polygenic nature of PM resistance have resulted in poor progress in breeding for Bgt resistance. Demand for wheat, which is important for human nutrition and food security, is increasing rapidly worldwide. In line with the increasing demand, new breeding strategies, technologies and tools are being used to urgently address yield and quality challenges associated with biotic and abiotic stresses such as climate change, pests and diseases. The pathogen can break these major gene resistance genes by developing new races, causing severe infections and increasing crop losses (McDonald and Linde, 2002b). Therefore, the development of new sources of resistance or new resistant varieties is of great importance in the control of the agent. The emergence of high-throughput phenotyping, genotyping and phenomic approaches through the use of biotechnological and molecular approach technologies is increasing the efficiency of targeted selection. These technological approaches can be used to complement traditional breeding methods.

REFERENCES

Abdullah, A. A. (2015). Relationship the study of epidemic diseaseand support the decision-making in production of wheat in egypt. B.Sc. Agriculture (Plant Pathology) Faculty of Agriculture, Suez Canal University, Egypt.

Agrios, G. N. (2005). Plant pathology. Elsevier. Important Cereal Diseases and Surveillance Methods. T.R. Ministry of Agriculture and Rural Affairs, General Directorate of Agricultural Research, Department of Plant Health Research, Ankara.

Aktaş, H. (2001). Önemli Hububat Hastalıkları ve Sürvey Yöntemleri. T.C. Tarım ve Köyişleri Bakanlığı Tarımsal Araştırma Genel Müdürlüğü Bitki Sağlığı Araştırmaları Daire Başkanlığı, Ankara.

Alam, M.A., Xue, F., Wang, C., Ji, W. (2011). Powdery mildew resistance genes in Wheat: identification and genetic analysis. J. Mol. Biotech. Res., 1:20-39

Alam, M. A., Wang, C., Ji, W. (2013). Chromosomal location and SSR markers of a powdery mildew resistance gene in common wheat line N0308. African journal of Microbiology Research, 7 (6), 477-482

Alam, M.A., Hongpo, W., Hong, Z., Ji, W.Q. (2014). Differential expression of resistance to powdery mildew at the early stage of development in wheat line N0308. Genet. Mol. Res., 13: 4289-4301.

Allard, R. W., Bradshaw, A. D. (1964). Implications of genotype-environmental interactions in applied plant breeding 1. Crop science, 4 (5), 503-508.

An, D., Han, G., Wang, J., Yan, H., Zhou, Y., Cao, L., Jin, Y., Zhang, X. (2022). Cytological and genetic analyses of a wheat-rye 2RL ditelosomic addition line with adult plant resistance to powdery mildew. Crop. J., 10, 911-916.

Arzani, A., Ashraf, M. (2017). Cultivated ancient wheats (*Triticum* spp.): A potential source of health-beneficial food products. Comprehensive Reviews in Food Science and Food Safety, 16(3), 477-488.

Asad, M.A., Bai, B., Lan, C., Yan, J., Xia, X., Zhang, Y., He, Z. (2014). Identification of QTL for adult-plant resistance to powdery mildew in Chinese wheat landrace Pingyuan 50. Crop J., 2, 308-314.

Babayants, O.V., Babayants, L.T., Traskovetskaya, V.A., Gorash, A.F., Saulyak, N.I., Galaev, A.V. (2015). Race composition of *Blumeria graminis* (DC) *Speer* f. sp. *tritici* in the south of Ukraine and effectiveness of Pm-genes in 2004-2013. Cereal Res. Commun., 43: 449-458.

Basandrai, A.K., Basandrai, D. (2017) Powdery mildew of wheat and its management. In: Singh DP (ed) Management of wheat and barley diseases. Apple Academic Press, Canada, pp 133-181.

Basandrai, A.K., Mehta, A., Basandrai, D. (2023). Virulence structure of wheat powdery mildew pathogen, *Blumeria graminis tritici*: a review. Indian Phytopathology 76, 21-45. https://doi.org/10.1007/s42360-022-00571-z

Blanco, A., Gadaleta, A., Cenci, A., Carluccio, A.V., Abdelbacki, A.M., Simeone, R. (2008). Molecular mapping of the novel powdery mildew resistance gene Pm36 introgressed from *Triticum turgidum* var. *dicoccoides* in durum wheat. Theor. App. Genet., 117, 135-142.

Bowen, K.L., Everts, K.L., Leath, S. (1991). Reduction in yield on winter wheat in North Carolina due to powdery mildew and leaf rust. Phytopathol, 81:503-511.

Cao, X., Luo, Y., Zhou, Y., Duan, X., Cheng, D. (2013). Detection of powdery mildew in two winter wheat cultivars using canopy hyperspectral reflectance. Crop Protection, 45, 124-131.

Cenci, A., D'ovidio, R., Tanzarella, O.A., Ceoloni, C., Porceddu, E. (1999). Identification of molecular markers linked to Pm13, an *Aegilops longissima* gene conferring resistance to powdery mildew in wheat. Theor. Appl. Genet., 98, 448-454.

Chen, G., Wei, B., Li, G., Gong, C., Fan, R., Zhang, X. (2018). TaEDS1 genes positively regulate resistance to powdery mildew in wheat. Plant Mol Biol., 96 (6): 607-625.

Chen, F., Jia, H., Zhang, X., Qiao, L., Li, X., Zheng, J., Guo, H., Powers, C., Yan, L., Chang, Z. (2019). Positional cloning of PmCH1357 reveals the origin and allelic variation of the Pm2 gene for powdery mildew resistance in wheat. Crop J., 7, 771-783.

Cowger, C., Mehra, L., Arellano, C., Meyers, E., Murphy, J. P. (2018). Virulence differences in *Blumeria graminis* f. sp. *tritici* from the central and eastern United States. Phytopathology, 108 (3), 402-411.

Crespo-Herrera, L.A., Crossa, J., Huerta-Espino, J., Vargas, M., Mondal, S., Velu, G., … Singh, P. (2018). Genetic gains for grain yield in CIMMYT's semi-arid wheat yield trials grown in suboptimal environments. Crop Science, 58 (5), 1890-1898. Doi: https://doi.org/10.2135/cropsci2018.01.0017

Cunfer, B.M. (2002). Powdery Mildew, Bread Wheat Improvement and Production, Curtis, B.C., Rjajaram, S., Gomez-Macpherson, H., Eds., FAO Plant Production and Protection Series No. 30, FAO: Rome, Italy.

Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., et al. (2012). The top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13, 414–430. doi: 10.1111/j.1364-3703.2011.00783.x

Draz, I.S., Esmail, S.M., Abou-Zeid, M.A.E., Essa, T.A.E. (2019) Powdery mildew susceptibility of spring wheat cultivars as a major constraint on grain yield. Annals Agri Sci., 64, 39-45. https://doi.org/10.1016/j.aoas.2019.05.007

Elkot, A. F. A., Chhuneja, P., Kaur, S., Saluja, M., Keller, B., Singh, K. (2015). Marker assisted transfer of two powdery mildew resistance genes PmTb7A. 1 and PmTb7A. 2 from *Triticum boeoticum* (Boiss.) to *Triticum aestivum* (L.). PloS one, 10 (6), e0128297.

Feng, W., Li, X., Liu, W.D., Wang, X.Y., Wang, C.Y., Guo, T.C. (2014). Effects of powdery mildew infection on grain quality traits and yield of winter wheat. J. Tritic Crops, 34, 1706-1712.

Flor, H.H. (1971). Current status of the gene-for -gene concept. Annu. Rev. Phytopathol., 9: 275-296.

Friebe, B., Heun, M., Tuleen, N., Zeller, F.J., Gill, B.S. (1994). Cytogenetically monitored transfer of powdery mildew resistance from rye into wheat. Crop Sci., 34, 621-625.

Fu, B., Chen, Y., Li, N., Ma, H., Kong, Z., Zhang, L., Jia, H., Ma, Z. (2013). PmX: A recessive powdery mildew resistance gene at the Pm4 locus identified in wheat landrace Xiaohongpi. Theor. Appl. Genet., 126, 913-921.

Fu, B., Liu, Y., Zhang, Q., Wu, X., Gao, H., Cai, S., Wu, J. (2017a). Development of markers closely linked with wheat powdery mildew resistance gene Pm48. Acta Agron. Sin., 43, 307-312.

Fu, B., Zhang, Z., Zhang, Q., Wu, X., Wu, J., Cai, S. (2017b). Identification and mapping of a new powdery mildew resistance allele in the Chinese wheat landrace Hongyoumai. Mol. Breed., 37, 133.

Fu, Y., Zhang, H., Mandal, S.N., Wang, C., Chen, C., Ji, W. (2016). Quantitative proteomics reveals the central changes of wheat in response to powdery mildew. J Proteomics., 130: 108-119.

Gao, H.D., Zhu, F.F., Jiang, Y.J., Wu, J.Z., Yan, W., Zhang, Q.F., Jacobi, A., Cai, S.B. (2012). Genetic analysis and molecular mapping of a new powdery mildew resistance gene Pm46 in common wheat. Theor. Appl. Genet., 125, 967-973.

Gao, H.Y., He, D.X., Niu, J.S., Wang, C.Y., Yang, X.W. (2014a). The effect and molecular mechanism of powdery mildew on wheat grain prolamins. J. Agric. Sci., 152, 239.

Gao, H., Niu, J., Yang, X., He, D., Wang, C. (2014b). Impacts of powdery mildew on wheat grain sugar metabolism and starch accumulation in developing grains. Starch-Stärke, 66, 947-958.

Golzar, H., Shankar, M., D'Antuono, M. (2016). Responses of commercial wheat varieties and differential lines to western Australian powdery mildew (*Blumeria graminis* f. sp. *tritici*) populations. Australian. Plant Pathol., 45, 347-355.

Griffey, C. A., Das, M. K. (1994). Inheritance of adult-plant reistance to powdery mildew in Knox 62 and Massey winter wheats. crop science, 34 (3), 641-646.

Hao, Y., Liu, A., Wang, Y., Feng, D., Gao, J., Li, X., Liu, S., Wang, H. (2008). Pm23: A new allele of Pm4 located on chromosome 2AL in wheat. Theor. Appl. Genet., 117, 1205-1212.

Hao, Y., Parks, R., Cowger, C., Chen, Z., Wang, Y., Bland, D., Murphy, J.P., Guedira, M., Brown-Guedira, G., Johnson, J. (2015). Molecular characterization of a new powdery mildew resistance gene Pm54 in soft red winter wheat. Theor. Appl. Genet., 128, 465-476.

Hao, M., Liu, M., Luo, J., Fan, C., Yi, Y., Zhang, L., Yuan, Z., Ning, S., Zheng, Y., Liu, D. (2018). Introgression of powdery mildew resistance gene Pm56 on rye chromosome arm 6RS into wheat. Front. Plant Sci., 9, 1040.

Hart, G. E. (2001). Molecular-marker maps of the cultivated wheats and other Triticum species. In DNA-Based Markers in Plants (pp. 421-441). Springer, Dordrecht.

He, R., Chang, Z., Yang, Z., Yuan, Z., Zhan, H., Zhang, X., Liu, J. (2009). Inheritance and mapping of powdery mildew resistance gene Pm43 introgressed from *Thinopyrum intermedium* into wheat. Theor. Appl. Genet., 118, 1173-1180.

He, H., Liu, R., Ma, P., Du, H., Zhang, H., Wu, Q., Yang, L., Gong, S., Liu, T., Huo, N., et al. (2021). Characterization of Pm68, a new powdery mildew resistance gene on chromosome 2BS of Greek durum wheat TRI 1796. Theor. Appl. Genet., 134, 53-62.

Heun, M., Friebe, B., Bushuk, W. (1990). Chromosomal location of the powdery mildew resistance gene of Amigo wheat. Phytopathology, 80 (10), 1129-1133.

Hsam, S.L.K., Huang, X.Q., Zeller, F.J. (2001). Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.) 6. Alleles at the Pm5 locus. Theor. Appl. Genet., 102, 127-133.

Hsam, S.L.K., Lapochkina, I.F., Zeller, F.J. (2003). Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.). 8. Gene Pm32 in a wheat-Aegilops speltoides translocation line. Euphytica, 133, 367-370.

Hu, P., Liu, J., Xu, J., Zhou, C., Cao, S., Zhou, W., Huang, Z., Yuan, S., Wang, X., Xiao, J., Zhang, R., Wang, H., Zhang, S., Xing, L., Cao, A. (2018). A malectin-like/leucine-rich repeat receptor protein kinase gene, RLK-V, regulates powdery mildew resistance in wheat. Mol Plant Pathol., 19 (12): 2561-2574.

Hua, W., Liu, Z., Zhu, J., Xie, C., Yang, T., Zhou, Y., Duan, X., Sun, Q., Liu, Z. (2009). Identification and genetic mapping of pm42, a new recessive wheat powdery mildew resistance gene derived from wild emmer (*Triticum turgidum* var. *dicoccoides*). Theor. Appl. Genet., 119, 223-230.

Huang, X., Wang, L., Xu, M., Röder, M. (2003). Microsatellite mapping of the powdery mildew resistance gene Pm5e in common wheat (*Triticum aestivum* L.). Theor. Appl. Genet., 106, 858-865.

Huang, X.Q., Hsam, S.L.K., Zeller, F.J., Wenzel, G., Mohler, V. (2000). Molecular mapping of the wheat powdery mildew resistance gene Pm24 and marker validation for molecular breeding. Theor. Appl. Genet., 101, 407-414.

Hunter, M. C., Smith, R. G., Schipanski, M. E., Atwood, L. W., Mortensen, D. A. (2017). Agriculture in 2050: recalibrating targets for sustainable intensification. Bioscience, 67 (4), 386-391.

Jakobson, I., Reis, D., Tiidema, A., Peusha, H., Timofejeva, L., Valárik, M., Kladivová, M., Šimková, H., Doležel, J., Järve, K. (2012). Fine mapping, phenotypic characterization and validation of non-race-specific resistance to powdery mildew in a wheat-*Triticum militinae* introgression line. Theor. Appl. Genet., 125, 609-623.

Jankovics, T., Komáromi, J., Fábián, A., Jäger, K., Vida, G., Kiss, L. (2015). New insights into the life cycle of the wheat powdery mildew: Direct observation of ascosporic infection in *Blumeria graminis* f. sp. *tritici*. Phytopathology, 105, 797-804.

Järve, K., Peusha, H.O., Tsymbalova, J., Tamm, S., Devos, K.M., Enno, T.M. (2000). Chromosomal location of a *Triticum timopheevii*-derived powdery mildew resistance gene transferred to common wheat. Genome, 43, 377-381.

Jia, J., Devos, K.M., Chao, S., Miller, T.E., Reader, S.M., Gale, M.D. (1996). RFLP-based maps of the homoeologous group-6 chromosomes of wheat and their application in the tagging of Pm12, a powdery mildew resistance gene transferred from Aegilops speltoides to wheat. Theor. Appl. Genet., 92, 559-565.

Jin, Y., Xue, F., Zhou, Y., Duan, X., Hu, J., Li, Y., Zhu, H., Sun, J. (2020). Fine-mapping of the powdery mildew resistance gene mlxbd in the common wheat landrace Xiaobaidong. Plant Dis., 104, 1231-1238.

Keller, M., Keller, B., Schachermayr, G., Winzeler, M., Schmid, J.E., Stamp, P., Messmer, M.M. (1999). Quantitative trait loci for resistance against powdery mildew in a segregating wheat×spelt population. Theor. Appl. Genet., 98, 903-912.

Kloppe, T., Boshoff, W., Pretorius, Z., Lesch, D., Akin, B., Morgounov, A., Shamanin, V., Kuhnem, P., Murphy, P., Cowger, C. (2022). Virulence of *Blumeria graminis* f. sp. *tritici* in Brazil, South Africa, Turkey, Russia, and Australia. Adv. Breed. Wheat. Dis. Resist., 13, 954958.

Koller, T., Brunner, S., Herren, G., Hurni, S., Keller, B. (2018). Pyramiding of transgenic Pm3 alleles in wheat results in improved powdery mildew resistance in the field. Theor. Appl. Genet., 131, 861-871.

Lagudah, E.S., Krattinger, S.G., Herrera-Foessel, S., Singh, R.P., Huerta-Espino, J., Spielmeyerm, W., Brown-Guedira, G., Selter, L.L., Keller, B. (2009). Gene-specific markers for the wheat gene Lr34/Yr18/Pm38 which confers resistance to multiple fungal pathogens. Theo. Appl. Genet., 119, 889-898.

Laloševi´c, M., Jevti´c, R., Župunski, V., Maširevi´c, S., Orbovi´c, B. (2022). Virulence structure of the wheat powdery mildew population in Serbia. Agronomy, 12:45.

Leonova, I.N. (2019). Genome-Wide Association study of powdery mildew resistance in Russian Spring Wheat (*T. aestivum* L.) Varieties. Russ. J. Genet., 55, 1360-1374.

Li, G., Cowger, C., Wang, X., Carver, B.F., Xu, X. (2019). Characterization of Pm65, a new powdery mildew resistance gene on chromosome 2AL of a facultative wheat cultivar. Theoret. Appl. Genet., 132 (9): 2625-2632.

Li, G., Fang, T., Zhang, H., Xie, C., Li, H., Yang, T., Nevo, E., Fahima, T., Sun, Q., Liu, Z. (2009). Molecular identification of a new powdery mildew resistance gene Pm41 on chromosome 3BL derived from wild emmer (*Triticum turgidum* var. *dicoccoides*). Theor. Appl. Genet., 119, 531-539.

Li, G., Xu, X., Bai, G., Carver, B.F., Hunger, R., Bonman, J.M. (2016). Identification of novel powdery mildew resistance sources in wheat. Crop Sci., 56, 1817-1830.

Li, H., Dong, Z., Ma, C., Xia, Q., Tian, X., Sehgal, S., Koo, D.H., Friebe, B., Ma, P., Liu, W. (2020a). A spontaneous wheat-Aegilops longissima translocation carrying Pm66 confers resistance to powdery mildew. Theor. Appl. Genet., 133, 1149-1159.

Li, Y., Shi, X., Hu, J., Wu, P., Qiu, D., Qu, Y., Xie, J., Wu, Q., Zhang, H., Yang, L., et al. (2020b). Identification of a Recessive Gene PmQ Conferring Resistance to Powdery Mildew. Plant. Dis., 1-41.

Li, S., Lin, D., Zhang, Y., Deng, M., Chen, Y., Lv, B., Li, B., Lei, Y., Wang, Y., Zhao, L., Liang, Y., Liu, J., Chen, K., Liu, Z., Xiao, J., Qiu, J., Gao, C. (2022a). Genome-edited powdery mildew resistance in wheat without growth penalties. Nature., 602 (7897): 455-460.

Li, Y., Wei, Z.Z., Sela, H., Govta, L., Klymiuk, V., Roychowdhury, R., Chawla, H.S., Ens, J., Wiebe, K., Bocharova, V., et al. (2022b). Long-read genome sequencing accelerated the cloning of Pm69 by resolving the complexity of a rapidly evolving resistance gene cluster in wheat. bioRxiv, 10.

Lili, Q., Peidu, C., Daun, L., Bo, Z., Shouzhong, Z., Baoqin, S., Qijun, X., Xiayu, D., Yilin, Z. (1995). The gene Pm21-a new source for resistance to wheat powdery mildew. Zuo Wu Xue Bao, 21, 257-262.

Lillemo, M., Asalf, B., Singh, R.P., Huerta-Espino, J., Chen, X.M., He, Z.H., Bjørnstad, Å. (2008). The adult plant rust resistance loci Lr34/Yr18 and Lr46/Yr29 are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theor. Appl. Genet., 116, 1155-1166.

Lipps, E. (2010). "Powdery Mildew of Wheat," The Ohio State University Extension. Retrieved from http://ohioline.osu.edu/ac-fact/0010.htmltm.

Liu, J., Zhang, T., Jia, J., Sun, J. (2016). The wheat mediator subunit TaMED25 interacts with the transcription factor TaEIL1 to negatively regulate disease resistance against powdery mildew. Plant Physiol., 170 (3): 1799-1816.

Liu, W. (2012). Studies of etiology, occurrence and control of *Camellia oleifera* anthracnose. Huazhong Agricultural University, China (MSc Thesis, in Chinese).

Liu, W., Koo, D.H., Xia, Q., Li, C., Bai, F., Song, Y., Friebe, B., Gill, B.S. (2017). Homoeologous recombination-based transfer and molecular cytogenetic mapping of powdery mildew-resistant gene Pm57 from Aegilops searsii into wheat. Theor. Appl. Genet., 130, 841-848.

Liu, Z., Sun, Q., Ni, Z., Nevo, E., Yang, T. (2002). Molecular characterization of a novel powdery mildew resistance gene Pm30 in wheat originating from wild emmer. Euphytica, 123, 21-29.

Luo, P.G., Luo, H.Y., Chang, Z.J., Zhang, H.Y., Zhang, M., Ren, Z.L. (2009). Characterization and chromosomal location of Pm40 in common wheat: A new gene for resistance to powdery mildew derived from *Elytrigia intermedium*. Theor. Appl. Genet., 118, 1059-1064.

Ma, H., Kong, Z., Fu, B., Li, N., Zhang, L., Jia, H., Ma, Z. (2011). Identification and mapping of a new powdery mildew resistance gene on chromosome 6D of common wheat. Theor. Appl. Genet., 123, 1099-1106.

Ma, P., Xu, H., Zhang, H., Li, L., Xu, Y., Zhang, X., An, D. (2015). The gene PmWFJ is a new member of the complex Pm2 locus conferring unique powdery mildew resistance in wheat breeding line Wanfengjian 34. Mol. Breed., 35, 210.

Mapuranga, J., Chang, J.Y., Yang, W.X. (2022). Combating powdery mildew: advances in molecular interactions between *Blumeria graminis* f. sp. *tritici* and wheat. Front Plant Sci., 13: 1102908.

Mazzucotelli, E., Rubiales, D., Keller, B., Cattivelli, L., Valè, G. (2021). Characterization of the Resistance to powdery mildew and leaf rust carried by the bread wheat cultivar. Victo. Int. J. Mol. Sci., 22: 3109.

McDonald, B. A., Linde, C. (2002a). Pathogen population genetics, evolutionary potential, and durable resistance. Annual review of phytopathology, 40 (1), 349-379.

Mc Donald, B.A., Linde, C. (2002b). The population genetics of plant pathogens and breeding strategies for durable resistance. Euphytica, 124: 163-180.

Mehta, A., Basandrai, A.K., Banyal, D.K., Basandrai, D. (2018). Effect of weather parameters on powdery mildew development of wheat at different location in Himachal Pradesh. Indian Phytopathol., 71: 349-353.

Mehta, A., Basandrai, A.K., Basandrai, D., Rana, V., Singh, P. (2022). Identification of field relevant powdery mildew and yellow rust resistant donors in some advanced breeding material of wheat through GGE biplot analysis. Indian Phytopathol., 75: 405-418.

Menardo, F., Wicker, T., Keller, B. (2017). Reconstructing the evolutionary history of powdery mildew lineages (*Blumeria graminis*) at different evolutionary time scales with NGS data. Genome Biol. Evol., 9, 446-456.

Menardo, F., Praz, C.R., Wyder, S., Ben-David, R., Bourras, S., Matsumae, H., McNally, K.E., Parlange, F., Riba, A., Roffler, S., et al. (2016). Hybridization of powdery mildew strains gives rise to pathogens on novel agricultural crop species. Nat. Genet., 48, 201-205.

Meyer, M., Bacha, N., Tesfaye, T., Alemayehu, Y., Abera, E., Hundie, B., Woldeab, G., Girma, B., Gemechu, A., Negash, T., et al. (2021). Wheat rust epidemics damage Ethiopian wheat production: A decade of field disease surveillance reveals national-scale trends in past outbreaks. PLoS ONE, 16, e0245697.

Miranda, L.M., Murphy, J.P., Marshall, D., Leath, S. (2006). Pm34: A new powdery mildew resistance gene transferred from Aegilops tauschii Coss. to common wheat (*Triticum aestivum* L.). Theor. Appl. Genet., 113, 1497-1504.

Miranda, L.M., Murphy, J.P., Marshall, D., Cowger, C., Leath, S. (2007). Chromosomal location of Pm35, a novel Aegilops tauschii derived powdery mildew resistance gene introgressed into common wheat (*Triticum aestivum* L.). Theor. Appl. Genet., 114, 1451-1456.

Mondal, S., Rutkoski, J. E., Velu, G., Singh, P. K., Crespo-Herrera, L. A., Guzman, C., ... Singh, R. P. (2016). Harnessing diversity in wheat to enhance grain yield, climate resilience, disease and insect pest resistance and nutrition through conventional and modern breeding approaches. Frontiers in plant science, 7, 991.

Morgounov, A., Tufan, H.A., Sharma, R., Akin, B., Bagci, A., Braun, H.J., Kaya, Y., Keser, M., Payne, T.S., Sonder, K., et al. (2012). Global incidence of wheat rusts and powdery mildew during 1969-2010 and durability of resistance of winter wheat variety Bezostaya 1. Europ. J. Plant Pathol., 132, 323-340.

Mwale, V.M., Chilembwe, E.H.C., Uluko, H.C. (2014). Wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*): Damage effects and genetic resistance developed in wheat (*Triticum aestivum*). Inter Res J Plant Sci 5:1-16

Mwale, V.M., Tang, X., Chilembwe, E. (2017). Molecular detection of disease resistance genes to powdery mildew (*Blumeria graminis* f. sp. *tritici*) in wheat (*Triticum aestivum*) cultivars. Afr. J. Biotech., 16, 22-31.

Nordestgaard, N.V., Thach, T., Sarup, P., Rodriguez-Algaba, J., Andersen, J.R., Hovmøller, M.S., Jahoor, A., Jørgensen, L.N., Orabi, J. (2021). Multi-parental populations suitable for identifying sources of resistance to powdery mildew in winter wheat. Front. Plant Sci., 11, 570863.

Perugini, L.D. (2007). Genetic characterization of wheat germplasm with resistance to *Fusarium* head blight (FHB) and powdery mildew.

Perugini, L.D., Murphy, J.P., Marshall, D., Brown-Guedira, G. (2008). Pm37, a new broadly effective powdery mildew resistance gene from *Triticum timopheevii*. Theor. Appl. Genet., 116, 417-425.

Petersen, S., Lyerly, J.H., Worthington, M.L., Parks, W.R., Cowger, C., Marshall, D.S., Brown-Guedira, G., Murphy, J.P. (2015). Mapping of powdery mildew resistance gene Pm53 introgressed from Aegilops speltoides into soft red winter wheat. Theor. Appl. Genet., 128, 303-312.

Peusha, H., Hsam, S.L., Zeller, F.J. (1996). Chromosomal location of powdery mildew resistance genes in common wheat (*Triticum aestivum* L. em. Thell.) 3. Gene Pm22 in cultivar Virest. Euphytica, 91, 149-152.

Peusha, H., Enno, T., Priilinn, O. (2000). Chromosomal location of powdery mildew resistance genes and cytogenetic analysis of meiosis in common wheat cultivar Meri. Hereditas, 132, 29-34.

Piarulli, L., Gadaleta, A., Mangini, G., Signorile, M.A., Pasquini, M., Blanco, A., Simeone, R. (2012). Molecular identification of a new powdery mildew resistance gene on chromosome 2BS from *Triticum turgidum* ssp. *dicoccum*. Plant Sci., 196, 101-106.

Pietrusińska, A., Czembor, J.H. (2017). Pyramiding winter wheat resistance genes (Pm21 + Pm34) of powdery mildew of cereals and grasses (*Blumeria graminis* f. sp. *tritici*). Prog. Plant Prot., 57, 41-46.

Pietrusińska, A., Tratwal, A. (2020). Characteristics of powdery mildew and its importance for wheat grown in Poland. Plant Prot. Sci., 56, 141-153.

Pietrusińska, A., Czembor, J.H., Czembor, P.C. (2011). Pyramiding two genes for leaf rust and powdery mildew resistance in common wheat. Cer. Res. Commun., 39, 577-588.

Pietrusińska, A., Żurek, M., Piechota, U., Słowacki, P., Smolińska, K. (2018). Searching for diseases resistance sources in old cultivars, landraces and wild relatives of cereals. A review. Ann. UMCS Sect. E Agric., 73, 45-60.

Purnhauser, L., Bóna, L., Láng, L. (2011). Occurrence of 1BL. 1RS wheat-rye chromosome translocation and of Sr36/Pm6 resistance gene cluster in wheat cultivars registered in Hungary. Euphytica, 179, 287-295.

Qi, L., Cao, M., Chen, P., Li, W., Liu, D. (1996). Identification, mapping, and application of polymorphic DNA associated with resistance gene Pm21 of wheat. Genome, 39, 191-197.

Ray, D.K., Mueller, N.D., West, P.C., Foley, J.A. (2013). Yield trends are insufficient to double global crop production by 2050. PLoS ONE. 8:e66428. Doi: 10.1371/journal.pone.0066428

Robe, P., Pavoine, M.T., Doussinault, G. (1996). Early assessment of adult plant reaction of wheat (*Triticum aestivum* L) to powdery mildew (*Erysiphe graminis* f sp *tritici*) at the five-leaf seedling stage. Agronomie, 16, 441-451.

Salgado, J.D., Paul, P.A. (2016). Powdery Mildew of Wheat. Ohio State University Extension. https://ohioline.osu.edu/factsheet/plpath-cer-11 (Access date: November 15, 2023)

Samobor, V., Vukobratović, M., Jošt, M. (2006). Effect of powdery mildew attack on quality parameters and experimental bread baking of wheat. Acta Agric. Slov, 87, 381-391.

Sánchez-Martín, J., Bourras, S., Keller, B., Oliver, R. (2018). Diseases Affecting Wheat and Barley: Powdery Mildew. Integrated Disease Management of Wheat and Barley, Burleigh Dodds Science Publishing Limited: Cambridge, UK, pp. 69-93.

Sánchez-Martín, J., Widrig, V., Herren, G., Wicker, T., Zbinden, H., Gronnier, J., Spörri, L., Praz, C.R., Heuberger, M., Kolodziej, M.C., Isaksson, J., Steuernagel, B., Karafiátová, M., Doležel, J., Zipfel, C., Keller, B. (2021). Wheat Pm4 resistance to powdery mildew is controlled by alternative splice variants encoding chimeric proteins. Nat Plants., 7 (3): 327-341.

Schneider, D.M., Heun, M., Fischbeck, G. (1991). Inheritance of the powdery mildew resistance gene Pm9 in relation to Pm1 and Pm2 of wheat. Plant Breed., 107, 161-164.

Schwessinger, B. (2017). Fundamental wheat stripe rust research in the 21st century. New Phytologist, 213 (4), 1625-1631.

Shahin, A.A., Ashmawy, M.A., Esmail, S.M., El-Moghazy, S.M. (2019). Biocontrol of wheat powdery mildew disease under field conditions in EGYPT. Zagazig J. Agric. Res., 46, 2255-2270.

Sharma, S.C., Basandrai, A.K., Aulakh, K.S. (1990). Virulence in *Erysiphe graminis* f. sp. *tritici* Causing Powdery Mildew in Wheat. Plant Dis. Res., 5: 115-117.

Shen, X. K., Ma, L. X., Zhong, S. F., Liu, N., Zhang, M., Chen, W. Q., et al., Bai, G. H. (2015). Identification and genetic mapping of the putative Thinopyrum intermedium-derived dominant powdery mildew resistance gene PmL962 on wheat chromosome arm 2BS. Theoretical and applied genetics, 128 (3), 517-528.

Shi, A.N., Leath, S., Murphy, J.P. (1998). A major gene for powdery mildew resistance transferred to common wheat from wild einkorn wheat. Phytopathology, 88, 144-147.

Shi, W., Gong, S., Zeng, F., Xue, M., Yang, L., Yu, D. (2016). Sexual reproduction and detection of mating-type of *Blumeria graminis* f. sp. *tritici* populations. Acta. Phytopath. Sin., 46, 645-652.

Simeone, R., Piarulli, L., Nigro, D., Signorile, M.A., Blanco, E., Mangini, G., Blanco, A. (2020). Mapping powdery mildew (*Blumeria graminis* f. sp. *tritici*) resistance in wild and cultivated tetraploid wheats. Internat. J. Mol. Sci., 21, 7910.

Singh, A., Breja, P., Khurana, J. P., and Khurana, P. (2016). Wheat brassinosteroid-Insensitive1 (TaBRI1) interacts with members of taserk gene family and cause early flowering and seed yield enhancement in Arabidopsis. PLoS One 11:e0153273. doi: 10.1371/journal.pone.0153273

Singrün, C.H., Hsam, S.L.K., Hartl, L., Zeller, F.J., Mohler, V. (2003). Powdery mildew resistance gene Pm22 in cultivar Virest is a member of the complex Pm1 locus in common wheat (*Triticum aestivum* L. em Thell.). Theor. Appl. Genet. 2003, 106, 1420-1424.

Sönmezoğlu, Ö. A., Yıldırım, A., Güleç, T. E., Kandemir, N. (2010). Markör Destekli Seleksiyonun Buğday Islahında Kullanımı. Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi, 2010 (1), 105-112.

Sönmezoğlu, Ö. A., Yıldırım, A., Türk, Ü., Yanar, Y. (2019). Bazı Yerel Makarnalık Buğday Çeşitlerinde Küllemeye (*Blumeria graminis* f. sp. *tritici*) Karşı Dayanıklılığın Belirlenmesi. Avrupa Bilim ve Teknoloji Dergisi, (17), 944-950.

Spielmeyer, W., Singh, R.P., McFadden, H., Wellings, C.R., Huerta-Espino, J., Kong, X., Appels, R., Lagudah, E.S. (2008). Fine scale genetic and physical mapping using interstitial deletion mutants of Lr34/Yr18: A disease resistance locus effective against multiple pathogens in wheat. Theor. Appl. Genet., 116, 481-490.

Sun, H., Hu, J., Song, W., Qiu, D., Cui, L., Wu, P., Zhang, H., Liu, H., Yang, L., Qu, Y., et al. (2018). Pm61: A recessive gene for resistance to powdery mildew in wheat landrace Xuxusanyuehuang identified by comparative genomics analysis. Theor. Appl. Genet., 131, 2085-2097.

Sun, H., Song, W., Sun, Y., Chen, X., Liu, J., Zou, J., Wang, X., Zhou, Y., Lin, X., Li, H. (2015). Resistance of 'Zhongmai 155'wheat to powdery mildew: Effectiveness and detection of the resistance gene. Crop Sci., 55, 1017-1025.

Tan, C., Li, G., Cowger, C., Carver, B.F., Xu, X. (2018). Characterization of Pm59, a novel powdery mildew resistance gene in Afghanistan wheat landrace PI 181356. Theor. Appl. Genet., 131, 1145-1152.

Tan, C., Li, G., Cowger, C., Carver, B.F., Xu, X. (2019). Characterization of Pm63, a powdery mildew resistance gene in Iranian landrace PI 628024. Theor. Appl. Genet., 132, 1137-1144.

Tang, X., Cao, X., Xu, X., Jiang, Y., Luo, Y., Ma, Z., Fan, J., Zhou, Y. (2017). Effects of climate change on epidemics of powdery mildew in winter wheat in China. Plant Dis., 101, 1753-1760.

TeBeest, D.E., Paveley, N.D., Shaw, M.W., Van, Den. B.F. (2008). Disease–weather relationships of powdery mildew and yellow rust on winter wheat. Phytopathol 98:609-617

Terefe, T. (2019). Wheat producers, treat fungicides with caution! Farmer's Wkly., 42-44.

TOB (2023). Pest Control Technical Instructions, Volume 1 (Ankara, 2008). https://www.tarimorman.gov.tr/TAGEM/Belgeler/Teknik%20tal%C4%B1matlar%202008/C%C4%B0LT%201.pdf (Acced date: 15. November. 2023)

Tosa, Y., Sakai, K. (1990). The genetics of resistance of hexaploid wheat to the wheatgrass powdery mildew fungus. Genome, 33, 225-230.

Tosa, Y., Nakamura, T., Kusaba, M. (1995). Distribution of genes for resistance to the wheatgrass mildew fungus in Japanese wheat cultivars and of their corresponding genes in the wheat mildew fungus. Jpn. J. Genet., 70, 119-126.

Vikas, V.K., Kumar, S., Archak, S., Tyagi, R.K., Kumar, J., Jacob, S., Sivasamy, M., Jayaprakash, P., Saharan, M.S., Basandrai, A.K., et al. (2020). Screening of 19,460 genotypes of wheat species for resistance to powdery mildew and identification of potential candidates using focused identification of germplasm strategy (FIGS). Crop Sci., 60, 2857-2866.

Walczak, F., Gałęzewski, M., Jakubowska, M., Skorupska, A., Tratwal, A., Wojtowicz, A., Złotowski, J. (2019). Zespół Zakładu Metod Prognozowania i Rejestracji Agrofagów oraz Zakład Badania Gryzoni Polnych IOR w Poznaniu. Available online: http://www.ior.poznan.pl/aktualizacja/data/pliki/263_Stan_fitosanitarny_2007.pdf (accessed on 15 November 2023).

Wang, Z., Li, H., Zhang, D., Guo, L., Chen, J., Chen, Y., Wu, Q., Xie, J., Zhang, Y., Sun, Q., et al. (2015). Genetic and physical mapping of powdery mildew resistance gene MlHLT in Chinese wheat landrace Hulutou. Theor. Appl. Genet., 128, 365-373.

Weise, M.V. (1977). Compendium of Wheat Diseases, American Phytopathological Society: St. Paul, MN, USA.

Wiersma, A.T., Pulman, J.A., Brown, L.K., Cowger, C., Olson, E.L. (2017). Identification of Pm58 from Aegilops tauschii. Theor. Appl. Genet., 130, 1123-1133.

Wricke, G., Dill, P., Senft, P. (1996). Linkage between a major gene for powdery mildew resistance and an RFLP marker on chromosome 1R of rye. Plant breeding, 115 (1), 71-73.

Wu, P., Hu, J., Zou, J., Qiu, D., Qu, Y., Li, Y., Li, T., Zhang, H., Yang, L., Liu, H., et al. (2019). Fine mapping of the wheat powdery mildew resistance gene Pm52 using comparative genomics analysis and the Chinese Spring reference genomic sequence. Theor. Appl. Genet., 132, 1451-1461.

Wu, X., Bian, Q., Gao, Y., Ni, X., Sun, Y., Xuan, Y., Cao, Y., Li, T. (2021a). Evaluation of resistance to powdery mildew and identification of resistance genes in wheat cultivars. PeerJ, 9, e10425.

Wu, Y., Yu, X., Zhang, X., Yan, L., Gao, L., Hao, Y., Wang, X., Xue, S., Qu, Y., Hu, T., et al. (2021b). Characterization of PmDGM conferring powdery mildew resistance in Chinese wheat landrace Duanganmang. Plant Dis., 105, 3127-3133.

Xiao, M., Song, F., Jiao, J., Wang, X., Xu, H., Li, H. (2013). Identification of the gene Pm47 on chromosome 7BS conferring resistance to powdery mildew in the Chinese wheat landrace Hongyanglazi. Theoretical and Applied Genetics, 126 (5), 1397- 1403.

Xie, C., Sun, Q., Ni, Z., Yang, T., Nevo, E., Fahima, T. (2004). Identification of resistance gene analogue markers closely linked to wheat powdery mildew resistance gene Pm31. Plant Breed., 123, 198-200.

Xie, W., Ben-David, R., Zeng, B., Distelfeld, A., Röder, M.S., Dinoor, A., Fahima, T. (2021). Identification and characterization of a novel powdery mildew resistance gene PmG3M derived from wild emmer wheat, *Triticum dicoccoides*. Theor. Appl. Genet., 124, 911-922.

Xue, F., Wang, C., Li, C., Duan, X., Zhou, Y., Zhao, N., Wang, Y., Ji, W. (2012). Molecular mapping of a powdery mildew resistance gene in common wheat landrace Baihulu and its allelism with Pm24. Theor. Appl. Genet., 125, 1425-1432.

Yahiaoui, N., Srichumpa, P., Dudler, R., Keller, B. (2004). Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene Pm3b from hexaploid wheat. Plant J., 37, 528-538.

Yang, X., Liu, L., Sun, D., Zhang, L. (2014). Genetic characteristics of wheat resistance gene Lr46/Yr29/Pm39, Sr2/Yr30 and Lr68 and association analysis of main agronomic traits. Acta Bot. Boreali-Occident. Sin., 34, 454-462.

Yang, L., Zhang, X., Zhang, X., Wang, J., Luo, M., Yang, M., Wang, H., Xiang, L., Zeng, F., Yu, D., et al. (2017). Identification and evaluation of resistance to powdery mildew and yellow rust in a wheat mapping population. PLoS ONE, 12, e0177905.

Yıldırım, A., Sakin, M., Karadag, Y., Gokmen, S., Kandemir, N., Akkaya, M. S., Yıldırım, F. (2004). Genetic marker mediated transfer of an alien gene, Pm21, into wheat conferring resistance to powdery mildew. Biotechnology & Biotechnological Equipment, 18 (2), 15-19.

Yıldırım, A., Ateş Sönmezoğlu, Ö., Sayaslan, A., Kandemir, N., Gökmen, S. (2019). Molecular Breeding of Durum Wheat for Pasta Quality. Quality Assurance and Safety of Crops & Foods, 11 (1), 15-21.

Zeller, F.J., Kong, L., Hartl, L., Mohler, V., Hsam, S.L.K. (2002). Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.) 7. Gene Pm29 in line Pova. Euphytica, 123, 187-194.

Zeybek, A., Khan, M.K., Pandey, A., Gunel, A., Erdogan, O., Akkaya, M.S. (2017). Genetic structure of powdery mildew disease pathogen *Blumeria graminis* f. sp. *hordei* in the barley fields of cukurova in turkey. Fresenius Environ. Bull., 26, 906-912.

Zhan, H., Li, G., Zhang, X., Li, X., Guo, H., Gong, W., Jia, J., Qiao, L., Ren, Y., Yang, Z., et al. (2014). Chromosomal location and comparative genomics analysis of powdery mildew resistance gene Pm51 in a putative wheat-*Thinopyrum ponticum* introgression line. PLoS ONE, 9, e113455.

Zhang, R., Sun, B., Chen, J., Cao, A., Xing, L., Feng, Y., Lan, C., Chen, P. (2016). Pm55, a developmental-stage and tissue-specific powdery mildew resistance gene introgressed from *Dasypyrum villosum* into common wheat. Theor. Appl. Genet., 129, 1975-1984.

Zhang, R., Fan, Y., Kong, L., Wang, Z., Wu, J., Xing, L., Cao, A., Feng, Y. (2018). Pm62, an adult-plant powdery mildew resistance gene introgressed from *Dasypyrum villosum* chromosome arm 2VL into wheat. Theor. Appl. Genet., 131, 2613-2620.

Zhang, D., Zhu, K., Dong, L., Liang, Y., Li, G., Fang, T., Guo, G., Wu, Q., Xie, J., Chen, Y., et al. (2019). Wheat powdery mildew resistance gene Pm64 derived from wild emmer

(*Triticum turgidum* var. *dicoccoides*) is tightly linked in repulsion with stripe rust resistance gene Yr5. Crop J., 7, 761-770.

Zhang, R., Xiong, C., Mu, H., Yao, R., Meng, X., Kong, L., Xing, L., Wu, J., Feng, Y., Cao, A. (2021). Pm67, a new powdery mildew resistance gene transferred from *Dasypyrum villosum* chromosome 1V to common wheat (*Triticum aestivum* L.). Crop J., 9, 882-888.

Zhang, X., Wang, W., Liu, C., Zhu, S., Gao, H., Xu, H., Zhang, L., Song, J., Song, W., Liu, K., et al. (2021). Diagnostic kompetitive allele-specific PCR markers of wheat broad-spectrum powdery mildew resistance genes Pm21, PmV, and Pm12 developed for high-throughput marker-assisted selection. Plant Dis., 105, 2844-2850.

Zhang, W., Yu, Z., Wang, D., Xiao, L., Su, F., Mu, Y., Zheng, J., Li, L., Yin, Y., Yu, T., et al. (2023). Characterization and identification of the powdery mildew resistance gene in wheat breeding line ShiCG15-009. BMC Plant Biol., 23, 113.

Zhao, F., Li, Y., Yang, B., Yuan, H., Jin, C., Zhou, L., Shen, Q.H. (2020). Powdery mildew disease resistance and marker-assisted screening at the Pm60 locus in wild diploid wheat *Triticum urartu*. Crop. J., 8 (2): 252-259.

Zhao, Z., Sun, H., Song, W., Lu, M., Huang, J., Wu, L., Wang, X., Li, H. (2013). Genetic analysis and detection of the gene MlLX99 on chromosome 2BL conferring resistance to powdery mildew in the wheat cultivar Liangxing 99. Theor. Appl. Genet., 126, 3081-3089.

Zheng H, Dong L, Han X, Jin H, Yin C, Han Y, Li B, Qin H, Zhang J, Shen Q, Zhang K, Wang D. (2020a). The TuMYB46L-TuACO3 module regulates ethylene biosynthesis in einkorn wheat defense to powdery mildew. New Phytol., 225 (6): 2526-2541.

Zheng, W., Li, S., Liu, Z., Zhou, Q., Feng, Y., Chai, S. (2020b). Molecular marker-assisted gene stacking for disease resistance and quality genes in the dwarf mutant of an elite common wheat cultivar Xiaoyan22. BMC Genet., 21, 45.

Zhu, Z., Zhou, R., Kong, X., Dong, Y., Jia, J. (2005). Microsatellite markers linked to 2 powdery mildew resistance genes introgressed from *Triticum carthlicum* accession PS5 into common wheat. Genome, 48, 585-590.

Zou, S., Wang, H., Li, Y., Kong, Z., Tang, D. (2018). he NB-LRR gene Pm60 confers powdery mildew resistance in wheat. New Phytol., 218 (1): 298-309.

Moringa oleifera (*Moringaceae*): A Review on Phytochemical Contents

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ABSTRACT: Moringa Oleifera is a plant belonging to the Moringaceae family and is an important agricultural product in Asia and Africa. It is considered one of the greatest health wonders in recent years, a plant species. This review examines some phytochemical compounds found in different parts of the *Moringa oleifera* plant. The phytochemical content of Moringa includes various compounds such as vitamins, proteins, phenolic compounds, carotenoids, and mineral substances. These components particularly support the plant's antioxidant, anti-inflammatory, antibacterial, and anti-diabetic properties in human health. Phytochemicals, carotenoids, and glucosinolates with high content are reported to be responsible for most of these activities, as documented in the literature. *Moringa* oleifera's leaves have been the most extensively studied part. The potential of the phytochemical substances in the content of *Moringa oleifera* on health continues to be a subject of great interest and research. For a better understanding of the benefits of this plant in the fields of health and nutrition, future studies should focus more on these essential compounds.

Keywords: *Moringa oleifera*, Phytochemical compounds, Nutritional components

INTRODUCTION

Today, there is an increased interest in the use and research of plants that are considered multipurpose. One of these plants, also known by various names worldwide such as the "drumstick tree" or "horseradish tree," "Behçet tree," or "Moringa tree," is Moringa (Moringa oleifera Lam.,) belonging to the Moringaceae family and consisting of 14 different species. Its native land is considered to be India (Anjorin et al., 2010: Jongrungruangchok et al., 2010). India is well known for the natural growth of this plant, especially in the northwest and northeast regions, where it thrives and exhibits various variations under different geographical conditions (Aja et al., 2013; Marrufo et al., 2013). However, despite its specific regions in India, this plant is widely cultivated in many places such as Africa, Central and South America, Sri Lanka, India, Mexico, Malaysia, Indonesia, and the Philippines (Anwar et al., 2007; Padayachee and Baijnath, 2012: Özcan, 2020).

In today's world, the interest in a healthy lifestyle and natural food sources is steadily increasing. In this context, *Moringa oleifera* has become a plant that attracts the attention of both traditional medicine and modern science due to its nutritional values, health benefits, biological properties, ease of cultivation, nutritional values, and positive effects on human health. *Moringa oleifera* has been used for centuries in traditional medical systems due to its various medicinal properties. Different parts of this plant, including its leaves, fruits, flowers, roots, and seeds, are used, and they can be consumed raw or dried and ground into powder. The most commonly used part of the plant is its leaves. Moringa tea, smoothies, meals, and dietary supplements are popular products that can be made using this plant (Vergara-Jimenez et al., 2017; Özcan, 2020; Milla et al., 2021).

Moringa leaves contain a variety of phytochemicals. They include phytochemical compounds such as flavonoids, carotenoids, polyphenols, alkaloids, glucosinolates, and isothiocyanates, as well as proteins, vitamins (especially A and C), minerals (such as calcium and iron), and antioxidants. Therefore, it has become a product that is consumed for health purposes as well as for medical use. Many different studies have demonstrated the beneficial effects of these compounds found in *Moringa oleifera* on humans (Leone et al., 2015; Saini et al., 2016). These include reported effects such as protection against stomach ulcers (Pal et al., 1995), antidiabetic properties (Oyedepo et al., 2013), and anti-inflammatory effects (Rao et al., 1998). Additionally, it has been shown to improve liver and kidney functions (Bennett et al., 2003) and regulate thyroid hormone status (Tahiliani et al., 2000). *Moringa oleifera* leaves have also been studied for their potential effects on oxidative stress (Anwar et al., 2007), inflammation (Mahajan et al., 2009), liver diseases (Hamza et al., 2010; Pari et al., 2002; Efiong et al., 2013), hypercholesterolemia (Okwari et al., 2013), bacterial activity (Walter et al., 2011), and cancer (Anwar et al., 2007; Vergara-Jimenez et al., 2021). It is especially considered as an alternative solution to food deficiency and health problems in developing countries.

In recent years, modern research has further supported the potential health benefits of Moringa, particularly its antioxidant and anti-inflammatory effects. This review provides information on some of the important phytochemicals contained in *Moringa oleifera*.

PHYTOCHEMICALS

1. Isothiocyanates

Moringa oleifera contains compounds like isothiocyanates. These compounds may have antioxidant and anti-inflammatory properties associated with health benefits. *Moringa oleifera* is a plant that contains bioactive compounds like isothiocyanates. Isothiocyanates are naturally occurring compounds in plants that could have health benefits. Research suggests that these compounds may have protective effects against cancer and possess antioxidant properties. Some studies on the isothiocyanates found in *Moringa oleifera* include:

Isothiocyanates are bioactive compounds that show anti-inflammatory and antioxidant activity due to their ability to activate detoxification enzymes (Maldini et al., 2014). Many authors have reported hypoglycemic, anti-inflammatory, anti-cancer, and antioxidant effects of *Moringa oleifera* glucosinolates and isothiocyanates through various in vitro and in vivo models (Guevara et al., 1999; Cheng et al., 2019). Given the benefits of these compounds in preventing diseases associated with oxidative stress, extraction methods become a crucial point. Different processes during harvesting and subsequent drying methods have been shown to affect the glucosinolate content of the plant (Ramabulana et al., 2017; Tetteh et al., 2019; Lopez-Rodriguez et al., 2020).

In some studies, on the isothiocyanates found in *Moringa oleifera*:

4-(α-L-Rhamnosyloxy)-benzyl isothiocyanate: This isothiocyanate compound is found in Moringa oleifera leaves. Various studies suggest that this compound possesses antioxidant and anti-inflammatory properties and may inhibit the growth of cancer cells (Park et al., 2011; Galuppo et al., 2015).

4-(4'-O-acetyl-α-L-rhamnosyloxy)-benzyl isothiocyanate: This compound has also been identified in Moringa oleifera leaves. Research indicates that this isothiocyanate has antioxidant activity and may reduce cell damage (Park et al., 2011; Galuppo et al., 2015).

2. Glucosinolates

Glucosinolates are compounds found in plants that can be converted into isothiocyanates. Moringa oleifera also contains glucosinolates. Moringa oleifera is a plant species naturally containing glucosinolates, which are a type of compound found in some plant species and are part of their natural defense mechanisms. These compounds may help plants protect themselves against harmful organisms and offer some health benefits. The glucosinolate content in Moringa oleifera can be a part of the potential effects associated with the plant's health benefits. However, the effects of these compounds on health are still under investigation and may require further research.

Glucosinolates (GL) are natural compounds synthesized by Moringa oleifera. GL is associated with isothiocyanates (ITC), which are secondary metabolites with the plant's biological properties [Cavaiuolo and Ferrante, 2014]. GLs are hydroxysulfate esters containing β-D-thioglucose in a Z-configuration and are also known as esters of cis-configured hydroximinosulfate. The main glucosinolate in Moringa oleifera is 4-[(α-L-rhamnosyloxy)-benzyl]-GL, known as glucomoringin [Förster et al., 2015]. Glucomoringin has been identified in seeds, leaves, flowers, stems, and roots. Some other Moringa tissues have also been shown to contain glukotropaeolin and glucoerucin [Amaglo et al., 2010]. The concentration of GLs varies depending on the plant tissue and maturity stage, with higher levels often found in seedlings rather than mature plants [Lopez-Rodriguez et al., 2020].

3. Flavonoids

Moringa oleifera is a rich source of various flavonoids. Flavonoids are a group of polyphenolic compounds found in plants, and they encompass a wide range of compounds that may possess potential bioactive properties such as antioxidants, anti-inflammatory effects, anti-cancer potential, and more (Aqil et al., 2006; Khalafalla et al., 2010; Atawodi et al., 2010; Moyo et al., 2012). The types and quantities of polyphenols in Moringa oleifera can vary depending on factors like the plant's growth conditions, soil quality, season, and harvesting methods. Some of the most commonly reported flavonoids in Moringa oleifera include:

Quercetin: Abundantly found in Moringa oleifera leaves, quercetin is known for its antioxidant properties that can neutralize free radicals and reduce inflammation (Bischoff et al., 2008; Atawodi et al., 2010).

Kaempferol: Another significant polyphenol found in Moringa oleifera leaves, kaempferol exhibits anti-inflammatory and antioxidant effects and may contribute to heart health (Lako et al., 2007).

Chlorogenic Acid: Present in Moringa oleifera leaves, chlorogenic acid can reduce cellular damage due to its antioxidant properties and potentially help control blood sugar (Karthikesan et al., 2010).

Rutin: A polyphenol found in Moringa oleifera leaves, rutin may assist in protecting blood vessels and supporting circulatory health (Vergara-Jimenez et al., 2017).

4. Carotenoids

Moringa oleifera is a rich source of various nutritious components, including carotenoids. Carotenoids are colorful pigments found in plants that not only give them their characteristic colors but also have antioxidant properties. Carotenoids are precursors of vitamin A, which is important for human health and can have various positive effects (Saucedo-Pompa et al., 2018;

Saini et al., 2013; Haroen et al., 2022). Some of the important carotenoids found in Moringa oleifera include:

Beta-Carotene: Abundantly present in Moringa oleifera, beta-carotene is a precursor to vitamin A, which supports vision, skin health, and the immune system. It can be converted into vitamin A in the body and has antioxidant properties (Kumar Saini et al., 2014; Padayachee and Baijnath, 2020).

Alpha-Carotene: Another important carotenoid, alpha-carotene, can be converted into vitamin A and has antioxidant properties that support the immune system (Kumar Saini et al., 2014; Padayachee and Baijnath, 2020).

Moringa leaves are the richest part of the plant in terms of carotenoid content, making them a valuable source for meeting the body's carotenoid requirements. Consuming carotenoids with a bit of fat or a fat-containing food can enhance their absorption

5. Vitamins

Numerous studies have been conducted on the vitamins found in *Moringa oleifera*. These studies demonstrate the wide range of vitamin content in the plant and its potential positive effects on health (Kamran et al., 2020; Özcan, 2020). Here are some important vitamins found in *Moringa oleifera* and related studies:

Vitamin A (Beta-Carotene): *Moringa oleifera* is a rich source of beta-carotene, particularly high in beta-carotene content. Beta-carotene is converted into vitamin A in the body and is essential for eye health, skin health, and the immune system. Several studies have highlighted Moringa's high beta-carotene content, suggesting it can help prevent vitamin A deficiency (Khawaja Tahir et al., 2010; Mukunzi et al., 2011; Lopez-Teros et al., 2017; Kashyap et al., 2022).

Vitamin C (Ascorbic Acid): *Moringa oleifera* is known for its high vitamin C content. Numerous studies have explored its vitamin C content. Vitamin C plays a crucial role in strengthening the immune system, providing antioxidant protection, and promoting collagen production. It can help enhance the immune system and offers antioxidant properties (Kamran et al., 2020; Özcan, 2020).

Vitamin E (Tocopherols and Tocotrienols): Moringa oleifera contains tocopherols and tocotrienols, which are derivatives of vitamin E. Vitamin E is a potent antioxidant that helps protect cells from damage. Studies have indicated that Moringa's vitamin E content contributes to its antioxidant activity (Efiong et al., 2013; Kashyap et al., 2022).

B Vitamins (Riboflavin, Niacin, Folic Acid, etc.): Moringa oleifera contains various B vitamins such as riboflavin (B2), niacin (B3), and folic acid (B9). These B vitamins are critical for energy production, cellular metabolism, and nervous system function. Studies on Moringa's B vitamin content suggest its potential in providing these essential vitamins (Yameogo et al., 2011; Borel et al., 2013).

6. Minerals

Every part of Moringa oleifera is like a storehouse of essential nutrients and minerals. Moringa leaves, in particular, are rich in minerals such as calcium, potassium, zinc, magnesium, iron, and copper (Kasolo et al., 2010). Moringa contains several minerals crucial for growth and development, with calcium being one of the most important for human growth. While 8 ounces of milk provide approximately 300-400 mg of calcium, Moringa leaves can offer more than 1000 mg, and Moringa powder can exceed 4000 mg. This makes Moringa powder a potential alternative to iron tablets and, thus, could serve as a treatment for anemia. For instance, while beef contains only 2 mg of iron, Moringa leaf powder contains 28 mg of iron. Moringa has been reported to contain more iron than spinach (Fuglie et al., 2005; Gopalakrishnan et al., 2016). Adequate dietary intake of zinc is required for the proper growth of sperm cells and for DNA and RNA synthesis. Moringa leaves contain approximately 25.5–31.03 mg of zinc per kilogram, equivalent to the daily recommended intake of zinc (Barminas et al., 1995; Abbas et al., 2018).

7. Protein

Moringa oleifera contains protein in various parts of the plant, including its leaves and seeds. Different parts of the plant may contain varying amounts of protein. Here is some information regarding the protein content in *Moringa oleifera*:

Leaves: Moringa leaves are the richest part of the plant in terms of protein content, containing approximately 20% to 30% protein. This makes Moringa leaves valuable as nutritional supplements or sources of nourishing foods (Richter et al., 2003; Kamran et al., 2020; Özcan, 2020).

Seeds: Moringa seeds are also rich in protein, containing approximately 35% to 40% protein. They are versatile in nutrition as they also contain fats and fiber (Anwar et al., 2006; Aja et al., 2013).

Roots and Other Parts: While other parts of the Moringa plant may contain protein, leaves and seeds are generally the primary sources of protein. A study has shown that immature pods contain approximately 46.78% fiber and about 20.66% protein. Pods contain about 30% amino acids, while leaves contain 44% and flowers contain 31% amino acids. (Sánchez-Machado et al., 2010). In a study conducted by Gopalakrishnan et al. in 2020, they found that the protein content in fresh Moringa leaves was 6.7%, and in dried leaves, it was 29.4%.

This high protein content makes Moringa especially valuable in nutrition. Moringa leaves and seeds can be used to meet protein needs, particularly in vegan and vegetarian diets

CONCLUSION

Moringa oleifera, one of the monogenic groups of the Moringaceae family, is the most commonly cultivated plant. This review highlights the latest scientific evidence on the bioactive profiles of Moringa leaves. The components of this tree are not only a rich source of essential minerals but also contain a variety of nutrients such as vitamins, β-carotene, protein, flavonoids, and various mineral elements. High contents of phytochemicals, carotenoids, and glucosinolates have been reported in the literature. Furthermore, there is an increasing interest in using Moringa as an added-value component in the development of functional foods. Efforts to identify and measure these beneficial compounds from Moringa leaves are still ongoing. Future studies should focus more on these important compounds to better understand the health and nutritional benefits of this plant.

Statement of Conflict of Interest

There are no conflicts of interest.

Authors' Contributions

I would like to express my gratitude for the preparation of the draft, making revisions, and necessary changes, as well as coordinating with all co-authors once the draft of the draft was ready.

REFERENCES

Abbas, R.K., Elsharbasy, F.S., Fadlelmula, A.A., 2018. Nutritional Values of Moringa oleifera, Total Protein, Amino Acid, Vitamins, Minerals, Carbohydrates, Total Fat and Crude

Fiber, under the Semi-Arid Conditions of Sudan. J Microb Biochem Technol 10: 56-58. doi: 10.4172/1948-5948.1000396

Aja, P.M., Ibiam, U.A., Uraku, A.J., Orji, O.U., Offor, C.E., Nwali, B.U., 2013. Comparative proximate and mineral composition of Moringa oleifera leaf and seed. Global Advanced Research the Journal of Agricultural Science 137–141.

Amaglo N.K.Ç, Bennett, R.N., Lo Curto R.B., Rosa, EAS., Lo Turco, V., Giuffrida A., Lo Curto A., Crea F., Timpo G.M., 2010. Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree Moringa oleifera L., grown in Ghana. Food Chem 122(4):1047–1054. https://doi.org/10.1016/j.foodchem.2010.03.073

Anwar, F., Bhanger, M.I., 2003. Analytical characterization of Moringa oleifera seed oil grown in temperate regions of Pakistan. Journal of Agricultural and Food Chemistry 51, 6558–6563.

Anwar, F., Latif, S., Ashraf, M., Gilani, A.H., 200). Moringa oleifera. A food plant with multiple medicinal uses. Phytother Res 21: 17–25.

Barminas, J.T., Charles, M., Emmanuel, D., 1998. Mineral composition of non-conventional leafy vegetables Plant Foods Hum. Nutr., 53 (1998), pp. 29-36.

Bennett, R.N. Mellon, F.A. Foidl, N. Pratt, J.H., Dupont, M.S., Perkins, L., Kroon, P.A., 2003. Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees Moringa oleifera L. (horseradish tree) and Moringa stenopetala L. J. Agric. Food Chem. 2003, 51, 3546–3553

Cavaiuolo, M., Ferrante, A., (2014) Nitrates and glucosinolates as strong determinants of the nutritional quality in rocket leafy salads. Nutrients 6(4):1519–1538. https://doi.org/10.3390/nu6041519.

Efiong, E.E. Igile, G.O. Mgbeje, B.I.A. Out, E.A. Ebong, P.E., 2013. Hepatoprotective and anti-diabetic effect of combined extracts of Moringa oleifera and Vernoniaamygdalina in streptozotocin-induced diabetic albino Wistar rats. J. Diabetes Endocrinol., 4, 45–50.

Förster, N., Ulrichs, C., Schreiner, M., Müller, C.T., Mewis, I., 2015. Development of a reliable extraction and quantification method for glucosinolates in Moringa oleifera. Food Chem 166:456–464. https://doi.org/10.1016/j.foodchem.2014.06.043

Gopalakrishnan, L., Doriya, K., Santhosh Kumar, D., 2016. Moringa oleifera: A review on nutritive importance and its medicinal application Food Science and Human Wellness

Volume 5, Issue 2, June 2016, Pages 49-56.

Jongrungruangchok, S., Bunrathep, S., and Songsak, T., 2010. Nutrıents And Mınerals Content of Eleven Dıfferent Samples of Morınga Oleıfera Cultıvated in Thailand. Journal of Health Research 2010, 24(3): 123-127

Hamza, A.A., 2010. Ameliorative effects of Moringa oleifera Lam seed extract on liver fibrosis in rats. Food Chem. Toxicol. 2010, 48, 345–355.

Leone, A. Spada, A. Battezzati, A. Schiraldi, A. Aristil, J. Bertoli, S., 2015. Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of Moringa oleifera Leaves: An overview. Int. J. Mol. Sci., 16, 12791–12835

Lopez-Rodriguez, N.A., Gaytán-Martínez, M., María de la Luz Reyes-Vega Loarca- Piña, G., (2020. Glucosinolates and Isothiocyanates from Moringa oleifera: Chemical and Biological Approaches Plant Foods Hum Nutr (2020) 75:447–457.

Mahajan, S. Banerjee, A. Chauhan, B. Padh, H. Nivsarkar, M. Mehta, A., 2009. Inhibitory effect of N-butanolfraction of Moringa oleifera Lam seeds on ovalbumin-induced airway inflammation in a guinea pig model of asthma. Int. J. Toxicol. 2009, 28, 519–527

Marrufo, T., Nazzaro, F., Mancini, E., Fratianni, F., Coppola, R., De Martino, L., Agostinho, A.B., De feo, V., 2013. Chemical composition and biological activity of the essential oil from leaves of Moringa oleifera Lam. cultivated in Mozambique. Molecules 18, 10,989–11,000.

Milla, P.G. Peñalver, R. Nieto, G., 2012. Health Benefits of Uses and Applications of Moringa oleifera in Bakery Products. Plants 2021, 10, 318. https://doi.org/10.3390/plants10020.

Okwari, O. Dasofunjo, K. Asuk, A. Alagwu, E. Mokwe, C., 2013. Anti-hypercholesterolemic and hepatoprotective effect of aqueous leaf extract of Moringa oleifera in rats fed with thermoxidized palm oil diet. J. Pharm. Biol. Sci. 2013, 8, 57–62.

Oyedepo, T.A. Babarinde, S.O. Ajayeoba, T.A., 2013. Evaluation of the antihyperlipidemic effect of aqueous leaves extract of Moringa oleifera in alloxan induced diabetic rats. Int. J. Biochem. Res. Rev. 2013, 3, 162–170.

Özcan M.M., 2020. Moringa spp: Composition and bioactive properties. South African Journal of Botany 129 (2020) 25–31

Padayachee, B. ve Baijnath, H., 2012. An overview of the medicinal importance of Moringaceae. J. Med. Plants Res.2012, 6, 5831–5839.

Pal, S.K.; Mukherjee, P.K.; Saha, B.P. (1995). Studies on the antiulcer activity of Moringa oleifera leaf extract on gastric ulcer models in rats. Phytother. Res. 1995, 9, 463–465.

Pari, L. Kumar, N.A., 2002. Hepatoprotective activity of Moringa oleifera on antitubercular drug-induced liver damage in rats. J. Med. Food 2002, 5, 171–177.

Saini, R.K. Sivanesan, I. Keum, Y.S., 2016. Phytochemicals of Moringa oleifera: A review of their nutritional, therapeutic and industrial significance. 6, Article number: 203

Sánchez-Machado, D.I., Núñez-Gastélum, J.A., Reyes-Moreno, C., Ramírez-Wong, B. López-Cervantes, J., 2010. Nutritional quality of edible parts of Moringa oleifera Food Anal. Methods, 3 2010, pp. 175-180

Sultana, S., 2020. Nutritional and functional properties of Moringa oleifera. Metabolism Open Volume 8, December 2020, 100061.

Tahiliani, P. Kar, A., 2000. Role of Moringa oleifera leaf extract in the regulation of thyroid hormone status in adult male and female rats. Pharmacol. Res. 2000, 41, 319–323.

Vergara-Jimenez, M., Almatrafi, M.M., and Fernandez, M.L., 2017. Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease Antioxidants 2017, 6, 91; doi:10.3390/antiox6040091.

Walter, A. Samuel, W., Peter, A. Joseph, O., 2011. Antibacterial activity of Moringa oleifera and Moringa stenopetala methanol and N-hexane seed extracts on bacteria implicated in water borne diseases. Afr. J. Microbiol. Res., 5, 153–157.

Chemical Composution of Black Cumin (*Nigella Sativa* L.) Seeds: A review

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ABSTRACT: Black seed (Nigella sativa L.) is a plant species with a long history in the fields of medicine and food, widely used in various cultures. This review focuses on the chemical composition of black seed. Through examinations, it has been determined that the volatile oils of black seed seeds contain the main component known as thymoquinone. Thymoquinone is known to possess antioxidant, anti-inflammatory, and anticancer properties. It contains significant components with various biological and pharmacological activities, with the most well-known and effective being the volatile oils. Additionally, other important components found in black seed seeds include carotenoids, flavonoids, proteins, minerals (such as potassium, magnesium, and calcium), vitamins (including the B vitamin complex and vitamin E), and fibers. The chemical composition of black seed is under investigation in various areas such as its use as a food additive, its application in alternative medicine, and its potential applications in the pharmaceutical industry. As a result, the rich chemical composition of black seed continues to be a subject of interest in the fields of health and medicine. The components of this plant may lead to further research and application opportunities in the future, contributing to the discovery of new treatment options.

INTRODUCTION

Black seed (*Nigella sativa* L.), commonly known as black cumin or black caraway, is an annual herbaceous plant of the Ranunculaceae family. It is believed to have originated in the Middle East and has been used by different civilizations in the region throughout history. Particularly, the ancient Egyptians, Greeks, and the Roman Empire used it for various medical and culinary purposes. It is also well-documented that black seed is widely used for medicinal and nutritional purposes in the Islamic world (Salem, 2005; Butt et al., 2010). Black seed has spread to different geographical regions beyond the Middle East and is currently cultivated worldwide. It is especially prevalent in countries such as Turkey, Iran, Iraq, and Saudi Arabia in the Middle East. In the Indian subcontinent, countries like India, Pakistan, and Bangladesh also cultivate black seed. In the Mediterranean region, black seed is grown in countries like Greece and Egypt and is popular as a spice. In Europe and America, black seed is also cultivated, though it is less common (Chaudhry et al., 2020; Hannan et al., 2021).

Today, the terms "nutraceuticals" or "functional foods" have become popular among health-conscious individuals, as some research has indicated a close relationship between a healthy lifestyle and an extended lifespan (Gupta and Prakash, 2015; Hannan et al., 2021). These concepts have attracted the attention of dietitians, nutrition experts, food scientists, doctors, and the food and pharmaceutical industries. As the global market for functional foods expands, extensive research is being conducted to discover traditional foods that offer health benefits (Embuscado, 2015; Jiang, 2015; Yimer et al., 2019). Many plants offered by nature have been used for centuries as health-supporting agents in traditional medical systems. One such plant, containing numerous phytochemical compounds, is black seed (Nigella sativa). The most commonly used part of black seed is its black seeds. Extensive research has been conducted by various researchers and doctors over the years to study the pharmacological effects and efficacy of these black seeds against various diseases. In these studies, black seed has been found to have various pharmacological effects, including antioxidant effects (Bourgou et al., 2012; Umar et al., 2012), antibacterial effects (Morsi, 2000; Hosseinzadeh et al., 2007; Hannan et al., 2008), antimicrobial effects (Forouzanfar et al., 2014), anti-inflammatory effects (Houghton et al., 1995; Mutabagani and El-Mahdy, 1997), analgesic effects (Khanna et al., 1993), antidermatophytic effects (Aljabre et al., 2005), anti-cancer effects (Shafi et al., 2009; Majdalawieh et al., 2010), central nervous system depressant effects, anxiolytic effects (Bin Sayeed et al., 2004), cardiovascular effects (Sultan et al., 2009), and effects on the immune system (Swamy and Tan, 2000).

Black seed exhibits a wide range of biological effects, including anti-inflammatory, anti-hyperlipidemic, antimicrobial, anticancer, antioxidant, antidiabetic, antihypertensive, and anticonvulsant effects, as well as effects on reproduction, digestion, immunity, and the central nervous system (Ermumcu and Şanlıer, 2017; Toma et al., 2010; Khan et al., 2011; Kooti et al., 2016; Sharma et al., 2009). This broad spectrum of effects supports various medical and health-related applications of black seed. However, it is essential to consult a healthcare professional before using black seed for such purposes, as there can be interactions and side effects.

Black seed seeds contain numerous phytochemical compounds, including flavonoids, carotenoids, polyphenols, alkaloids, glucosinolates, isothiocyanates, proteins, vitamins (especially A and C), minerals (such as calcium and iron), and antioxidants. As a result, black seed has become a product consumed for health purposes, in addition to its use in traditional medicine.

As mentioned earlier, the nutritional value of black seed has been the subject of numerous epidemiological studies, which is due to its high nutrient content and the presence of various active phytochemical compounds. The phytochemical composition of black seed can vary depending on the regions where it is grown, the stage of maturity, processing methods, and isolation techniques. The purpose of this review is to provide information about some of the important phytochemicals found in black seed.

The phytochemical composition of black seed, which is predominantly comprised of black seeds, is divided into different chemical classes, consisting of large and small secondary metabolites. These include:

PHYTOCHEMICAL PROFILES

1. Volatile Compounds

Various studies have demonstrated the presence of various natural compounds in the volatile oil of Nigella sativa, commonly known as black cumin or black seed. These compounds include monoterpenes, diterpenes, sesquiterpenes, monoterpenoid alcohols, and ketones. Volatile oils generally constitute a complex mixture, and the analysis of these unique compounds with distinct chemical structures and properties is a common practice (Dalli et al., 2022). Among the mentioned compounds are components like thymoquinone (TQ), thymohydroquinone, thymol, carvacrol, phellandrene, α-pinene, and β-pinene, which collectively belong to the terpenes and terpenoids family. This family represents the primary chemical group of black cumin (*Nigella sativa*) (Kabir et al., 2020; Dalli et al., 2021; Hannan et al., 2021).

Thymoquinone (TQ)

Thymoquinone is the most significant and active compound found in the black seeds of *Nigella sativa*. This compound is a synthetic form of thymoquinone, which is a phenolic monoterpenoid. Thymoquinone is a volatile oil responsible for the characteristic aroma of black cumin. It contains a phenolic ring and a quinone structure, and it plays a crucial role in the biological activities exhibited by *Nigella sativa*. It has been reported to range from 18% to 48% in different studies (Ayhan, 2012; Ahmad et al., 2013; Ulus et al., 2018; Güzelsoy et al., 2018). Thymoquinone and its associated compounds, particularly thymoquinone (TQ), have been the focus of numerous medical research studies. These compounds are believed to be responsible for various biological activities, including antioxidant, anti-inflammatory, liver-protective, analgesic, antineoplastic (anti-cancer), antimutagenic, kidney-protective, immune-boosting, hypoglycemic (blood sugar-lowering), ulcer-preventing, antimicrobial, and antiparasitic effects, among others (Ghedira, 2006; Kumar et al., 2017; Alhmied et al., 2021; Hannan et al., 2021; Hossain et al., 2021; Shahid et al., 2021; Albakry et al., 2022; Dalli et al., 2022). Research on thymoquinone and other compounds in black cumin is still ongoing.

T*hymohydroquinone*

Thymohydroquinone, like thymoquinone, possesses antioxidant properties and can be found in the volatile oil of *Nigella sativa*.

Thymol

Thymol is a component that contributes to the characteristic taste and aroma of the volatile oil of *Nigella sativa*. It has antimicrobial and anti-inflammatory properties.

p-Cymene (P-Cymene)

Another important component found in black cumin seeds is p-cymene, which makes up about 7-15% of the black seeds' composition. It exhibits antimicrobial properties (Ahmad et al., 2013; Güzelsoy et al., 2018; Hannan et al., 2021).

Carvacrol

Carvacrol is another volatile compound found in the black cumin oil and has antimicrobial and antioxidant effects. It is present in black seeds at levels of 6-12% (Ahmad et al., 2013).

Phellandrene

Phellandrene is a terpene compound found in various plants and is present in the volatile oil of *Nigella sativa*. It has a pleasant odor.

α-Pinene and β-Pinene

These two pinene compounds are commonly found in trees and some plants. It is likely that they are present in the volatile oil of *Nigella sativa* and may contribute to various aromas.

2. Alkaloids

Different alkaloids have been isolated and identified from black cumin seeds in various studies. The alkaloids of black cumin can be classified based on alkaloid skeletons. These include nigellisine, which consists of an indazole core, nigellimine, which is an isoquinoline molecule, and pyrazole or indazole alkaloids such as nigellidine and nigellissine. Moreover, alkaloids specific to black cumin, called nigellamines, belong to the diterpene family and have been reported to exhibit potent lipid metabolism-enhancing activities. Additionally, another molecule recently identified in the upper part of black cumin is magnoflorin. Nigellone and nigellin have antioxidant and anti-inflammatory effects (Akram Khan and Afzal, 2016; Parveen et al., 2020; Dalli et al., 2021).

3. Flavonoids and Polyphenols

Nigella sativa contains various biochemical components, and some of them are flavonoids and polyphenols. These compounds are known for their antioxidant properties, offering protection against free radicals. Flavonoids have also been reported to reduce the adhesion of inflammatory cells to the endothelium, which leads to a decrease in inflammatory response. Inhibition of peroxidase activity by flavonoids is another beneficial effect, as it prevents the formation of reactive oxygen species. Several studies have identified phenolic compounds such as quercetin, kaempferol, gallic acid, ferulic acid, caffeic acid, vanillic acid, p-coumaric acid, chlorogenic acid, catechin, quercetin, apigenin, rutin, myricetin, fisetin, patuletin, luteolin, nigelflavonoside B, flavon, and apigenin in *Nigella sativa* seeds. Among these compounds, quercetin and kaempferol have been reported as the most abundant flavonoids in black cumin (Khan et al., 2011; Toma et al., 2015; Anand David et al., 2016; Kumar, 2017; Dabeek and Marra, 2019; Parveen et al., 2020; Dalli et al., 2021; Hannan et al., 2021).

4. Fatty Acids

Black seed (*Nigella sativa*) is rich in terms of fatty acids and contains various types of fatty acids, as indicated in some studies using GC-MS techniques. However, these studies are limited and insufficient. These fatty acids contribute to the plant's health-related properties. Black seed oil contains a higher amount of unsaturated fatty acids compared to other vegetable oils, indicating its potential health benefits (Rouhou et al., 2007; Bayati et al., 2020; Albakry et al., 2022; Zarifikhosroshahi et al., 2022).

In the composition of black seed oil, the primary composition of saturated and unsaturated fatty acids varies between approximately 32% to 40%. Linolenic acid, an unsaturated fatty acid (omega-6), is the dominant and most abundant acid in black seed, constituting over 50% of the composition. It is followed by oleic acid at around 20% and palmitic acid at approximately 10%. Other fatty acids like arachidonic acid, eicosadienoic acid, stearic acid, myristic acid, linoleic acid, and palmitoleic acid have also been reported in some studies (Nickavar et al., 2003; El-Tahir et al., 2006; Rouhou et al., 2007; Khoddami et al., 2011; Aziz et al., 2017; Güzelsoy et al., 2018; Bayati et al., 2020; Tiji et al., 2021; Zarifikhosroshahi et al., 2022; Dalli et al., 2022). Among the most common unsaturated fatty acids, linoleic acid (omega-6) and linolenic acid (omega-3) have been proven to reduce blood pressure and the risk of vascular diseases, while oleic acid has unique properties for reducing blood cholesterol levels (Ali and Blunden, 2003; Beyzi et al., 2019; Hosseini et al., 2019; Zarifikhosroshahi et al., 2022). Therefore, black seed oil is considered a valuable and nutritious source as a rich supplier of essential fatty acids, with potential to serve as an alternative to traditional oils.

5. Protein

Proteins found in black seed are one of the significant nutritional components present in the seeds of the plant. These proteins consist of various amino acids and can serve as an important protein source for humans. The composition of proteins in black seed varies, with some studies indicating it falls within the range of approximately 15% to 30% (Al-Gaby et al., 1998; Nickavar et al., 2003; Rouhou et al., 2007; Ahmad et al., 2013; Fidan et al., 2019). Some of these proteins contribute to the nutritional value of the plant and can be beneficial for health.

6. Minerals

Black seed (*Nigella sativa*) seeds are rich in various minerals and contain significant amounts of minerals. These minerals play important roles in various biological functions in the body and can contribute to human nutrition. Some studies have reported that black seed contains potassium, phosphorus, magnesium, sodium, iron, manganese, selenium, aluminum, zinc, copper, and other minerals. Analysis of these studies indicates variations in the dominant minerals depending on the region. In certain areas, such as Turkey and Yemen, seeds are predominantly rich in calcium and potassium, while in India and Ethiopia, magnesium is found to be the dominant element (Al-Jassir et al., 1992; Özcan et al., 2004; Al-Naqeep et al., 2009; Sultan et al., 2009; Herlina et al., 2017; Mamun and Absar, 2018; Thilakarathna and Nawarathna, 2018; Fidan et al., 2019; Kabir et al., 2019; Albakry et al., 2022; Imperl et al., 2023).

7. Vitamins

Vitamins are biologically active substances that control the health and growth of an organism. These substances often occur alongside compounds with similar chemical properties. Human nutrition must provide an appropriate amount of vitamins for the fundamental functions of the body. A lack of systematic vitamins in human nutrition can result in inadequate growth and development (Karrar et al., 2018; Albakry et al., 2022). Some studies have indicated that black seed contains Vitamin E (α-tocopherol), β-tocopherol, and γ-tocopherol, niacin (Vitamin

B3), Vitamin A (retinol), D2, and K1. Upon examination, different researchers have reported varying quantities of dominant vitamins. Vitamin E (α-tocopherol) and niacin (Vitamin B3) have been observed as the most abundant vitamins in different regions (Nergiz and Ötleş, 1993; Wakai et al., 2005; Al-Saleh et al., 2006; Fidan et al., 2019; Vatansev et al., 2013; Albakry et al., 2022).

CONCLUSION

This article reviewed studies related to the chemical composition of black seed (*Nigella sativa* L.) seeds and presented significant findings. Literature research indicates that black seed seeds are rich in various chemical components. Among these components, the most remarkable one is the major compound known as thymoquinone. It has been determined that thymoquinone possesses antioxidant, anti-inflammatory, and anticancer properties. Additionally, black seed seeds contain other components like carotenoids, flavonoids, protein, minerals (such as potassium, magnesium, and calcium), vitamins (B vitamin complex, Vitamin E, etc.), and fibers. These results can help us better understand the potential health benefits of black seed. Black seed can be an important resource in alternative medicine and the food industry. Furthermore, potential applications in the pharmaceutical industry may require further research. In conclusion, the chemical composition of black seed seeds is an interesting and significant topic in the fields of health, medicine, and nutrition. This study has contributed to a better understanding of the potential benefits of black seed for health and may guide future research.

Statement of Conflict of Interest

There are no conflicts of interest.

Authors' Contributions

I would like to express my gratitude for the preparation of the draft, making revisions, and necessary changes, as well as coordinating with all co-authors once the draft of the draft was ready.

REFERENCES

Abat, J. K., Kumar, S., & Mohanty, A. (2017). Ethnomedicinal, phytochemical and ethnopharmacological aspects of four medicinal plants of Malvaceae used in Indian traditional medicines: A review. Medicines, 4(4), 75.

Abat, J. K., Kumar, S., & Mohanty, A. (2017). Ethnomedicinal, phytochemical and ethnopharmacological aspects of four medicinal plants of Malvaceae used in Indian traditional medicines: A review. Medicines, 4(4), 75.

Ahmad, A., Husain, A., Mujeeb, M., Khan, S. A., Najmi, A. K., Siddique, N. A., ... & Anwar, F. (2013). A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pacific journal of tropical biomedicine, 3(5), 337-352.

Akram Khan, M., & Afzal, M. (2016). Chemical composition of *Nigella sativa* Linn: part 2 recent advances. Inflammopharmacology, 24, 67-79.

Albakry, Z., Karrar, E., Ahmed, I. A. M., Oz, E., Proestos, C., El Sheikha, A. F., ... & Wang, X. (2022). Nutritional composition and volatile compounds of black cumin (*Nigella sativa* L.) seed, fatty acid composition and tocopherols, polyphenols, and antioxidant activity of its essential oil. Horticulturae, 8(7), 575.

Al-Gaby, A. M. A. (1998). Amino acid composition and biological effects of supplementing broad bean and corn proteins with *Nigella sativa* (black cumin) cake protein. Food/Nahrung, 42(05), 290-294.

Alhmied, F., Alammar, A., Alsultan, B., Alshehri, M., & Pottoo, F. H. (2021). Molecular mechanisms of thymoquinone as anticancer agent. Combinatorial Chemistry & High Throughput Screening, 24(10), 1644-1653.

Ali, B. H., & Blunden, G. (2003). Pharmacological and toxicological properties of *Nigella sativa*. Phytotherapy Research: An international journal devoted to pharmacological and toxicological evaluation of natural product derivatives, 17(4), 299-305.

Aljabre, S. H. M., Randhawa, M. A., Akhtar, N., Alakloby, O. M., Alqurashi, A. M., & Aldossary, A. (2005). Antidermatophyte activity of ether extract of *Nigella sativa* and its active principle, thymoquinone. Journal of Ethnopharmacology, 101(1-3), 116-119.

Al-Jassir, M. S. (1992). Chemical composition and microflora of black cumin (*Nigella sativa* L.) seeds growing in Saudi Arabia. Food Chemistry, 45(4), 239-242.

Al-Naqeep, G. N., Ismail, M. M., Al-Zubairi, A. S., & Esa, N. M. (2009). Nutrients composition and minerals content of three different samples of *Nigella sativa* L. cultivated in Yemen. Asian Journal of Biological Sciences, 2(2), 43-48.

Al-Saleh, I. A., Billedo, G., & El-Doush, I. I. (2006). Levels of selenium, dl-α-tocopherol, dl-γ-tocopherol, all-trans-retinol, thymoquinone and thymol in different brands of *Nigella sativa* seeds. Journal of Food Composition and Analysis, 19(2-3), 167-175.

Aziz, S. A., Kurniawati, A., & Faridah, D. N. (2017). Changes of thymoquinone, thymol, and malondialdehyde content of black cumin (*Nigella sativa* L.) in response to Indonesia tropical altitude variation. Hayati Journal of Biosciences, 24(3), 156-161.

Bayati, P., Karimmojeni, H., & Razmjoo, J. (2020). Changes in essential oil yield and fatty acid contents in black cumin (*Nigella sativa* L.) genotypes in response to drought stress. Industrial Crops and Products, 155, 112764.

Bayati, P., Karimmojeni, H., & Razmjoo, J. (2020). Changes in essential oil yield and fatty acid contents in black cumin (*Nigella sativa* L.) genotypes in response to drought stress. Industrial Crops and Products, 155, 112764.

Beyzi, E., Gunes, A., Beyzi, S. B., & Konca, Y. (2019). Changes in fatty acid and mineral composition of rapeseed (Brassica napus ssp. oleifera L.) oil with seed sizes. Industrial Crops and Products, 129, 10-14.

Bourgou, S., Pichette, A., Marzouk, B., & Legault, J. (2012). Antioxidant, anti-inflammatory, anticancer and antibacterial activities of extracts from Nigella sativa (black cumin) plant parts. Journal of Food Biochemistry, 36(5), 539-546.

Butt, M. S., & Sultan, M. T. (2010). Nigella sativa: reduces the risk of various maladies. Critical reviews in food science and nutrition, 50(7), 654-665.

Chaudhry, Z., Khera, R. A., Hanif, M. A., Ayub, M. A., & Sumrra, S. H. (2020). Cumin. In Medicinal plants of South Asia (pp. 165-178). Elsevier.

Cheikh-Rouhou, S., Besbes, S., Hentati, B., Blecker, C., Deroanne, C., & Attia, H. (2007). Nigella sativa L.: Chemical composition and physicochemical characteristics of lipid fraction. Food chemistry, 101(2), 673-681.

Dabeek, W. M., & Marra, M. V. (2019). Dietary quercetin and kaempferol: Bioavailability and potential cardiovascular-related bioactivity in humans. Nutrients, 11(10), 2288.

Dalli, M., Azizi, S. E., Benouda, H., Azghar, A., Tahri, M., Bouammali, B., ... & Gseyra, N. (2021). Molecular composition and antibacterial effect of five essential oils extracted from Nigella sativa L. seeds against multidrug-resistant bacteria: a comparative study. Evidence-Based Complementary and Alternative Medicine, 2021.

Dalli, M., Bekkouch, O., Azizi, S. E., Azghar, A., Gseyra, N., & Kim, B. (2021). Nigella sativa L. phytochemistry and pharmacological activities: a review (2019–2021). Biomolecules, 12(1), 20.

Dalli, M., Bekkouch, O., Azizi, S. E., Azghar, A., Gseyra, N., & Kim, B. (2021). Nigella sativa L. phytochemistry and pharmacological activities: a review (2019–2021). Biomolecules, 12(1), 20.

David, A. V. A., Arulmoli, R., & Parasuraman, S. (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. Pharmacognosy reviews, 10(20), 84.

El-Tahir, K. E. D. H., & Bakeet, D. M. (2006). The black seed Nigella sativa Linnaeus-A mine for multi cures: a plea for urgent clinical evaluation of its volatile oil. Journal of Taibah University Medical Sciences, 1(1), 1-19.

Embuscado, M. E. (2015). Spices and herbs: Natural sources of antioxidants–a mini review. Journal of functional foods, 18, 811-819.

Fidan, H., Stankov, S., Daraba, A., Dogan, H., Alexieva, I., Stoyanova, A., & Ercisli, S. Phytochemical composition of black cumin (Nigella sativa L.) seeds from Turkey as an unconventional source for the food industry.

Forouzanfar, F., Bazzaz, B. S. F., & Hosseinzadeh, H. (2014). Black cumin (Nigella sativa) and its constituent (thymoquinone): a review on antimicrobial effects. Iranian journal of basic medical sciences, 17(12), 929.

Ghedira, K. (2006). La nigelle cultivée: Nigella sativa L.(Ranunculaceae). Phytothérapie, 4(5), 220-226.

Gupta, C., & Prakash, D. (2015). Nutraceuticals for geriatrics. Journal of traditional and complementary medicine, 5(1), 5-14.

Güzelsoy, P., Aydın, S.,Başaran, N. (2018). Çörek otunun (Nigella sativa L.) aktif bileşeni timokinonun insan sağlığı üzerine olası etkileri. Literatür Eczacılık Bilimleri Dergisi, 7(2), 118-135.

Hannan, A., Saleem, S., Chaudhary, S., Barkaat, M., & Arshad, M. U. (2008). Anti bacterial activity of Nigella sativa against clinical isolates of methicillin resistant Staphylococcus aureus. J Ayub Med Coll Abbottabad, 20(3), 72-74.

Hannan, M. A., Rahman, M. A., Sohag, A. A. M., Uddin, M. J., Dash, R., Sikder, M. H., ... & Kim, B. (2021). Black cumin (Nigella sativa L.): A comprehensive review on phytochemistry, health benefits, molecular pharmacology, and safety. Nutrients, 13(6), 1784.

Hannan, M. A., Rahman, M. A., Sohag, A. A. M., Uddin, M. J., Dash, R., Sikder, M. H., ... & Kim, B. (2021). Black cumin (Nigella sativa L.): A comprehensive review on phytochemistry, health benefits, molecular pharmacology, and safety. Nutrients, 13(6), 1784.

Hossain, M. S., Sharfaraz, A., Dutta, A., Ahsan, A., Masud, M. A., Ahmed, I. A., ... & Ming, L. C. (2021). A review of ethnobotany, phytochemistry, antimicrobial pharmacology and toxicology of Nigella sativa L. Biomedicine & Pharmacotherapy, 143, 112182.

Hosseini, S. S., Rezadoost, H., Nadjafi, F., & Asareh, M. H. (2019). Comparative essential oil composition and fatty acid profiling of some Iranian black cumin landraces. Industrial Crops and Products, 140, 111628.

Hosseinzadeh, H., Fazly Bazzaz, B. S., & Haghi, M. M. (2007). Antibacterial activity of total extracts and essential oil of Nigella sativa L. seeds in mice. Pharmacologyonline, 2, 429-435.

Imperl, J., Kolar, M., Petrova, P., & Chochkova, M. (2023). Mineral content estimation of Black cumin seeds. Journal of Chemical Technology and Metallurgy, 58(5), 859-864.

Jiang, T. A. (2019). Health benefits of culinary herbs and spices. Journal of AOAC International, 102(2), 395-411.

Kabir, Y., Akasaka-Hashimoto, Y., Kubota, K., & Komai, M. (2020). Volatile compounds of black cumin (Nigella sativa L.) seeds cultivated in Bangladesh and India. Heliyon, 6(10).

Kabir, Y., Shirakawa, H., & Komai, M. (2019). Nutritional composition of the indigenous cultivar of black cumin seeds from Bangladesh. Prog. Nutr, 21, 428-434.

Karrar, E., Sheth, S., Navicha, W. B., Wei, W., Hassanin, H., Abdalla, M., & Wang, X. (2019). A potential new source: Nutritional and antioxidant properties of edible oils from cucurbit seeds and their impact on human health. Journal of food biochemistry, 43(2), e12733.

Khan, A., Chen, H. C., Tania, M., & Zhang, D. Z. (2011). Anticancer activities of Nigella sativa (black cumin). African Journal of Traditional, Complementary and Alternative Medicines, 8(5S).

Khoddami, A., Ghazali, H. M., Yassoralipour, A., Ramakrishnan, Y., & Ganjloo, A. (2011). Physicochemical characteristics of nigella seed (Nigella sativa L.) oil as affected by different extraction methods. Journal of the American Oil Chemists' Society, 88, 533-540.

Majdalawieh, A. F., Hmaidan, R., & Carr, R. I. (2010). Nigella sativa modulates splenocyte proliferation, Th1/Th2 cytokine profile, macrophage function and NK anti-tumor activity. Journal of ethnopharmacology, 131(2), 268-275.

Malik, S., & Zaman, K. (1992). Nigellimine: a new isoquinoline alkaloid from the seeds of Nigella sativa. Journal of Natural Products, 55(5), 676-678.

Malik, S., Hasan, S. S., Choudhary, M. I., Ni, C. Z., & Clardy, J. (1995). Nigellidine—a new indazole alkaloid from the seeds of Nigella sativa. Tetrahedron letters, 36(12), 1993-1996.

Morsi, N. M. (2000). Antimicrobial effect of crude extracts of Nigella sativa on multiple antibiotics-resistant bacteria. Acta Microbiologica Polonica, 49(1), 63-74.

Nergiz, C., & Ötleş, S. (1993). Chemical composition of Nigella sativa L. seeds. Food chemistry, 48(3), 259-261.

Nergiz, C., & Ötleş, S. (1993). Chemical composition of Nigella sativa L. seeds. Food chemistry, 48(3), 259-261.

Nickavar, B., Mojab, F., Javidnia, K., & Amoli, M. A. R. (2003). Chemical composition of the fixed and volatile oils of Nigella sativa L. from Iran. Zeitschrift für Naturforschung C, 58(9-10), 629-631.

Nickavar, B., Mojab, F., Javidnia, K., & Amoli, M. A. R. (2003). Chemical composition of the fixed and volatile oils of Nigella sativa L. from Iran. Zeitschrift für Naturforschung C, 58(9-10), 629-631.

Özcan, M. (2004). Mineral contents of some plants used as condiments in Turkey. Food chemistry, 84(3), 437-440.

Parveen, A., Farooq, M. A., & Kyunn, W. W. (2020). A new oleanane type saponin from the aerial parts of nigella sativa with anti-oxidant and anti-diabetic potential. Molecules, 25(9), 2171.

Parveen, A., Farooq, M. A., & Kyunn, W. W. (2020). A new oleanane type saponin from the aerial parts of nigella sativa with anti-oxidant and anti-diabetic potential. Molecules, 25(9), 2171.

Sayeed, M. S. B., Shams, T., Hossain, S. F., Rahman, M. R., Mostofa, A. G. M., Kadir, M. F., ... & Asaduzzaman, M. (2014). Nigella sativa L. seeds modulate mood, anxiety and cognition in healthy adolescent males. Journal of Ethnopharmacology, 152(1), 156-162.

Shafi, G., Munshi, A., Hasan, T. N., Alshatwi, A. A., Jyothy, A., & Lei, D. K. (2009). Induction of apoptosis in HeLa cells by chloroform fraction of seed extracts of Nigella sativa. Cancer Cell International, 9, 1-8.

Shahid, M. A., Rahim, A., Chowdhury, M. A., & Kashem, M. A. (2022). Development of antibacterial nanofibrous wound dressing and conceptual reaction mechanism to deactivate the viral protein by Nigella sativa extract. Advances in Traditional Medicine, 22(2), 283-291.

Sultan, M. T., Butt, M. S., Anjum, F. M., & Jamil, A. (2009). Influence of black cumin fixed and essential oil supplementation on markers of myocardial necrosis in normal and diabetic rats. Pakistan Journal of Nutrition, 8(9), 1450-1455.

Sultan, M. T., Butt, M. S., Anjum, F. M., Jamil, A., Akhtar, S., & Nasir, M. (2009). Nutritional profile of indigenous cultivar of black cumin seeds and antioxidant potential of its fixed and essential oil. Pak. J. Bot, 41(3), 1321-1330.

Thilakarathne, R. C. N., & Navaratne, S. B. (2018). Comparison of Physico-chemical and microbial properties of indian and ethiophian Origin black cumin (Nigella sativa) seeds.

Tiji, S., Benayad, O., Berrabah, M., El Mounsi, I., & Mimouni, M. (2021). Phytochemical profile and antioxidant activity of Nigella sativa L growing in Morocco. The Scientific World Journal, 2021, 1-12.

Toma, C. C., Olah, N. K., Vlase, L., Mogoșan, C., & Mocan, A. (2015). Comparative studies on polyphenolic composition, antioxidant and diuretic effects of Nigella sativa L.(black cumin) and Nigella damascena L.(lady-in-a-mist) seeds. Molecules, 20(6), 9560-9574.

Ulus, C. A., Sökülmes, P. K., Taşcı, B. Çörek Otu (Nigella Sativa L.) Tohumunun Kimyasal Bileşimi ve insan Sağlığındaki Yeri.

Umar, S., Zargan, J., Umar, K., Ahmad, S., Katiyar, C. K., & Khan, H. A. (2012). Modulation of the oxidative stress and inflammatory cytokine response by thymoquinone in the collagen induced arthritis in Wistar rats. Chemico-biological interactions, 197(1), 40-46.

Vatansev, H., Ciftci, H., Ozkaya, A., Ozturk, B., Evliyaoglu, N., & Kiyici, A. (2013). Chemical composition of Nigella sativa L. seeds used as a medical aromatic plant from East Anatolia Region, Turkey.

Yimer, E. M., Tuem, K. B., Karim, A., Ur-Rehman, N., & Anwar, F. (2019). Nigella sativa L.(black cumin): a promising natural remedy for wide range of illnesses. Evidence-Based Complementary and Alternative Medicine, 2019.

Zarifikhosrashani, M., Erkoyuncu, M. T., Ergun, Z. (2022). The fatty acid composition of Nigella sativa from Turkey. International Journal of Agriculture Forestry and Life Sciences, 6(2), 39-43.

Microwave Aided Green Synthesis of Silver Nanoparticles Using Green Tea Leaves (*Camellia sinensis*)

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ABSTRACT: Nanometallic materials are metals and alloys that form nanocrystalline particles with a particle size ranging from approximately 5 to 100 nm. Nano-scale metals synthesized by chemical methods can lead to undesirable consequences such as environmental pollution, high energy consumption, and potential health issues. Green synthesis, a novel approach to the preparation of nanoparticles from biological sources, is more advantageous than traditional chemical synthesis routes due to its low cost and greater safety for both the environment and human health. Silver nanoparticles (AgNPs) are utilized in various fields including industrial applications, biology and medicine. In this study, it was aimed to synthesize AgNP with the help of microwave using green tea infusion and silver nitrate ($AgNO_3$). For this purpose, different concentrations of infusion obtained from green tea leaves (1%, 2% and 3%), different concentrations of $AgNO_3$ (1mM, 2mM and 3mM), different pH levels (pH 7.0, 8.0 and 9.0), different powers (300, 450 and 600 Watts) and different durations (0-9 minutes) were optimized. The characterization of the obtained AgNPs was determined by taking the spectrum (800-300 nm) using UV-Vis spectrophotometer. According to the findings; The optimum conditions were determined as 2% infusion concentration, 3mM AgNO3, pH:9.0, power 450 Watt and duration 9 minutes. Determining the sizes of the obtained AgNPs and investigating their biological activities could be considered as further studies.

Keywords: Nanoparticle, Green synthesis, Green tea, $AgNO_3$

INTRODUCTION

Today, nanoparticles have a wide range of applications in different fields, such as industry, biotechnology, physics, chemistry, and electronics. Nanoscale metals have a larger surface area compared to metals. In addition, they differ from the metals that form them and show unique physical and chemical properties. There are many types of nanoscale metals that have wide applications in many fields, such as medicine, biology, engineering, etc (Zhao et al., 2016). Examples of these are gold nanoparticles (AuNPs) have biological applications for enzyme activity regulation, antimicrobial, and muscle relaxant activities (Islam et al., 2015). Silver nanoparticles (AgNP) inhibit the growth and activities of both gram-positive and gram-negative bacteria (Ajitha et al., 2014). Iron nanoparticles (FeNP) inhibit bacterial growth (Naseem and Farrukh, 2015).

Nowadays, there are an increasing number of studies on the synthesis of nanoscale metals using chemical, physical, and green synthesis methods (Horwat et al., 2011). Green synthesis methods are replacing physical and chemical methods due to problems such as the large energy release of toxic and harmful chemicals, the use of complex equipment and synthesis conditions, and consumption (Salem and Fouda, 2021). On the contrary, metal nanoparticle synthesis with the green synthesis method is natural and environmentally friendly. Basically, various microorganisms and whole plants and their parts such as leaves, flowers, bark, roots, fruits and seeds are used in green synthesis (Ying et al., 2022).

The tea plant belongs to the *Camellia* genus (*Camellia sinensis* or *Thea sinensis*) of the Theaceae family (Liu et al., 2020). The tea plant is a short, perennial, shrub-like tree that grows in humid climates and is evergreen (Sun et al., 2021). Polyphenols constitute one third of the 4000 bioactive compounds found in tea (Namita et al., 2012). Thanks to the rich bioactive components it contains, it has a healing effect against coronary heart diseases, arthritis, and various types of cancer, and has antihypertensive, antioxidant, antiviral, and anti-inflammatory properties (Üstün and Demirci, 2013).

In this study, the optimization of microwave-aided silver nanoparticle synthesis with tea plant extract and its characterization with UV-vis spectrophotometry were aimed.

MATERIAL AND METHOD

Preparation of Green Tea Infusion

The ground leaves of the plant were used for extraction. 50 grams of the plant sample was weighed and placed in one-liter Erlenmeyer bottles, and then 1000 mL of deionized water was added. This extract was stirred for 30 minutes on the heater set at 100 ^{o}C and 1000 rpm. Infusion was obtained by passing the extract through filter paper.

Preparation of Microwave Aided Green Synthesis of Silver Nanoparticles and Optimization

$AgNO_3$ was added to the plant extract, and the final volume was completed to 100 ml. After mixing for 5 minutes, the pH was adjusted. After the nanoparticle was formed in the microwave oven, measurements were taken with a UV-vis spectrophotometer in the wavelength range of 300-800 nm.

To determine the plant concentration, 1%, 2% and 3% concentrations were prepared from the resulting infusion. Plant extracts containing 1mM, 2mM and 3mM $AgNO_3$ were prepared.

Three different solutions were prepared as pH:7, pH:8 and pH:9. The solution was optimized by applying 300, 450 and 600 Watts and a duration of 0-9 minutes.

RESULTS AND DISCUSSION

Determination of extract concentration: In the extracts whose spectrum (800-300 nm) was read on the UV-Vis spectrophotometer, the best result was observed in 2% extract (Figure 1).

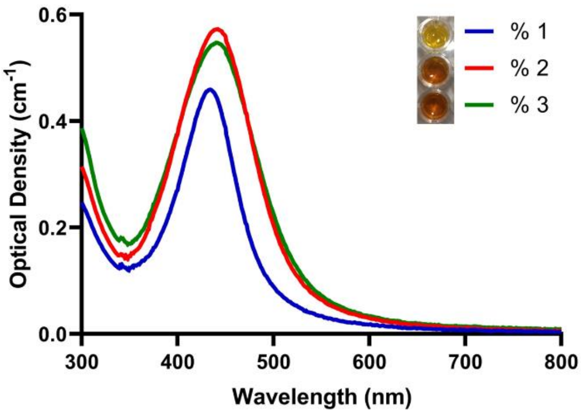

Figure 1. Effects of extract concentration on AgNP

Determination of $AgNO_3$ concentration: In 2% plant extracts containing 1, 2 and 3 mM $AgNO_3$, the result was determined as 3 mM (Figure 2).

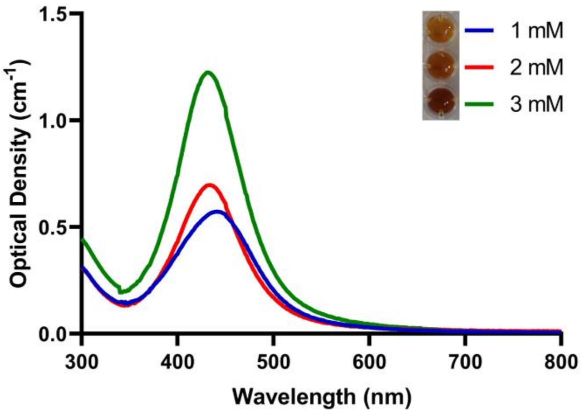

Figure 2. Effects of $AgNO_3$ concentration on AgNP

Determination of pH: The pH was determined as 9 in 2% plant extracts containing 3 mM $AgNO_3$ (Figure 3).

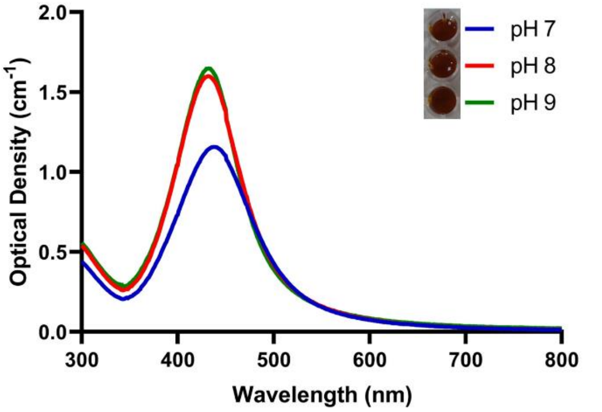

Figure 3. Effects of pH on AgNP

Determination of Force: The power was determined as 450 Watt in 2% plant extracts containing 3 mM $AgNO_3$ at pH 9 (Figure 4).

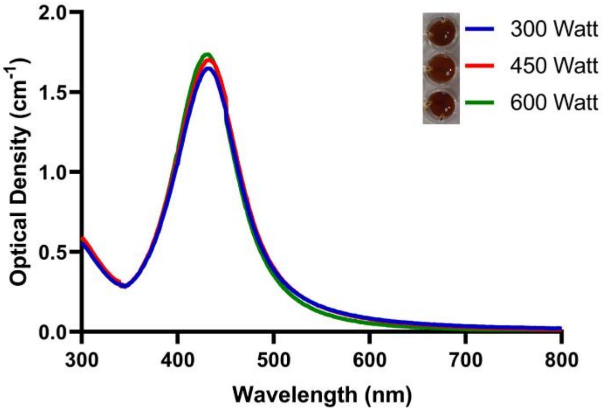

Figure 4. Effects of force on AgNP

Determination of Exposure Time: The best result was observed in the ninth minute (Figure 5).

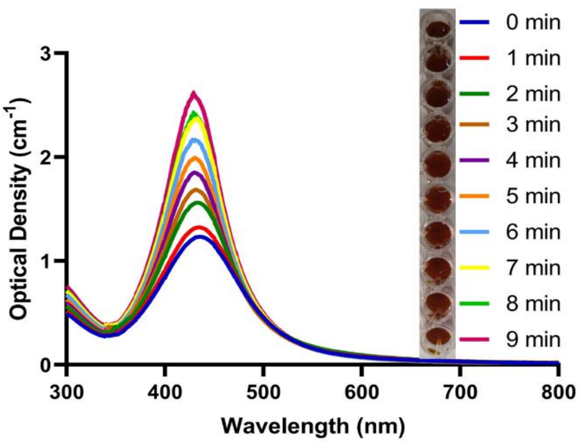

Figure 5. Effects of exposure time on AgNP

According to the findings obtained as a result; optimum conditions were determined as 2% infusion concentration, 3 mM $AgNO_3$, pH: 9.0, power 450 Watt and duration 9 minutes (Figure 6).

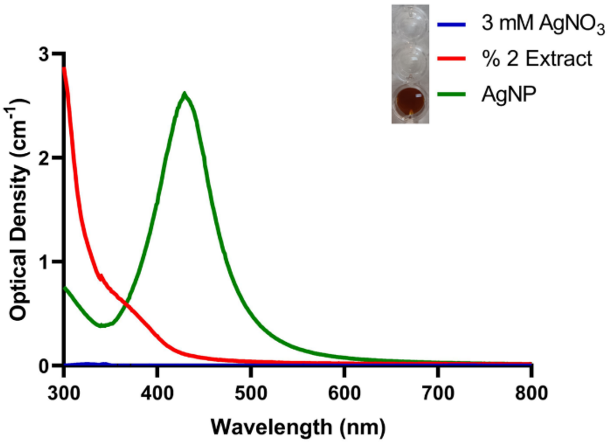

Figure 6. UV-VİS spectrum of AgNP under optimal conditions (green line), extract (red line), $AgNO_3$ (blue line)

CONCLUSION

The results showed that AgNPs were produced using green tea extract and this was confirmed using UV-visible spectroscopy. We observed that the Surface Plasmon Resonance peak corresponding to AgNPs appears at a wavelength of 429 nm. As a result, the optimal conditions for AgNPs prepared with microwave-assisted green tea extract were determined as 2% infusion concentration, 3 mM $AgNO_3$, pH: 9.0, power 450 Watt, and duration 9 minutes. Determining the sizes of the obtained AgNPs and investigating their biological activities can be considered as further studies.

Statement of Conflict of Interest

The authors declare no conflict of interest.

Authors' Contributions

ÖB and FD designed and analyzed the research, ÖB and FD studies arranged. ÖB and FD worked on the preparation of pictures and tables. All authors contributed to the writing of the article and took part in the process of publication of the article and read and approved it.

REFERENCES

Ajitha, B., Reddy, Y.A.K., Reddy, P.S., 2014. Biogenic nano-scale silver particles by *Tephrosia purpurea* leaf extract and their inborn antimicrobial activity. Spectrochim. Acta A Mol. Biomol. Spectrosc., 121: 164-172.

Horwat, D., Zakharov, D. I., Endrino, J. L., Soldera, F., Anders, A., Migot, S., Pierson, J.F., 2011. Chemistry, phase formation, and catalytic activity of thin palladium-containing oxide films synthesized by plasma-assisted physical vapor deposition. Surf. Coat. Technol., 205: 171-177.

Islam, N.U., Jalil, K., Shahid, M., Muhammad, N., Rauf, A., 2019. *Pistacia integerrima* gall extract mediated green synthesis of gold nanoparticles and their biological activities. Arab. J. Chem., 12(8): 2310-2319.

Liu, Y., Zhao, G., Li, X., Shen, Q., Wu, Q., Zhuang, J., Xia, T., 2020. Comparative analysis of phenolic compound metabolism among tea plants in the section Thea of the genus Camellia. Food Res. Int., 135: 109276.

Namita, P., Mukesh, R., Vijay, K.J., 2012. *Camellia sinensis* (green tea): a review. Glob. J. Pharmacol., 6(2): 52-59.

Naseem, T., Farrukh, M.A., 2015. Antibacterial activity of green synthesis of iron nanoparticles using *Lawsonia inermis* and *Gardenia jasminoides* leaves extract. J. Chem., 2015.

Salem, S.S., Fouda, A., 2021. Green synthesis of metallic nanoparticles and their prospective biotechnological applications: an overview. Biol. Trace Elem. Res., 199: 344-370.

Sun, Y., Zhou, J., Guo, J., 2021. Advances in the knowledge of adaptive mechanisms mediating abiotic stress responses in *Camellia sinensis*. Front. Biosci. - Landmark., 26(12), 1714-1722.

Üstün, Ç., Demirci, N., 2013. Çay bitkisinin (*Camellia sinensis* L.) tarihsel gelişimi ve tıbbi açıdan değerlendirilmesi. Mersin Üniversitesi Tıp Fakültesi Lokman Hekim Tıp Tarihi ve Folklorik Tıp Dergisi, 3(3): 5-12.

Ying, S., Guan, Z., Ofoegbu, P. C., Clubb, P., Rico, C., He, F., Hong, J. (2022). Green synthesis of nanoparticles: Current developments and limitations. Environ. Technol. Innov., 26: 102336.

Zhao, X., Liu, W., Cai, Z., Han, B., Qian, T., Zhao, D., 2016. An overview of preparation and applications of stabilized zero-valent iron nanoparticles for soil and groundwater remediation. Water Res., 100: 245-266.

Melatonin: Importance in Horticulture Plants

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ABSTRACT: More recently most of the horticultural plants including fruits, vegetables, grapes etc. are under stress, especially due to the negative environmental conditions that have emerged in recent years and resulted a negative impact on their productivity and quality. Those stress factors including salinity, drought, heavy metal toxicity, extreme high and low temperatures etc. For this reason, plant breeders and farmers are making efforts to reduce the effects of the stress factors mentioned above and, in other words, to achieve more successful crop cultivation. At the forefront of these efforts is the development of varieties resistant to different abiotic and biotic stress conditions, as well as the use of certain stress relieving or preventive substances. One of these kinds of promising substances widely used in horticulture is melatonin. The studies revealed that melatonin has multiple protective mechanisms, including scavenging reactive oxygen species (ROS), enhancing antioxidant defense systems, regulating stomatal closure, modulating gene expression, and promoting osmotic adjustment in most of the economically important horticulture plants. Studies also showed that melatonin in mitigating the adverse effects of various abiotic stressors and enhanced plant growth and physiological functions in horticultural plants.

Keywords: Melatonin, Stress, Horticulture plants

INTRODUCTION

Plant kingdom is an important part of human life and they have many functions in ecosystem. However, they can be sometimes experience undesirable difficulties in their environment and can survive under certain conditions. The world face to global warming and climate change in recent two decades in particular. Its impact on the whole world in recent years are visible. The effect of this phenomena is quite clear for mankind that causing glaciers to melt, cause many disasters such as flood, erosion, drought etc. Finally, as a result of such negativities, abiotic and biotic stressors including microorganism damage, salinity, pollution can be more effective on living things on Earth. It disrupts the balance of the ecosystem by negatively affecting assets. When evaluated from an agricultural point of view, plants undergo germination, development and maturation phases, and this type of stress seriously affects physiological activities and finally resulting in reduced product quality and productivity.

The stress factors in plants can be describe as abiotic and biotic. Abiotic stress factors are also further described as physical (drought, salinity, extreme temperatures, radiation, flood, mechanical effects including wind and snow) and chemical (air pollution, pesticides, plant nutrient substances, toxins, pH) stressors. The major biotic stress factors are disease agents, wild plants (invasive plants), insects, microorganisms and animals.

Plants require a number of internal and external events in order to grow and develop. They are under the influence of many abiotic and biotic stress factors. Thus, suitable environment for a plant to grow conditions are needed. Stress-related damages on plants depend on the type of plant, tolerance and adaptation ability. Considering the stress phenomenon in plants and the species and varieties that can eliminate stress in the environment development becomes very important.

What is melatonin?

Recent studies showed that some important phytohormones such as abscisic acid (ABA), jasmonic acid (JA), gibberellic acid (JA), salicylic acid (SA), brassinosteroids (BRs), strigolactone (SL) etc. play key roles in plant growth and development under normal and abiotic stress conditions in different plant groups (Alhaithloul et al., 2020; Khan et al., 2020; Rhaman et al., 2020).

In addition to those substances, studies also showed that melatonin (MT) (Figure 1) and polyamines (PA) are also play an important role in abiotic stress tolerance (Wang et al., 2018; Zhao et al., 2022). Previous studies indicated that Melatonin (MT), derived from the essential amino acid tryptophan, and was found only in animals and contribute reproductive physiology, redox homeostasis, temperature homoeostasis and antioxidative defense enhancement (Kul et al., 2019; Khan et al., 2020). However, the recent studies confirmed the presence of melatonin in higher plants (Kanwar et al., 2018; Arnao and Hernández-Ruiz, 2020). After this finding, several field crops and horticultural plants including wheat, rice, tobacco, barley, corn, carrot, oat, cherries, cucumber and apple revealed that including melatonin (Shibaeva et al., 2018; Tripathi et al., 2021; Zhao et al., 2021). A number of studies have shown that melatonin is involved in regulating seed germination, flower development, photosynthesis and generates signaling responses to environmental stimuli in various plants (Arnao and Hernández-Ruiz, 2018; Shibaeva et al., 2018; Altaf et al., 2021).

Figure 1. The formula of melatonin

Role of melatonin in plants

In recent years, melatonin has emerged as a popular research topic in the field of plant biology. Since melatonin is implicated in numerous plant developmental processes and stress responses, the exploration of its roles in regulating various physiological and biochemical processes contributing to plant growth and development under normal or stress conditions has become a rapidly progressing field (Figure 2).

It is a very important biomolecule in plant growth and development. Melatonin promotes root and hypocotyl growth and promotes seedling growth with its auxin-like functions and increases its biomass (Byeon and Back, 2016).

Melatonin is also an important plant growth and development promoter. It is a hormone and is found in the root system of many plants. Like cucumber and red cabbage, application of melatonin in plants, seeds, seedlings and root systems promotes growth. It has been determined that it increases and improves germination (Wei et al., 2019). Additionally, melatonin where the concentration is high and seed under adverse conditions, it was found that the seed germination rate increased (Huang et al., 2019).

Moreover, melatonin increases the expression of genes related to ABA and gibberellic acid (GA) biosynthesis. It has been reported that it plays an important role in the GA and ABA biosynthesis pathway by regulating the biosynthesis (Zhang et al., 2014; Li et al., 2019). Melatonin is present in plants under various abiotic stresses, including salinity stress. It is involved in the modulation of defense mechanisms (Imran et al., 2021; Zhang et al., 2021; Li et al., 2022). Transcriptome analysis results show that melatonin is primarily affects plant hormone pathways of signal transduction and biosynthesis of secondary metabolites. The biosynthesis of melatonin in plants depends on serotonin. It starts with the converted tryptophan to serotonin and between tryptophan and melatonin, hydroxyindole-Omethyltransferase and caffeic acid O-methyltransferase (ASMT/COMT) enzymes and it catalyzes a reaction that produces an intermediate molecule called 5-methoxytritamine (Zhao et al., 2019).

Melatonin, as a powerful antioxidant, protects plants against ROS, reactive nitrogen species (RAT) and it has the ability to detoxify various chemical pollutants. Melatonin inducing enzymatic activity and scavenging antioxidant oxidative stress caused by excess cadmium in tomatoes (Hasan et al., 2015).

In research on the functions of melatonin in plants, it has been shown that melatonin is plays a very important role in plant growth and development under abiotic stress conditions. Melatonin increases higher plant tolerance under salinity stress, ionic homeostasis (especially whole plant Na+ ion channel genes to maintain the /K+ ratio), improves photosynthetic capacity, preserves chlorophylls and carotenoids and it is known to be associated with its properties of reducing photorespiration (Li et al., 2019).

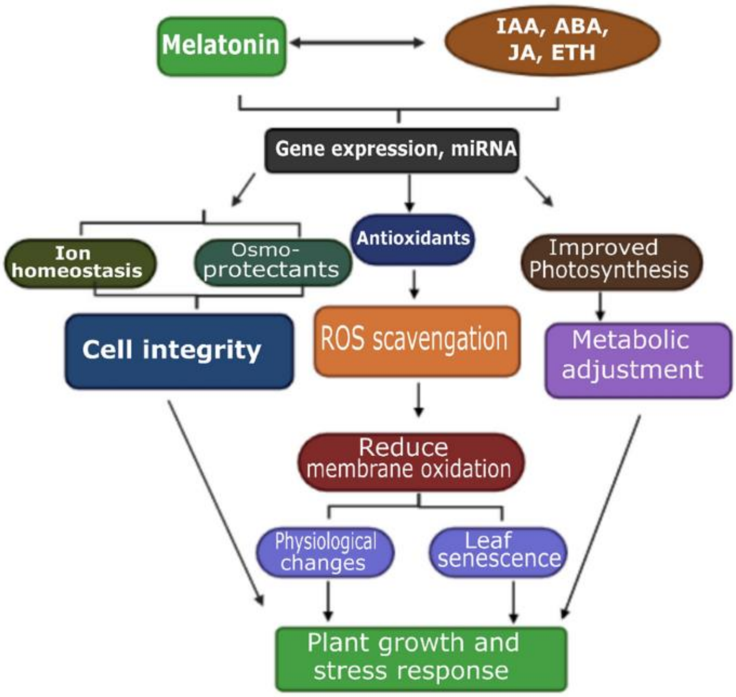

Figure 2. The role of melatonin in plants

REFERENCES

Alhaithloul, H.A.S., Abu-Elsaoud, A.M., Soliman, M.H. (2020). Abiotic stress tolerance in crop plants: role of phytohormones. In: Fahad S, Saud S, Chen Y, Wu C, Wang D (eds) Abi- otic Stress in Plants. Intecopen, London, p 233.

Altaf, M.A., Shahid, R., Ren, M.X., Mora-Poblete, F., Arnao, M.B., Naz, S., Anwar, M., Altaf, M.M., Shahid, S., Shakoor, A. (2021). Phytomelatonin: an overview of the importance and mediating functions of melatonin against environmental stresses. Physiol. Plant. 172(2):820-846.

Arnao, M.B., Hernández-Ruiz, J. (2020). Is phytomelatonin a new plant hormone? Agronomy 10(1):95.

Byeon, Y., Back, K. (2016). Low melatonin production by sup- pression of either serotonin N-acetyltransferase or N-acetylserotonin methyltransferase in rice causes seedling growth retardation with yield penalty, abiotic stress susceptibility, and enhanced coleoptile growth under anoxic conditions. J. Pineal. Res. 60 (3):348-359.

Hasan, M., Ahammed, G.J., Yin, L., Shi, K., Xia, X., Zhou, Y., Zhou, J. (2015). Melatonin mitigates cadmium phytotoxicity through modulation of phytochelatins biosynthesis, vacuolar sequestration, and antioxidant potential in *Solanum lycopersicum* L. Front. Plant Sci. 6, 601.

Huang, B., Chen, Y.E., Zhao, Y.Q., Ding, C.B., Liao, J.Q., Hu, C., Yuan, M. (2019). Exogenous melatonin alleviates oxidative damages and protects photosystem II in maize seedlings under drought stress. Front. Plant Sci. 10:677.

Imran, M., Latif Khan, A., Shahzad, R., Aaqil Khan, M., Bilal, S., Khan, A., Lee, I.J. (2021). Exogenous melatonin induces drought stress tolerance by promoting plant growth and antioxidant defense system of soybean plants. AoB Plants, 13(4):plab026.

Kanwar, M.K., Yu, J., Zhou, J. (2018). Phytomelatonin: recent advances and future prospects. J. Pineal. Res. 65(4):e12526.

Khan, T.A., Fariduddin, Q., Nazir, F., Saleem, M. (2020). Melatonin in business with abiotic stresses in plants. Physiol. Mol. Biol. Plants 26 (10):1931-1944.

Kul, R., Esringü, A., Dadasoglu, E., Sahin, Ü., Turan, M., Örs, S., Ekinci, M., Agar, G., Yildirim, E. (2019). Melatonin: role in increasing plant tolerance in abiotic stress conditions. Abiot. Biot. Stress Plants 1:19.

Li, J., Liu, J., Zhu, T., Zhao, C., Li, L., Chen, M. (2019). The role of melatonin in salt stress responses. Int. J. Mol. Sci. 20 (7):1735.

Li, J., Liu, Y., Zhang, M., Xu, H., Ning, K., Wang, B., Chen, M. (2022). Melatonin increases growth and salt tolerance of *Limonium bicolor* by improving photosynthetic and antioxidant capacity. BMC Plant Biol. 22 (1):1-14.

Rhaman, M.S., Imran, S., Rauf, F., Khatun, M., Baskin, C.C., Murata, Y., Hasanuzzaman, M. (2020). Seed priming with phytohormones: an effective approach for the mitigation of abiotic stress. Plants 10(1):37.

Shibaeva, T., Markovskaya, E., Mamaev, A. (2018). Phytomelatonin: a review. Biol. Bull. Rev. 8(5):375-388.

Tripathi, G.D., Javed, Z., Mishra, M., Fasake, V., Dashora, K. (2021). Phytomelatonin in stress management in agriculture. Heliyon 7(3):e06150.

Wang, Y., Reiter, R.J., Chan, Z. (2018). Phytomelatonin: a universal abiotic stress regulator. J. Exp. Bot. 69 (5):963-974.

Wei, Z., Li, C., Gao, T., Zhang, Z., Liang, B., Lv, Z., Ma, F. (2019). Melatonin increases the performance of *Malus hupehensis* after UV-B exposure. Plant Physiol. Biochem. 139:630-641.

Zhang, N., Zhang, H.J., Zhao, B., Sun, Q.Q., Cao, Y.Y., Li, R., Guo, Y.D. (2014). The RNA-seq approach to discriminate gene expression profiles in response to melatonin on cucumber lateral root formation. J. Pineal Res. 56 (1):39-50.

Zhang, Y., Fan, Y., Rui, C., Zhang, H., Xu, N., Dai, M., Ye, W. (2021). Melatonin improves cotton salt tolerance by regulating ROS scavenging system and Ca^{+2} signal transduction. Front. Plant Sci. 12:1239.

Zhao, D., Yu, Y., Shen, Y., Liu, Q., Zhao, Z., Sharma, R., Reiter, R.J. (2019). Melatonin synthesis and function: evolutionary history in animals and plants. Front. Endocrinol. 10: 249.

Zhao, Y-Q., Zhang, Z-W., Chen, Y-E., Ding, C-B., Yuan, S., Reiter, R.J., Yuan, M. (2021). Melatonin: a potential agent in delaying leaf senescence. Crit. Rev. Plant Sci. 40(1):1-22.

Zhao, C., Nawaz, G., Cao, Q., Xu, T. (2022). Melatonin is a potential target for improving horticultural crop resistance to abiotic stress. Sci. Hortic. 291:110560.

Effect of Different Solubilization Treatments on the Chemical Structures of Exopolysaccharides Produced by Lactic Acid Bacteria Isolated from Sourdough and Tarhana

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ABSTRACT: In this study, exopolysaccharide (EPS)-positive strains were isolated from sourdough and tarhana, and the effects of different treatments (shaking, ultrasonication, and ionic strength) on the solubility and chemical structure of purified EPSs from these strains were examined. The findings indicated that the physical and chemical treatments affected the EPS's water solubility and, as a result, the total carbohydrate concentration. Sonication and ionic strength treatments increased the solubility of EPSs almost fourfold compared to shaking, and this increase was found to be statistically significant (p<0.001). This situation varied with the EPSs produced by different strains. The impact of these treatments on the chemical structure of EPSs was determined by FTIR spectroscopy, comparing the functional groups of EPSs. Except for a few changes in peak intensities, all EPSs exhibited similar FTIR spectra, proving that the applied treatments did not have an effect on the chemical structure.

Keywords: Exopolysaccharide, Lactic acid bacteria, Solubilization, Ultrasonication, Ionic strength, Chemical structure

INTRODUCTION

Food fermentation, a part of human life since ancient times, is an effective and simple biotechnology process for protecting the safety and nutritional value of foods and guaranteeing the long shelf life of perishable products. These unique features of fermentation are greatly dependent on the microbial transformations that occur in fermented foods. Lactic acid bacteria (LAB) are the most important bacterial group that ensures microbial transformations in fermenting food as part of traditional fermentations (de Souza et al., 2023; Yildiz, 2011)

LAB are Gram-positive, catalase-negative, non-spore-forming (except *Lactobacillus inulinus*), facultative anaerobic organisms, and generally described as considered safe (GRAS) (Yildiz, 2011). LAB produces a number of metabolites, such as exopolysaccharides (EPS), bacteriocines, organic acids, reuterin, and short-chain fatty acids, that have the potential to be used as natural additives and have positive contributions to human health. Among these metabolites, EPSs are the most remarkable and commercially important microbial products. Some LABs secrete extracellular polysaccharides called EPS. EPSs secreted by LAB under suitable growth conditions have the function of controlling the physicochemical properties of the cell surface and protecting bacterial cells from environmental stress. EPSs are also responsible for important bioactivities such as antimicrobial, antioxidant, immunomodulatory, prebiotic, and cholesterol-lowering effects. Moreover, they can act as emulsifiers, gelling agents, stabilizers, texture improvers, and water-binding agents in the product to which they are added (Rahnama Vosough et al., 2022; Riaz Rajoka et al., 2020; Yildiz & Karataş, 2018).

Sourdough, a mixture of flour and water, is a fermented product consisting of lactic acid bacteria (LAB) and yeasts. The metabolites that are produced during fermentation by the sourdough microflora enhance the functional properties of the dough and final product (Boyaci Gunduz et al., 2022; Dertli et al., 2016). Tarhana is a traditional Turkish fermented food that has a recipe of mixing cereal flours, yogurt, chickpeas, vegetables (red pepper, tomatoes, and onions), and spices (salt, mint, and paprika), and this recipe might vary from region to region. The interaction between LAB and yeast is responsible for the characteristic acidic and sour taste of tarhana (Cankurtaran Kömürcü & Bilgiçli, 2022). Both sourdough and tarhana are especially valuable sources of LAB strains that have the possibility of producing a high amount of EPS.

Due to EPS's limited solubility in water or other polar solvents, only a small number of EPS, despite having been completely identified or characterized, have been commercialized. The bioactivities of EPS are affected by both the biochemical composition and the solubility (Jaroszuk-´Sciseł et al., 2020). Therefore, the solubilization of EPSs is a crucial step before carrying out bioactivity experiments. The aim of this study is to determine the effects of different processes (shaking, ultrasonication, and ionic strength) on the solubility and chemical structures (Fourier-transform infrared spectroscopy) of LAB-EPS isolated from sourdough and tarhana samples.

MATERIAL AND METHOD

Material

Samples of traditional Turkish sourdough (7 samples) were received from local bakeries located in Vakfıkebir-Trabzon, Türkiye, and samples of traditional Turkish homemade tarhana (5 samples) were received from local markets located in Gülsehir-Nevsehir, Türkiye.

Isolation of LAB

Serial dilutions of samples were prepared using physiologic-peptone water, and MRS agar (Merck, Darmstadt, Germany), M17 agar (Merck, Darmstadt, Germany), and GM17 agar plates were used for the isolation of LAB. The plates were incubated under the following conditions: MRS agar plates at 30 °C for 72 h under anaerobic conditions in a sealed jar using Anaerocoult A packs (Merck, Germany); M17 and GM17 agar plates at 37 °C for 72 h under aerobic conditions. Cycloheximide was added to all media at a final concentration of 0.1 mg/mL. Isolates that had different colony morphologies were chosen randomly from each plate, and a total of 372 isolates were obtained. Each isolate was subsequently streaked on the proper medium for purification. The isolates that were Catalase-negative and Gram-positive were chosen as lactic acid bacteria, and purified LAB were stored at -80 °C in a 20% glycerol medium (Yildiz, 2011).

Selection of EPS-producing LAB strain

EPS-producing LAB strain was selected with i) the detection of "ropy" phenotype and "mucoide non-ropy" phenotype, and ii) using ruthenium-red milk agar plates, which stains the cell wall of non-ropy colonies to red; however the cell wall of the ropy colonies stay white. The EPS-producing LAB strains were chosen and cultivated repeatedly (at least 5 times) to select the strain that maintained a stable EPS-producing phenotype (Reale et al., 2020; Ruas-Madiedo & De Los Reyes-Gavilán, 2005).

Production, isolation and purification of EPS

Fresh cultures were inoculated into the proper broth medium and incubated for 48 h at proper incubation conditions. The cells were harvested by centrifugation (8000xg, 20 min, 4 °C, Hanil Science Industrial Combi 514R, Korea) and resuspended in sterile physiologic water to a density that matches 0.5 MacFarland. The suspension was used to inoculate 200 mL of proper modified broth medium (without yeast extract) to a final density of 10^6 CFU/mL and incubated at proper conditions for 48 hours. After incubation, cells were separated by centrifugation at 8000xg for 20 min at 4 °C. The trichloroacetic acid solution (Sigma-Aldrich, Germany) was added to the supernatant to a final concentration of 10% (w/v) and incubated at 4 °C for 12 h. The solutions were centrifugated at 10,000xg for 25 min at 4 °C to get rid of the precipitated proteins. The supernatant was mixed with 1:2 volumes of cold ethanol (95%, Merch, Germany) and stored at 4 °C for 12 h. EPS were collected by centrifuged at 10,000xg for 30 min at 4 °C and lyophilized (FDU-8612, Operon Co., Ltd., Gimpo, Korea) for further study (Wang et al., 2023; Zhou et al., 2021).

Solubilization of EPS

The lyophilized EPS was solubilized using three different methods. *Method I-Shaking*: The lyophilized EPS was resuspended with the addition of dH_2O to a final concentration of 4.25 mg/mL and incubated in an orbital shaker incubator (JSR, JSSI-300C, Korea) at 50 °C, 200 rpm, 12 h. *Method II-Ultrasonication*: The lyophilized EPS was resuspended with the addition of dH_2O to a final concentration of 4.25 mg/mL and sonicated at 40 W for 150 sec using an ultrasonicator (Isolab, Germany). *Method III-Ionic strength*: The lyophilized EPS was resuspended with the addition of 0.85% NaCl (14.5 M) to a final concentration of 4.25 mg/mL and vortexed (Heidolph, MR Hei-Standard, Germany). All suspensions were filtered using a sterile syringe filter with a 0.45 µm pore size.

Determination of total carbohydrate content of crude EPS

The phenol–sulfuric acid method suggested by Dubois was used to determine the total sugar content of EPS. Briefly, 200 µL of 5% phenol were added to 200 µL of the sample, then 1000 µL of 95% H_2SO_4 was added immediately and incubated for 60 minutes at room temperature. The absorbance of the sample was read at 490 nm (Shimadzu UV-1208, Japan). The total carbohydrate content of EPS was calculated from a standard curve that was created using glucose as a standard (Dubois et al., 1956).

Fourier-transform infrared spectroscopy

To identify the functional groups in the structure of the EPS, an ATR-Frouier-transform infrared spectrometer (Perkin Elmer Spektrum 100, Waltham, Massachusetts, USA) was used. The spectrum of EPS was recorded in the range of 4,500–500 cm^{-1} with a total of 32 scans (Zhou et al., 2021).

Statistical analysis

The findings were presented as mean values with standard errors from the three replications. The effects of various factors were examined using a one-way analysis of variance (ANOVA). Mean values were compared using the Duncan multiple comparison test, and variations between mean values were deemed significant when $p < 0.05$.

RESULTS AND DISCUSSION

EPSs from LAB have drawn attention due to their positive effects on human health and their wide range of applications in different industries. However, the limited solubility of EPS, which directly affects bioactivity, restricted the potential application areas. The present study aimed to increase the solubility of EPSs by applying both physical (shaking, ultrasonication, and chemical (ionic strength) methods.

Isolation of LAB and selection of EPS-producing LAB

In the scope of this study, a total of 372 LAB strains, which were catalase negative and Gram positive, were isolated from sourdough and tarhana samples (156 and 216 strains, respectively). The distribution of isolated strains according to the media and samples used is presented in Table 1.

Table 1. The distribution of isolated strains according to the media and samples

Samples	MRS (15 °C)	MRS (30 °C)	MRS (40 °C)	M17	GM17	Total
Sourdough	16	45	29	37	29	156
Tarhana	41	43	53	44	35	216
Total isolate	57	88	82	81	64	372

Mucoid and ropy characteristics and Ruthenium red positive colony properties were factors that were considered for the selection of EPS-producing strains. Four EPS-producing strains among 372 isolates were chosen for this study, and the characteristics of these strains are presented in Table 2.

Table 2. The properties of the strains

Strain No	Source	Colony Morphology	Microscopic Morphology	Ruthenium Red
39.2 (GM17)	Sourdough	Cream color, shiny, mucoid	Rod	Positive-strong
40.1 (MRS 15 ℃)	Sourdough	White color, shiny, mucoid	Rod	Positive-strong
41.3 (MRS 15 ℃)	Tarhana	Mucoid	Rod	Positive-strong
41.4 (MRS 15 ℃)	Tarhana	Yellow color, mucoid	Rod	Positive-strong

Solubilization of EPS

The selected isolates were incubated in 200 mL of the relevant modified broth media for 48 hours, and then EPSs were extracted by cold-ethanol precipitation and lyophilized. To prevent contamination of EPS from yeast extract in the broth medium, modified broth media that did not contain yeast extract were used. The lyophilized EPS was solubilized using three different methods: method I-shaking, method II-ultrasonication, and method III-ionic strength. After solubilization, all samples were filtered using a sterile syringe filter with a 0.45 µm pore size. EPS solubility was checked by determining the total carbohydrates in the samples using the phenol-sulfuric acid method, and the results are presented in Fig. 1.

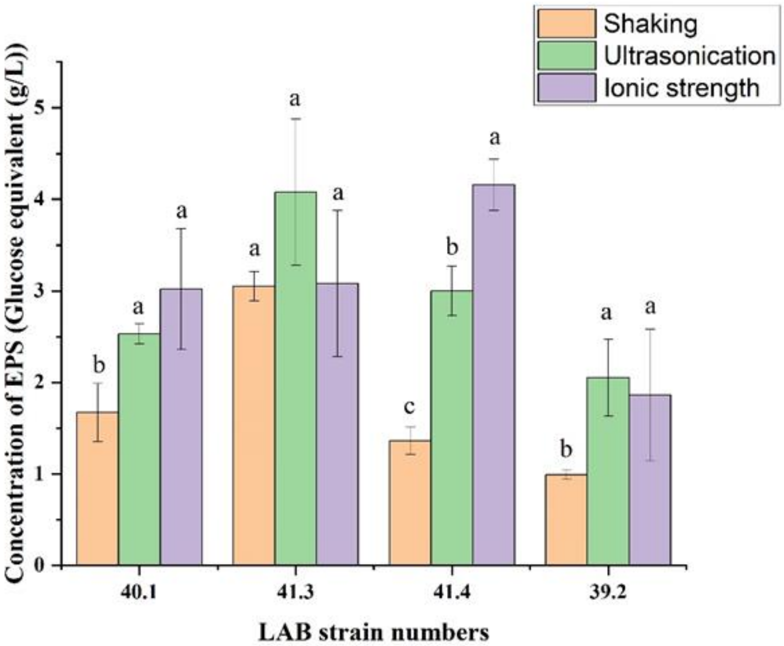

Figure 1. The effect of different treatments on EPS solubility and total carbohydrate concentration. Different lowercase letters indicate that different solubilization treatments had a significant difference in the total concentration of carbohydrates in EPSs (p <.001).

The results demonstrated that the applied physical and chemical processes affected the water solubility of EPS and, consequently, the total carbohydrate concentration. The results showed that shaking was not sufficient to completely solubilize EPS, while ultrasonication and ionic strength were found to be more effective in solubilizing EPS. In fact, it has been determined that these two methods increase the EPS concentration almost fourfold compared to the concentration obtained in the shaking method.

The effect of different teratment of chemical structure of EPS

To determine the effect of different treatments on the chemical structure of EPS, the functional groups were analyzed using FTIR. All EPS represented similar FTIR spectra, except for some variations in the peak intensities (Figs. 2 and Fig.3). As shown in Fig. 2., a broad absorption peak was observed at the region of 3300 cm^{-1} which represented -OH stretching vibrations (Fig. 2). The bands at around 2910 cm^{-1} indicated the aliphatic C-H stretching vibrations which is a characteristic peak of polysaccharides. Another characteristic peak for polysaccharides was observed in the region of around 1650 cm^{-1} which corresponds to the C=O and carboxyl groups. The existence of a strong band at around 1020 cm^{-1} referred to the presence of C-O-C groups, which indicated that the sugars exist mainly in the pyranose form. The peaks in the region of 880-820 cm^{-1} showed the presence of glycosidic connections (Fig. 2).

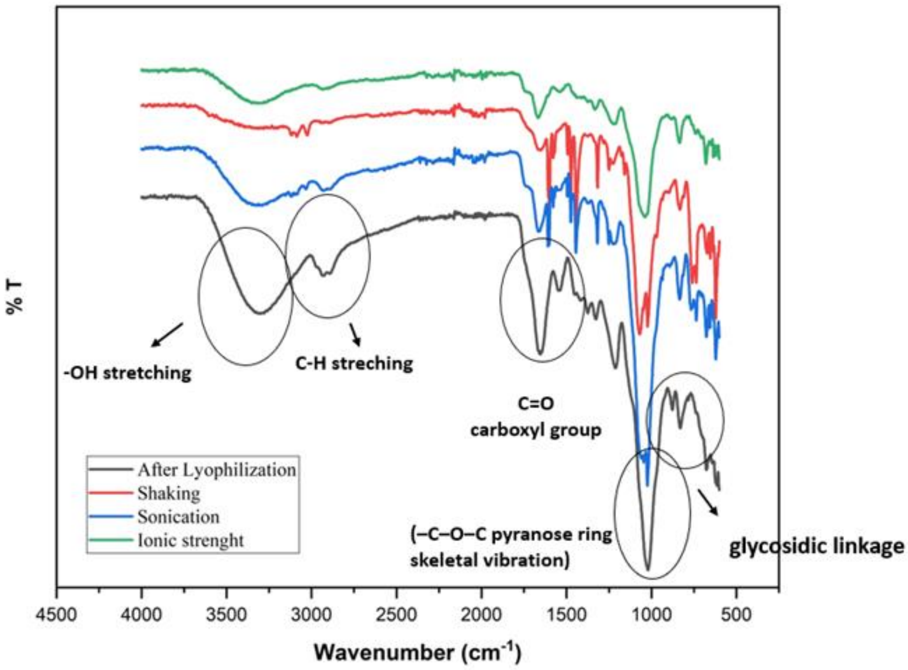

Figure 2. FTIR spectra of the purified EPSs produced by the LAB strain 40.1

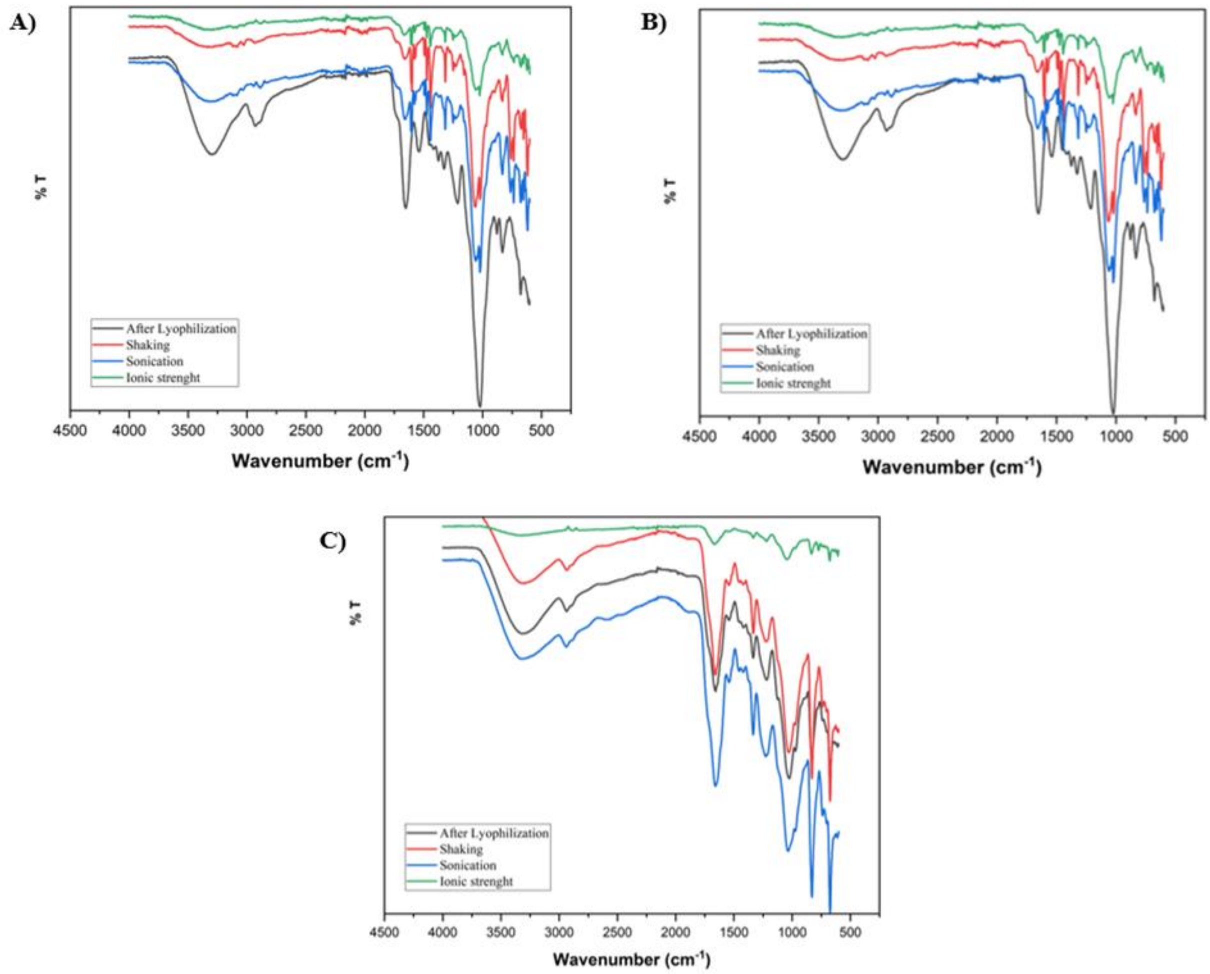

Figure 3. FTIR spectra of the purified EPSs produced by (A) the LAB strain 41.3; (B) the LAB strain 41.4; (C) the LAB strain 39.2.

CONCLUSION

The efficiency was very low when EPSs were dissolved only by shaking. Sonication and ionic strength applications increased the solubility of EPS almost fourfold. Since the applied processes had no effect on the chemical structure, it was decided to apply the ultrasonication process for the solubilization of EPS. On the other hand, the decision regarding the use of ionic strength will be made after bioactivity experiments.

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Statement of Conflict of Interest

The authors declare that they are no conflict of interest.

Authors' Contributions

Zeynep Gizem Taşkıran, Hilal Yıldız and Bahar Tuba Findik designed and analyzed the research, Hilal Yildiz and Bahar Tuba Findik worked on the preparation of pictures and tables. All authors contributed to the writing of the article and took part in the process of publication of the article and read and approved it.

REFERENCES

Boyaci Gunduz, C. P., Agirman, B., Gaglio, R., Franciosi, E., Francesca, N., Settanni, L., & Erten, H. (2022). Evaluation of the variations in chemical and microbiological properties of the sourdoughs produced with selected lactic acid bacteria strains during fermentation. *Food Chemistry: X*, *14*, 100357. https://doi.org/10.1016/J.FOCHX.2022.100357

Cankurtaran Kömürcü, T., & Bilgiçli, N. (2022). Effect of ancient wheat flours and fermentation types on tarhana properties. *Food Bioscience*, *50*, 101982. https://doi.org/10.1016/J.FBIO.2022.101982

de Souza, E. L., de Oliveira, K. Á., & de Oliveira, M. E. (2023). Influence of lactic acid bacteria metabolites on physical and chemical food properties. *Current Opinion in Food Science*, *49*, 100981. https://doi.org/10.1016/J.COFS.2022.100981

Dertli, E., Mercan, E., Arici, M., Yilmaz, M. T., & Sağdiç, O. (2016). Characterisation of lactic acid bacteria from Turkish sourdough and determination of their exopolysaccharide (EPS) production characteristics. *LWT - Food Science and Technology*, *71*, 116–124. https://doi.org/10.1016/J.LWT.2016.03.030

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *Anal. Chem.*, *28*(3), 350–356. https://pubs.acs.org/sharingguidelines

Jaroszuk-´ Sciseł, J., Nowak, A., Komaniecka, I., Choma, A., Jarosz-Wilkołazka, A., Osí Nska-Jaroszuk, M., Ty´skiewiczty´skiewicz, R., Wiater, A., Rogalski, J., & Leon, F. (2020). Differences in Production, Composition, and Antioxidant Activities of Exopolymeric Substances (EPS) Obtained from Cultures of Endophytic Fusarium culmorum Strains with Different Effects on Cereals. *Molecules*, *25 (3)*(616), 1–33. https://doi.org/10.3390/molecules25030616

Rahnama Vosough, P., Edalatian Dovom, M. R., Habibi Najafi, M. B., Javadmanesh, A., & Mayo, B. (2022). Biodiversity of exopolysaccharide-producing lactic acid bacteria from Iranian traditional Kishk and optimization of EPS yield by *Enterococcus* spp. *Food Bioscience*, *49*, 101869. https://doi.org/10.1016/J.FBIO.2022.101869

Reale, A., Zotta, T., Ianniello, R. G., Mamone, G., & Di Renzo, T. (2020). Selection criteria of lactic acid bacteria to be used as starter for sweet and salty leavened baked products. *LWT*, *133*, 110092. https://doi.org/10.1016/J.LWT.2020.110092

Riaz Rajoka, M. S., Wu, Y., Mehwish, H. M., Bansal, M., & Zhao, L. (2020). *Lactobacillus* exopolysaccharides: New perspectives on engineering strategies, physiochemical functions, and immunomodulatory effects on host health. *Trends in Food Science & Technology*, *103*, 36–48. https://doi.org/10.1016/J.TIFS.2020.06.003

Ruas-Madiedo, P., & De Los Reyes-Gavilán, C. G. (2005). Invited Review: Methods for the Screening, Isolation, and Characterization of Exopolysaccharides Produced by Lactic Acid Bacteria. *Journal of Dairy Science*, *88*(3), 843–856. https://doi.org/10.3168/JDS.S0022-0302(05)72750-8

Wang, L., Tian, H., Liu, W., Zheng, H., Wu, H., Guan, Y., Lu, Q., & Lv, Z. (2023). Effects of EPS-producing *Leuconostoc mesenteroides* XR1 on texture, rheological properties, microstructure and volatile flavor of fermented milk. *Food Bioscience*, *56*, 103371. https://doi.org/10.1016/J.FBIO.2023.103371

Yildiz, H. (2011). *Isolation-identification of lactic acid bacteria and yeasts from pickle and olive and determination of some properties of isolates* [PhD Thesis]. Institute of Science and Technology, Ataturk University.

Yildiz, H., & Karataş, N. (2018). Microbial exopolysaccharides: Resources and bioactive properties. *Process Biochemistry*, *72*, 41–46. https://doi.org/https://doi.org/10.1016/j.procbio.2018.06.009

Zhou, Y., Cui, Y., Suo, C., Wang, Q., & Qu, X. (2021). Structure, physicochemical characterization, and antioxidant activity of the highly arabinose-branched exopolysaccharide EPS-M2 from *Streptococcus thermophilus* CS6. *International Journal of Biological Macromolecules*, *192*, 716–727. https://doi.org/10.1016/J.IJBIOMAC.2021.10.047.

Bibliometric Analyses of Gene Editing in Livestock

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ABSTRACT: The article conducts bibliometrics (scientometrics, informetrics) analyses on gene editing studies in farm animals, for this purpose, publications on the subject published in WOS (https://www.webofscience.com) and Scopus (https://www.scopus.com/search) databases were searched using the keywords gene editing on farm animals (Livestock). Utilizing VOSviewer software, the study generates a report outlining publication distributions. As a result of our bibliometric analyses, most of the gene editing studies in farm animals are at the level of original articles and 378 publications were found in Scopus database and 359 publications were found in WOS database. It was observed that the publications were mostly made by scientists and teams in Republic of China and USA. It has been revealed that gene editing studies have been carried out in order to reproduce breeds that are resistant to diseases, high productivity, adaptable to different breeding conditions, especially in pig, cattle and sheep goat breeds, and to produce new organs by transplantation and to produce tissues and organs compatible with human health. This bibliometric study concluded that gene editing studies should be intensified in order to reproduce animals with high economic efficiency values in farm animals, to increase animal welfare and to increase the number of farm animals.

Keywords: Genome editing, Gene editing, Livestock, Crispr, Cas9

INTRODUCTION

Gene editing, also known as genome editing or genetic engineering, is the method of gene editing that stems from the identification of repair mechanisms of double-stranded DNA chains, allowing scientists to delete, insert, replace or modify DNA in the genome of a living organism. In recent years, the emergence of highly versatile genome editing technologies has provided researchers with the ability to rapidly and economically make sequence-specific changes to the genome of a wide range of cell types, organs and tissues (Camargo and Pereira, 2022; Park, 2023; Raza et al., 2023). Transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), homing endonucleases or meganucleases, and clustered regularly interspaced short palindromic repeats CRISPR-associated protein 9 (Cas9) are commonly used methods in the field of gene editing (Li et al., 2020; Gaj et al., 2016; Wang et al., 2022). Many genome editing studies have been conducted on farm animal pigs improve resistance to viruses, strengthen immunity against diarrhea disease (Watanabe et al., 2010), protection against influenza virüs (Whitworth et al., 2016), reduce pregnancy mortality, xenotransplantation applications (Hauschild et al., 2011; Yan et al., 2018), modification of the MSTN gene and uncoupling protein 1 (UCP1) gene to increase meat yield by producing pigs carrying the gene (Qian et al., 2015; Yugo et al., 2018; Whitworth et al., 2017; Tu et al., 2019; Lee et al., 2020). In cattle, studies have demonstrated that cattle resistant to Mycobacterium bovis infection could be produced via genome editing (Alberio and Wolf, 2021; Gao et al., 2017). With the majority of cattle breeds using the CRISPR technique, the main goals have been to increase resistance against the causative agent of pasteurellosis in calves, P. haemolytica virus (Shanthalingam et al., 2016), prevent mastitis (Liu et al., 2014; Mallikarjunappa et al., 2020), and provide resistance against tuberculosis and pneumonia (Gao et al., 2017, Ikeda et al., 217). Cattle breeding has involved gene editing for various purposes such as hornless cattle rearing (Carlson et al., 2016; Schuster et al., 2020), enhancing milk quality and meat quantity (Wang X. et al., 2015; Zhou et al., 2017; Koloskova et al., 2021), regulating body temperature by editing the slick hair coat (SLICK) locus in the prolactin hormone receptor (PRLR) gene (Huson et al., 2014), preventing the formation of black spots on the skin by editing the pre-melanosome protein 17 gene deletion in cattle breeds to develop heat-resistant breeds (Laible et al., 2020). Furthermore, gene editing has been carried out to silence the MSTN gene in cattle, leading to hyperplasia and hypertrophy of muscle fibers and resulting in double muscling (Alberio and Wolf, 2021; Raza et al., 2022).

Gene editing has been applied in sheep and goat breeds by transferring myostatin (MSTN) gene in order to increase meat and milk yield (Ni et al., 2014, Crispo et al., 2015, Li et al., 2016, Zhou et al., 2017, Kalds et al., 2021), improve wool quality (Hu et al., 2017, Zhang et al., 2020, Wang et al., 2015, Li X. et al., 2019), and increase reproductive abilities (Zhou et al., 2019, Niu et al., 2018). Similarly, different gene editing techniques have been applied to increase resistance to diseases, prevent diseases and strengthen the immune system (Vilarino et al., 2017, Niu Y. et al., 2017, Fan et al., 2018). In chickens, the CRISPR technique has been applied to eliminate the effects of allergens by targeting the genes encoding ovalbumin and ovomucoid, which cause egg allergies (Oishi et al., 2016). Additionally, CRISPR/Cas9 editing of the G0/G1 switch gene 2 (G0S2) has prevented triacylglycerol synthesis in chickens, thereby preventing fattening (Park et al., 2019, Popova et al., 2023), while also enabling the breeding of chickens resistant to avian leukosis virus (ALV) (Koslová et al., 2018, Lee et al., 2022). Metric studies use techniques such as bibliometrics, scientometrics, webometrics-cybermetrics, informatrics in libraries, and altmetrics (article-level metrics) that vary from one another using statistical and mathematical methods to analyze and scale publication successes, author performances, and the number of studies conducted in the scientific field (Chellappandi and Vijayakumar 2018, Tomaszewski 2023, Gomis et al., 2023). In general, bibliometrics is defined as quantitative methods in which the number of citations and data of any scientific work, including articles, reviews, books and other publications published in the scientific field, are analysed by mathematical and statistical techniques (Broadus, 1987; Jones, 2015; Pesta et al., 2018; Tomaszewski, 2023). Bibliometric analyzes accurately describes and classifies large amounts of unstructured data, facilitating the cumulative accumulation of scientific knowledge, enable the precise, transparent and objective assessment of the universal impact of scientific studies in multidisciplinary areas through appropriately recorded citation numbers. They facilitate comparisons between scientific papers, enable the storage and classification of studies in databases, and improve access to research results by targeting precise citation counts (Van Raan, 2014; Rousseau and Rousseau 2017; Meija et al., 2021; Donthu et al., 2021). The various uses of gene editing technology in livestock production are demonstrated by these examples, which include enhancing reproductive performance, improving animal welfare, boosting meat, milk, and structural yields, as well as improving health and disease resistance. The aim of our study was to conduct metric analyzes of studies conducted in the field of gene editing in livestock using bibliometric data analysis programs.

MATERIAL AND METHOD

The bibliographic analysis of gene or genome editing in farm animals from 1962 to 2023 was conducted during the study. Searches were performed in the Scopus database (Elsevier B.V., Amsterdam, The Netherlands, https://www.scopus.com) and Web of Science (WoS) database (https://www.webofscience.com) using the keywords "Gene editing in livestock". These databases' original articles, reviews, book chapters, and proceeding papers were exported in CSV format for the Scopus database and Tab Delimited format for WoS.

Bibliographic analyses considering citation indices, publication numbers, authors, most prolific countries, research areas with high searches terms and affiliations have been carried out in these databases. We used VOSviewer version 1.6.20, which was designed in 2010 by Van Eck and Waltman at ‘’www.vosviewer.com’’ to display and analyse the data. The findings from the analyses have been presented through tables and graphical formats.

RESULTS AND DISCUSSION

The Scopus and Web of Science Databases (WOS) were searched by entering the keywords "gene editing in livestock. As a result of the research, it appears that from 1962, when the first publications in the relevant field began, to 2023 there were 358 publications in the Scopus database and 563 publications in the Web of Science.

In the book ''Improvement of Livestock'' written by Richard L. Willham in 1962, it is seen that gene editing studies in farm animals started with the experiments carried out in mice in order to detect the mutation caused by *Peromyscus maniculatus sonoriensis* fungus, which is the hair loss agent in deer (Rıchard L.W. 1962). As a result of the VOSviewer analyzes we conducted between 1962 and 2023 using data from WOS data banks, it appears that the most intensive studies cover the years 2014-2023, while the fewest studies cover the years 1962-2012 were carried out. Between 2023 and 2021, intensive research on farm animals was conducted in an average of 50 studies. The most intensive studies in this area were published in 2020 with 85 different publications, including original articles, reviews, book chapters and other publications. An average of 50 studies were conducted between 2023 and 2021, with intensive studies conducted on farm animals. Between 1962 and 2023, 440 original articles, 113 review studies and 14 book chapters were found in the Web of Science database.

The majority of studies in the field of gene editing in livestock are in the fields of agriculture, dairy science (113), followed by genetic inheritance (77), veterinary science (72), biotechnology, applied microbiology (64) and other fields, the top ten Fields of study are listed in **Figure 1**.

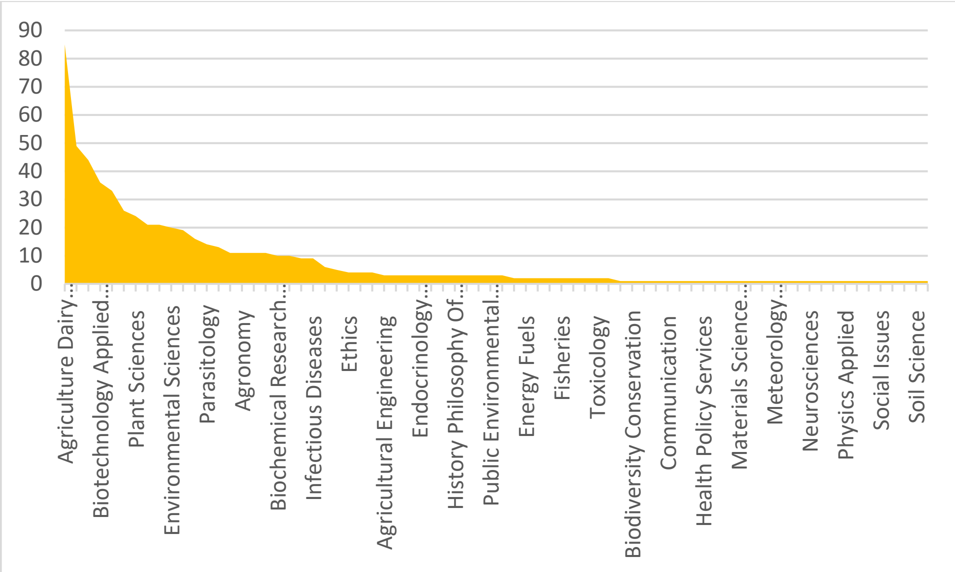

Figure 1. The majority of studies in the field of gene editing in livestock are in the fields

Analysis of Keywords

Among the 563 publications we examined, 1682 keywords were found among the most repeated words in publications and citations, and the most commonly used common words among these were genome editing (66), crıspr (55), gene editing (50), livestock (43), cas9 (33), crıspr-cas9 (31) cattle (31), pigs (22). Among the least used and cited publications, the words used at least once are black rust, domestic animals, sire pedigree, pre-pubertal, population personalization (**Figure 2**).

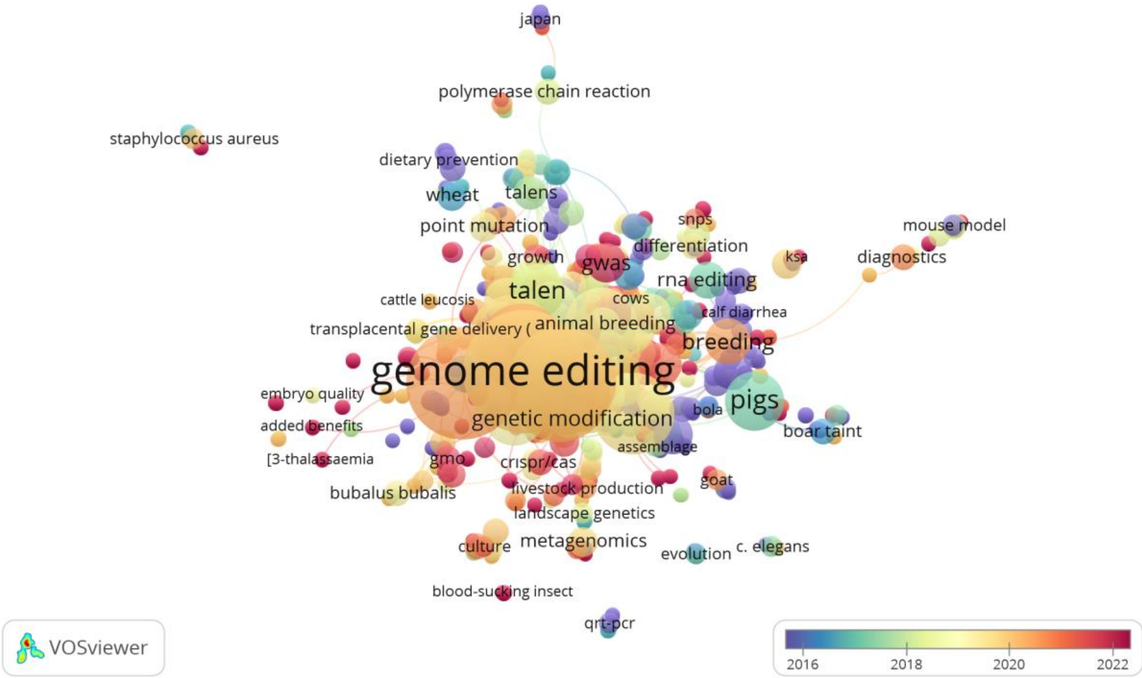

Figure 2. Among the 563 publications Founding 1682 keywords

When the keywords repeated at least 5 times in the publications are analysed, it is seen that 47 keywords are used and the majority of these words are genome editing (66), crıspr (55), gene editing (50), livestock (43) and cattle (29), which are widely repeated especially in review and original articles (**Figure 3**).

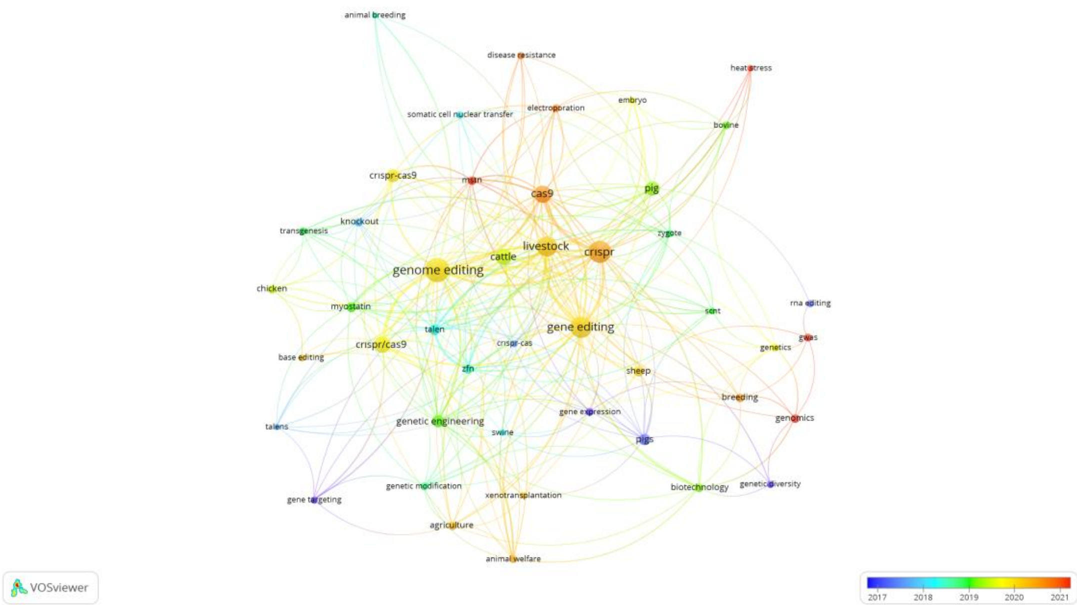

Figure 3. Keywords repeated at least 5 times

When the repeated words in gene editing studies are analysed, it is seen that studies on cattle, pigs and sheep are intensively used in farm animal species, and among the techniques used, CRISPR / Cas9, talen, ZFN, rna editing techniques are intensively used.

Most Prolific Authors

The 10 authors who published intensive studies on gene editing in farm animals between 1962 and 2023 are presented in the table. A total of 3209 authors participated in the study of Gene Editing in livestock and 5 authors published more than 33 articles. Petersen B has been the author with the most publications submitting 17 different publications until 2023 on gene editing studies in farm animals, which they started in 2015 with the study 'Molecular scissors and their application in genetically modified farm animals' (Petersen B., Niemann, H. 2015). Of the 17 studies conducted by Petersen B. and his team, 11 were published as original articles, 4 as reviews, and the other 3 studies were published as book chapters. The author's initial research publication reveals that he used the CRISPR/Cas9 technology to do gene editing experiments on on different animals, mainly pigs and sheep (Zhou et al.2023, Kurtz and Petersen 2019, Zhou et al.2022, Zhou et al.2020).

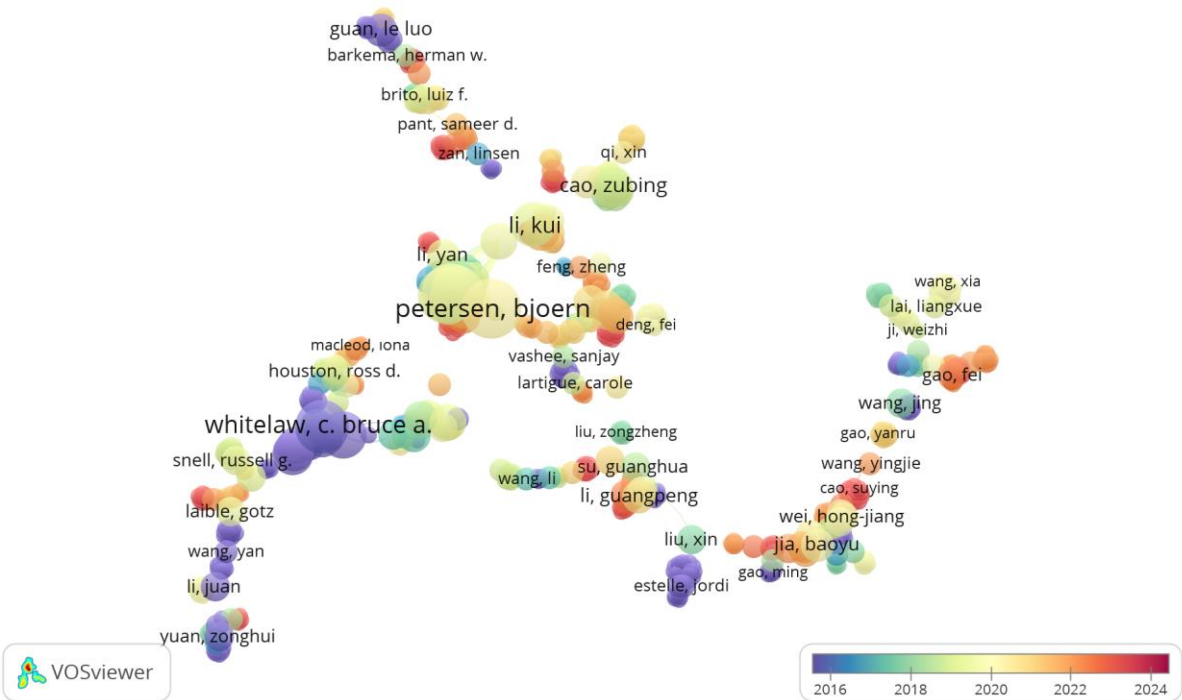

Figure 4. Network map of study authors. Of the 3209 authors

The second ranked belongs to Wang Xian et al. with 12 publications on Gene editing in different farm animals. The CRISPR/Cas9 method of gene knocking in sheep and goat breeds is the subject of Wang Xian and colleagues' other eleven articles, one of which is a book chapter (Wang and Betersen 2022). Other authors and the number of publications they have made are given in **Figure 4**. In the vos wiwer analyses we conducted, the co-authors involved related studies were grouped into different clusters of the same color (**Figure 5**).

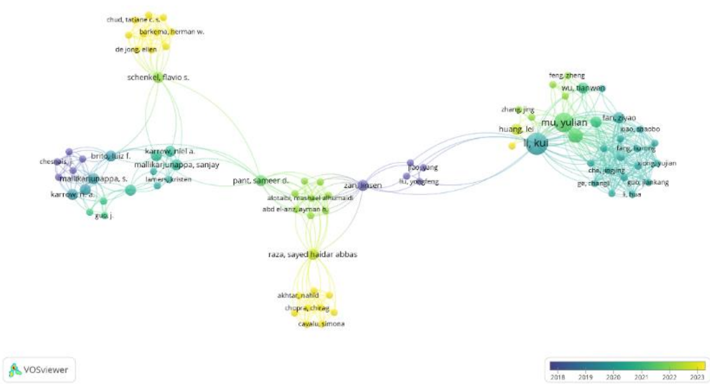

Figure 5. The writers and their teams with the highest publication counts are grouped together using color scales.

Countries and Collaborations

Peoples R China leads with 182 publications among 563 publications in 69 countries in the field of gene editing in livestock (32.327%). In second place is USA with 136 publications (24.156%), followed by Japan with 47 (8.348%), Germany 45 (7.993%), Indıa 35 (6.217%). The top 10 countries according to the number of publications in this field are presented in **Table 1.**

Table 1: Countries with the most publications in gene editing in livestock, number of publications.

Countries/Regions	Record Count	% of 563
PEOPLES R CHINA	113	31.476%
USA	79	22.006%
GERMANY	31	8.635%
JAPAN	25	6.964%
CANADA	23	6.407%
INDIA	21	5.850%
AUSTRALIA	19	5.292%
SCOTLAND	19	5.292%
BRAZIL	11	3.064%
ENGLAND	11	3.064%

Among these countries, 182 of the studies conducted in China have 158 original article publications, 23 of them are in the form of review and one publication is in the form of book chapter. In the list of authors with the most publications in gene editing studies in farm animals, Chen, Yulin (11), Zhou, Shiwei (10), Wang Xiaojuan (9 publications) working at Northwest A&F University, Gu Jie (9 publications) working at Jiangnan University School of Biotechnology, and Huang, Xingxu (8 publications) conducting academic research at Chinese Academy of Agricultural Sciences have brought the Republic of China to the first place on a country basis with their studies (**Figure 6**). Between 2019 and 2023, research on gene editing intensified, building on the foundation of molecular carcinogenesis studies in China in 2010. Using CRISPR and TALENT techniques, studies were conducted on various livestock to improve animal health, increase muscle development, and produce more meat.

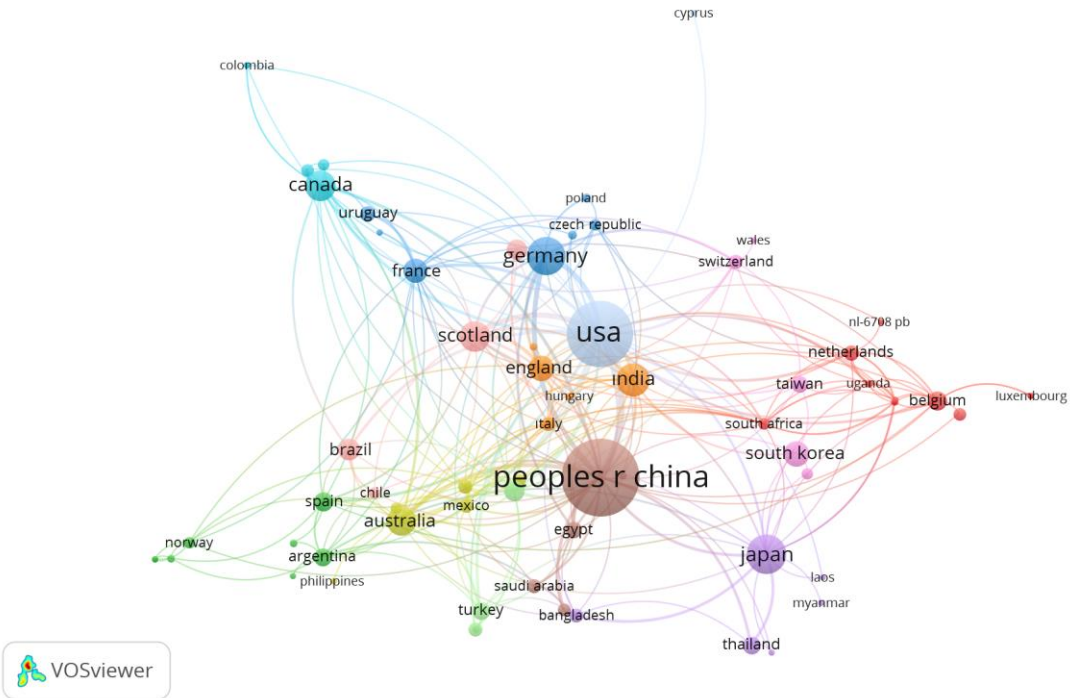

Figure 6: Countries and networks with the most publications in gene editing in livestock.

Gene editing studies started in the USA in 2001 with the study on silencing of disease-causing virus proteins in pigs (Bowden et al. 2001), and then studies in farm animals, especially in genetic heredity and Biotechnology applied microbiology, Agricultural dairy animal science and multidisciplinary fields, intensified between 2015 and 2023 (**Figure 7**). In the USA, gene editing studies started in 2001 with the study on silencing of disease-causing virus proteins in pigs (Bowden et al. 2001), and then gene knockout studies were intensified between 2015 and 2023 in farm animals, especially in genetic heredity and Biotechnology applied microbiology, Agricultural dairy animal science and multidisciplinary fields. In the USA, in the field of gene editing, CRISPR/Cas9 technique as well as TALEN techniques have been applied, especially in the fields of embryonic manipulations in different farm animal species, increasing reproductive functions in animal breeding, and preventing virological diseases.

Among 908 affiliations with 563 publications, Northwest A F Unıversıty China 34 studies, Unıversıty Of California System 29, Bıological Sciences Research Council (BBSRC), Unıversıty Of Edınburgh and UK Research Innovatıon (UKRI) published 28 studies Mınıstry Of Agriculture Rural Affairs 26 studies on gene editing in farm animals. Other affliation sites and their published numbers are given in the **Table 2**.

Table 2. Most Affiliations publishing records

Affiliations	Record Count	% of 563
NORTHWEST A F UNIVERSITY CHINA	34	6.039%
UNIVERSITY OF CALIFORNIA SYSTEM	29	5.151%
BIOTECHNOLOGY AND BIOLOGICAL SCIENCES RESEARCH COUNCIL BBSRC	28	4.973%
UK RESEARCH INNOVATION UKRI	28	4.973%
UNIVERSITY OF EDINBURGH	28	4.973%
MINISTRY OF AGRICULTURE RURAL AFFAIRS	26	4.618%
ROSLIN INSTITUTE	25	4.440%
CHINESE ACADEMY OF SCIENCES	23	4.085%
NATIONAL AGRICULTURE FOOD RESEARCH ORGANIZATION JAPAN	21	3.730%
HINESE ACADEMY OF AGRICULTURAL SCIENCES	20	3.552%

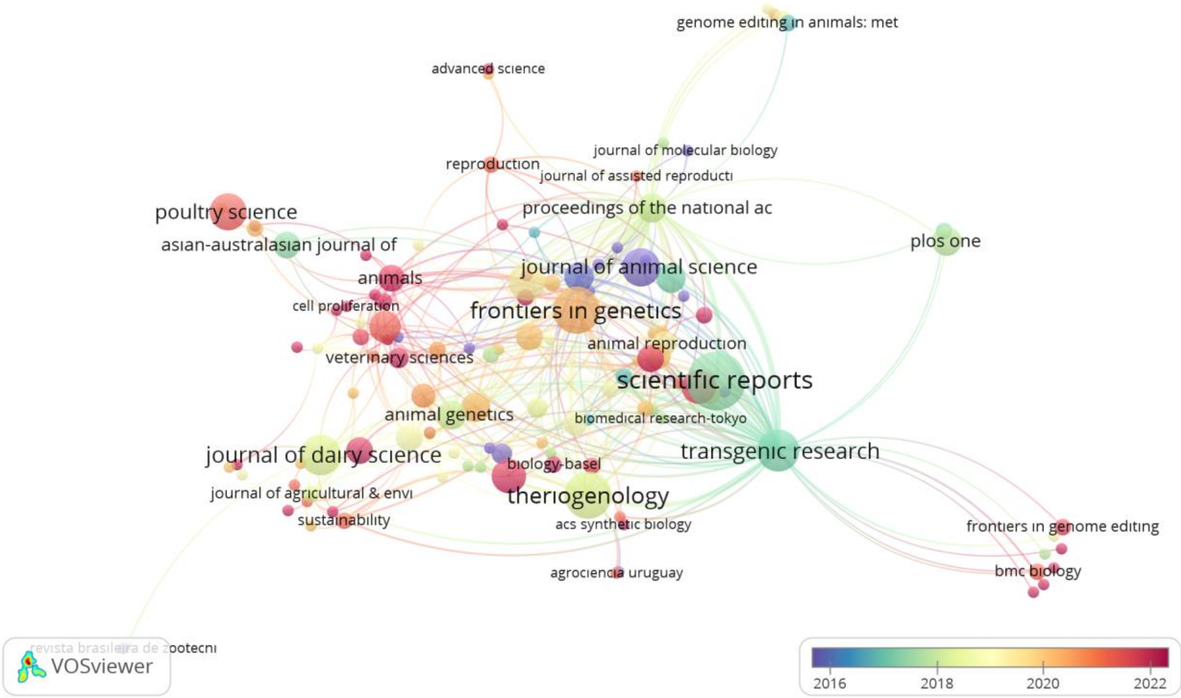

Figure 7. Most Affiliations networks

CONCLUSION

In conclusion the peresent study used bibliometric methods to identify research in the field of Gene Editing in Livestock over the first publicution 1962 and 2023 years. As a result of our analyses, it was concluded that gene editing studies in farm animals are important in increasing animal health and welfare, increasing the quantity and quality of yield, improving human health and nutrition, breeding quality breeds and that studies in this field should be increased.

REFERENCES

Alberio, R., Wolf, E., 2021. 25th Anniversary of cloning by somatic-cell nuclear transfer: Nuclear transfer and the development of genetically modified/gene edited livestock. Reproduction 162, F59–F68.

Bowden, T. R., Westenberg, M., Wang, L. F., Eaton, B. T., & Boyle, D. B. (2001). Molecular characterization of Menangle virus, a novel paramyxovirus which infects pigs, fruit bats, and humans. Virology, 283(2), 358-373.

Broadus, R.N. Toward a definition of “bibliometrics”. Scientometrics 12, 373–379 (1987). https://doi.org/10.1007/BF02016680

Carlson, D. F., Lancto, C. A., Zang, B., Kim, E. S., Walton, M., Oldeschulte, D., ... & Fahrenkrug, S. C. (2016). Production of hornless dairy cattle from genome-edited cell lines. Nature biotechnology, 34(5), 479-481.

Chellappandi, P., & Vijayakumar, C. S. (2018). Bibliometrics, Scientometrics, Webometrics/Cybermetrics, Informetrics and Altmetrics--An Emerging Field in Library and Information Science Research. Shanlax International Journal of Education, 7(1), 5-8.

Crispo, M., Mulet, A. P., Tesson, L., Barrera, N., Cuadro, F., dos Santos-Neto, P. C., ... & Menchaca, A. (2015). Efficient generation of myostatin knock-out sheep using CRISPR/Cas9 technology and microinjection into zygotes. PloS one, 10(8), e0136690.

de Almeida Camargo, L. S., & Pereira, J. F. (2022). Genome-editing opportunities to enhance cattle productivity in the tropics. CABI Agriculture and Bioscience, 3(1), 8.

Donthu, N., Kumar, S., Mukherjee, D., Pandey, N., & Lim, W. M. (2021). How to conduct a bibliometric analysis: An overview and guidelines. Journal of business research, 133, 285-296.

Fan, Z., Perisse, I. V., Cotton, C. U., Regouski, M., Meng, Q., Domb, C., et al. (2018). A sheep model of cystic fibrosis generated by CRISPR/Cas9 disruption of the CFTR gene. JCI Insight 3:e123529.

Gaj, T., Sirk, S. J., Shui, S. L., & Liu, J. (2016). Genome-Editing Technologies: Principles and Applications. Cold Spring Harbor perspectives in biology, 8(12), a023754. https://doi.org/10.1101/cshperspect.a023754

Gao, Y., Wu, H., Wang, Y., Liu, X., Chen, L., Li, Q., Cui, C., Liu, X., Zhang, J., Zhang, Y., 2017. Single Cas9 nickase induced generation of NRAMP1 knockin cattle with reduced off-target effects. Genome Biology. 18, 13.

Gomis, M. K. S., Oladinrin, O. T., Saini, M., Pathirage, C., & Arif, M. (2023). A scientometric analysis of global scientific literature on learning resources in higher education. Heliyon, 9(4).

Hauschild, J., Petersen, B., Santiago, Y., Queisser, A. L., Carnwath, J. W., Lucas-Hahn, A., ... & Niemann, H. (2011). Efficient generation of a biallelic knockout in pigs using zinc-finger nucleases. Proceedings of the National Academy of Sciences, 108(29), 12013-12017.

Hu, R., Fan, Z. Y., Wang, B. Y., Deng, S. L., Zhang, X. S., Zhang, J. L., ... & Lian, Z. X. (2017). RAPID COMMUNICATION: Generation of FGF5 knockout sheep via the CRISPR/Cas9 system. Journal of animal science, 95(5), 2019-2024.

Huson, H. J., Kim, E. S., Godfrey, R. W., Olson, T. A., McClure, M. C., Chase, C. C., ... & Sonstegard, T. S. (2014). Genome-wide association study and ancestral origins of the slick-hair coat in tropically adapted cattle. Frontiers in Genetics, 5, 101.

Ikeda, M., Matsuyama, S., Akagi, S., Ohkoshi, K., Nakamura, S., Minabe, S., et al. (2017). Correction of a disease mutation using CRISPR/Cas9-assisted genome editing in Japanese black cattle. Sci. Rep. 7:17827

Jones, A. (2015). Forensic Journals: Bibliometrics and Journal Impact Factors. Encyclopedia of Forensic and Legal Medicine (Second Edition), 528-538. https://doi.org/10.1016/B978-0-12-800034-2.00181-6

Kalds, P., Crispo, M., Li, C., Tesson, L., Anegon, I., Chen, Y., ... & Menchaca, A. (2022). Generation of Double-Muscled Sheep and Goats by CRISPR/Cas9-Mediated Knockout of the Myostatin Gene. Applications of Genome Modulation and Editing, 2495, 295-323.

Koloskova, E., Ezerskiy, V., Ostrenko, K., (2021). Modifications of the betalactoglobulin gene in bovine and goats for correction of milk composition using CRISPR/Cas9 technology. J. Livestock Sci. (ISSN online 2277–6214) 12, 8– 16

Koslová, A., Kučerová, D., Reinišová, M., Geryk, J., Trefil, P., & Hejnar, J. (2018). Genetic resistance to avian leukosis viruses induced by CRISPR/Cas9 editing of specific receptor genes in chicken cells. Viruses, 10(11), 605.

Kurtz, S., & Petersen, B. (2019). Pre-determination of sex in pigs by application of CRISPR/Cas system for genome editing. Theriogenology, 137, 67-74.

Laible, G., Cole, S. A., Brophy, B., Wei, J., Leath, S., Jivanji, S., ... & Wells, D. N. (2020). Holstein Friesian dairy cattle edited for diluted coat color as adaptation to climate change. BioRxiv, 2020-09.

Lee, J., Kim, D. H., Karolak, M. C., Shin, S., & Lee, K. (2022). Generation of genome-edited chicken and duck lines by adenovirus-mediated in vivo genome editing. Proceedings of the National Academy of Sciences, 119(45), e2214344119.

Lee, K., Uh, K., & Farrell, K. (2020). Current progress of genome editing in livestock. Theriogenology, 150, 229-235.

Li, H., Wang, G., Hao, Z., Zhang, G., Qing, Y., Liu, S., ... & Wei, H. J. (2016). Generation of biallelic knock-out sheep via gene-editing and somatic cell nuclear transfer. Scientific reports, 6(1), 33675.

Li, H., Yang, Y., Hong, W. et al. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. Sig Transduct Target Ther 5, 1 (2020). https://doi.org/10.1038/s41392-019-0089-y

Li, X., Hao, F., Hu, X., Wang, H., Dai, B., Wang, X., et al. (2019). Generation of Tb4 knock-in Cashmere goat using CRISPR/Cas9. Intl. J. Biol. Sci. 15, 1743–1754. doi: 10.7150/ijbs.34820

Liu, X., Wang, Y., Tian, Y., Yu, Y., Gao, M., Hu, G., ... & Zhang, Y. (2014). Generation of mastitis resistance in cows by targeting human lysozyme gene to β-casein locus using zinc-finger nucleases. Proceedings of the Royal Society B: Biological Sciences, 281(1780), 20133368. doi: 10.1098/rspb.2013.3368

Mallikarjunappa, S., Shandilya, U. K., Sharma, A., Lamers, K., Bissonnette, N., Karrow, N. A., & Meade, K. G. (2020). Functional analysis of bovine interleukin-10 receptor alpha in response to Mycobacterium avium subsp. paratuberculosis lysate using CRISPR/Cas9. BMC genetics, 21, 1-11.

McGrail, M., Sakuma, T. & Bleris, L. Genome editing. Sci Rep 12, 20497 (2022). https://doi.org/10.1038/s41598-022-24850-x

Mejia, C., Wu, M., Zhang, Y., & Kajikawa, Y. (2021). Exploring topics in bibliometric research through citation networks and semantic analysis. Frontiers in Research Metrics and Analytics, 6, 742311.

Navarro-Serna, S., Vilarino, M., Park, I., Gadea, J., & Ross, P. J. (2020). Livestock gene editing by one-step embryo manipulation. Journal of equine veterinary science, 89, 103025.

Ni, W., Qiao, J., Hu, S., Zhao, X., Regouski, M., Yang, M., ... & Chen, C. (2014). Efficient gene knockout in goats using CRISPR/Cas9 system. PloS one, 9(9), e106718.

Niu, Y., Jin, M., Li, Y., Li, P., Zhou, J., Wang, X., et al. (2017). Biallelic b-carotene oxygenase 2 knockout results in yellow fat in sheep via CRISPR/Cas9. Anim. Genet. 48, 242–244. doi: 10.1111/age.12515

Niu, Y., Zhao, X., Zhou, J., Li, Y., Huang, Y., Cai, B., et al. (2018). Efficient generation of goats with defined point mutation (I397V) in GDF9 through CRISPR/Cas9. Reprod. Fertil. Dev. 30, 307–312. doi: 10.1071/rd17068

Oishi, I., Yoshii, K., Miyahara, D., Kagami, H., & Tagami, T. (2016). Targeted mutagenesis in chicken using CRISPR/Cas9 system. Scientific reports, 6(1), 23980.

Park T. S. (2023). - Invited Review - Gene-editing techniques and their applications in livestock and beyond. Animal bioscience, 36(2), 333–338. https://doi.org/10.5713/ab.22.0383

Park, T. S., Park, J., Lee, J. H., Park, J. W., & Park, B. C. (2019). Disruption of G0/G1 switch gene 2 (G0S2) reduced abdominal fat deposition and altered fatty acid composition in chicken. The FASEB Journal, 33(1), 1188-1198.

Perisse, I. V., Fan, Z., Singina, G. N., White, K. L., & Polejaeva, I. A. (2021). Improvements in gene editing technology boost its applications in livestock. Frontiers in genetics, 11, 614688.

Pesta, B., Fuerst, J., & W. Kirkegaard, E. O. (2018). Bibliometric Keyword Analysis across Seventeen Years (2000–2016) of Intelligence Articles. Journal of Intelligence, 6(4). https://doi.org/10.3390/jintelligence6040046

Petersen, B., Niemann, H. Molecular scissors and their application in genetically modified farm animals. Transgenic Res 24, 381–396 (2015). https://doi.org/10.1007/s11248-015-9862-z

Popova, J., Bets, V., & Kozhevnikova, E. (2023). Perspectives in Genome-Editing Techniques for Livestock. Animals, 13(16), 2580.

Pritchard, A. 1969, Statistical bibliography or bibliometrics? Journal of Documentation 24, 348-349.

Qian, L., Tang, M., Yang, J., Wang, Q., Cai, C., Jiang, S., ... & Cui, W. (2015). Targeted mutations in myostatin by zinc-finger nucleases result in double-muscled phenotype in Meishan pigs. Sci Rep 5: 14435.

Raza, S. H. A., Hassanin, A. A., Pant, S. D., Bing, S., Sitohy, M. Z., Abdelnour, S. A., Alotaibi, M. A., Al-Hazani, T. M., Abd El-Aziz, A. H., Cheng, G., & Zan, L. (2022). Potentials, prospects and applications of genome editing technologies in livestock production. Saudi Journal of Biological Sciences, 29(4), 1928-1935. https://doi.org/10.1016/j.sjbs.2021.11.037

Richard L. Willham, IMPROVEMENT OF LIVESTOCK, Journal of Heredity, Volume 53, Issue 4, July 1962, Page 194, https://doi.org/10.1093/oxfordjournals.jhered.a107169

Rousseau, S., & Rousseau, R. (2017). Being metric-wise: Heterogeneity in bibliometric knowledge. Profesional de la información, 26(3), 480-487. doi:10.3145/epi.2017.may.14

Schuster, F., Aldag, P., Frenzel, A., Hadeler, K. G., Lucas-Hahn, A., Niemann, H., & Petersen, B. (2020). CRISPR/Cas12a mediated knock-in of the Polled Celtic variant to produce a polled genotype in dairy cattle. Scientific reports, 10(1), 13570.

Tomaszewski, R. (2023). Visibility, impact, and applications of bibliometric software tools through citation analysis. Scientometrics, 1-22.

Tu, C. F., Chuang, C. K., Hsiao, K. H., Chen, C. H., Chen, C. M., Peng, S. H., ... & Chen, C. M. (2019). Lessening of porcine epidemic diarrhoea virus susceptibility in piglets after editing of the CMP-N-glycolylneuraminic acid hydroxylase gene with CRISPR/Cas9 to nullify N-glycolylneuraminic acid expression. PLoS One, 14(5), e0217236.

Van Eck, N. J., & Waltman, L. (2010). Software survey: VOSviewer, a computer program for bibliometric mapping. Scientometrics, 84(2), 523–538

Van Raan, A. F. (2014). Advances in bibliometric analysis: research performance assessment and science mapping. Bibliometrics Use and Abuse in the Review of Research Performance, 87, 17-28.

Vilarino, M., Rashid, S. T., Suchy, F. P., Mcnabb, B. R., Van Der Meulen, T., Fine, E. J., et al. (2017). CRISPR/Cas9 microinjection in oocytes disables pancreas development in sheep. Sci. Rep. 7:17472.

Vilarino, M., Soto, D. A., Bogliotti, Y. S., Yu, L., Zhang, Y., Wang, C., ... & Ross, P. J. (2020). Derivation of sheep embryonic stem cells under optimized conditions. Reproduction, 160(5), 761-772.

Wang, S., Qu, Z., Huang, Q., Zhang, J., Lin, S., Yang, Y., ... & Zhang, K. (2022). Application of gene editing technology in resistance breeding of livestock. Life, 12(7), 1070.

Wang, X., & Petersen, B. (2022). More abundant and healthier meat: will the MSTN editing epitome empower the commercialization of gene editing in livestock? Science China Life Sciences, 65(2), 448-450.

Wang, X., Yu, H., Lei, A., Zhou, J., Zeng, W., Zhu, H., ... & Chen, Y. (2015). Generation of gene-modified goats targeting MSTN and FGF5 via zygote injection of CRISPR/Cas9 system. Scientific reports, 5(1), 13878.

Watanabe, M., Umeyama, K., Matsunari, H., Takayanagi, S., Haruyama, E., Nakano, K., ... & Nagashima, H. (2010). Knockout of exogenous EGFP gene in porcine somatic cells using zinc-finger nucleases. Biochemical and biophysical research communications, 402(1), 14-18.

Whitworth, K. M., Benne, J. A., Spate, L. D., Murphy, S. L., Samuel, M. S., Murphy, C. N., ... & Wells, K. D. (2017). Zygote injection of CRISPR/Cas9 RNA successfully modifies the target gene without delaying blastocyst development or altering the sex ratio in pigs. Transgenic research, 26, 97-107.

Whitworth, K. M., Rowland, R. R., Ewen, C. L., Trible, B. R., Kerrigan, M. A., Cino-Ozuna, A. G., ... & Prather, R. S. (2016). Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. Nature biotechnology, 34(1), 20-22.

Yan, S., Tu, Z., Liu, Z., Fan, N., Yang, H., Yang, S., ... & Li, X. J. (2018). A huntingtin knockin pig model recapitulates features of selective neurodegeneration in Huntington's disease. Cell, 173(4), 989-1002.

Yugo, D. M., Heffron, C. L., Ryu, J., Uh, K., Subramaniam, S., Matzinger, S. R., ... & Meng, X. J. (2018). Infection dynamics of hepatitis E virus in wild-type and immunoglobulin heavy chain knockout JH−/− gnotobiotic piglets. Journal of virology, 92(21), 10-1128.

Zhou, S., Ding, Y., Liu, J., Liu, Y., Zhao, X., Li, G., ... & Chen, Y. (2020). Highly efficient generation of sheep with a defined FecBB mutation via adenine base editing. Genetics Selection Evolution, 52(1), 1-7.

Zhou, S., Kalds, P., Luo, Q., Sun, K., Zhao, X., Gao, Y., ... & Wang, X. (2022). Optimized Cas9: sgRNA delivery efficiently generates biallelic MSTN knockout sheep without affecting meat quality. BMC genomics, 23(1), 348.

Zhou, S., Lenk, L. J., Gao, Y., Wang, Y., Zhao, X., Pan, M., ... & Wang, X. (2023). Generation of sheep with defined FecBB and TBXT mutations and porcine blastocysts with KCNJ5G151R/+ mutation using prime editing. BMC genomics, 24(1), 1-9.

Zhou, W., Wan, Y., Guo, R., Deng, M., Deng, K., Wang, Z., ... & Wang, F. (2017). Generation of beta-lactoglobulin knock-out goats using CRISPR/Cas9. PloS one, 12(10), e0186056.

Zhou, X., Xin, J., Fan, N., Zou, Q., Huang, J., Ouyang, Z., et al. (2015). Generation of CRISPR/Cas9-mediated gene-targeted pigs via somatic cell nuclear transfer. Cell Mol. Life. Sci. 72, 1175–1184. doi: 10.1007/s00018-014- 1744-7.

POSTER PRESENTATIONS

Oil and Fatty Profile on The Film from The Pistacia Region of Collo

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ABSTRACT: The objective of this study is the determination of the analytical parameters and the fatty acid composition of the film from *pistacia lentiscus*, the extraction was carried out by soxhlet using an apolar solvent which is hexane, the chemical composition of fatty acids was performed by chromatography alone and coupled to mass spectroscopy (CGC, GC/MS) this study identified 7 constituents representing 86.81% the compounds the major compounds are palmitic acid 28.15%, oleic 26.56% and linoleic 24.57%.

Keyword: Pistacia, Extraction, Oil, Fatty acids, CG/MS

INTRODUCTION

The *Lentiscus pistachio L* is a shrub ofsize ranging from 2m to 6m which spreads throughout the Algerian land with a high density in the forest areas and also in the fresh countryside and belongs to the family of anacardiaceae.1 Its fruits have a spherical flattened shape of small dwarf at the beginning its color is green then turns to the black color, this fruit is covered by a soft zest then comes a hard layer containing a pulp of good taste and an embalmed odor (IRN BITAR).In the literature several works have been done on the oil extracted from the mesocarp and epicarp mixture of the wall fruit proving that it contains saturated and unsaturated fatty acids.2-3 Bibliographie investigations have shown no study has been done on the determination of the parameters and fatty acids of the epicarp. We were interested in this work which consists of an extraction, isolation and identification by chromatography coupled to mass spectroscopy.

MATERIALS AND METHODS

Table 1. The conditions for harvesting the fruit in the following table

Botanical name	Date of Harvest	Location	Stage of development	Vegetative
Pistacia	December 2016	Collo	Fruit Walls	Foret

The harvested fruit is separated after peeling for two part mesocarp and epicarp film is the latter which will be the subject of our work in the following partof the film then dipped in liquid nitrogen for stabilization from the chemical point of view.cryogynation and primordial in the conservation of vegetable matter, it is crushed to obtain a powder which will be put in the freezer at -4 ° C until analysis.

Extraction of the oil: The fruit is dried in an oven at 80 ° C for 10H the oil obtained after 16H extraction with hexane in a continuous extractor sohxlet after removal of the solvent by evaporation in vacuo, recovers oil qi is yellow and a very strong scented odor and solidifies at room temperature.

Analysis of analytical parameters: the determination of main chemical characteristics is made according to standardized standard methods.4

Statistical Analysis

All the experiments underwent three repetitions using the analysis of the variance (ANOVA) the values were calculated by comparison of the averages.

Preparation of methyl esters of fatty acids: The method we used is the cold transesterification using a methanoidal sodium hydroxide solution, in a 10 ml screw tube are introduced 0.5 g of oil then 10 mldd heptane and The mixture is stirred and then 0.1 ml of 2N sodium methanoic is added, poured and stirred very hard and then decanted to recover the upper layer containing the methyl esters [the reaction was followed by IR to confirm the existence ofaband) at 1750cm-1

GC analysis: The COG analyzes were carried out on AGITANTTECHNRLOGIE 6890 equipment equipped with a flame ionization detector FID) of an injecter and a HP5 capillary column (30 x 0.32 mm, film thickness 0,25 m) the carrier gas is helium, the temperature is 270 °C., the temperature program of the oven consists of an isothermal 80 °/min followed by a temperature lamp at 50 ° / min up to 310 ° / 2min, the injection is done by SPLILESS mode, the injected volume of 1ml.

Analysis bv GPC-SMRH: The analyzes were carried out on equipment AGITENT type TECHNOLOGY 6890 dote an automatic injector and a capillary column HPS [30Mfois 0.32 mm film thickness 0.25 microns] coupled to a mass spectroscopyAUTOSPEC 610 the ionization mode is the electronic impact at 70W, the detection is done by HRMS analyzer [high resoultion mass spectrometry) of EBE type in the mass range from 50 to 800Da, the carrier gas is helium with a flow rate of 1ml / min, the programming of the temperature is identical to that used previousiy for detection by FID, the injection is done by the Splytiess mode the spectra obtained hâve been identified by comparison with the spectrum database ofknown NIST compounds [5].

Qualitative and quantitative analysis: For each of the compounds, the retention indices are calculated from the retention times of a standard range ofC8-C30 aïkanes (KOVATS indices) analyzed in the mining conditions chroma graphies cited above the calculation of relative percentages of thèse compounds was performed on the chromatograms obtained by FID.

RESULTS AND DISCUSSIONS

The results of our experiments on the determination of the analytical parameters are shown in Table 2.

Table 2. Characterization of film and fat

Water content	24,50 ± 0,21
Fat extracted with hexane	68,50 ± 0,05
Cendre	2,70 ± 0,01
Protéine **N x 6,25**	10,50 ± 0,41

Mineral elements (mg / 100g of dry matter)

K*	Fe	P	Ça	Zn**	Mn	Mg	Na*	Cu
9,07	165	103,7	1,287	22,9	30,50	3,201	86	11,4

* Indicates by emission of the flame

** refers to atomic absorption

Table 3. Phosphorus was measured by ascorbic acid calorimetric method and 820nm ammonium

Indice de réfraction	1.4 ± 0.2
Density	1,3 ±0,5
Standard Saponification Index (T60206)	191.90 ±0.5
lodine value(wijs)	CT60206)
Acid value(AOCS)	2.7 ±0.21
Unsaponifiable (hexane method)	3.14 ±0.15
Peroxide value (mmol /kg)	3.8 ±0.2
lovibond color	Blue= 0.6 Red=2.7 Yellow = 80.7
Melting point	27.5 ± 0.1

The high value found for the content in eu lights us on its water richness which is higher compared to that of rapeseed (17,64%) and sunflower (19,77%) concerning the content of ash which is about 2.7% confirms that our sample does not contain toxic elements, the low protein content shows us that the amino acids they contain are verylow, for the mineral elements there is no evidence of toxic elements since the ash rate is very low. The high unsaponifiable content corresponds to an oil rather to be useful as an interesting raw material in cosmetics according to (OLLE 2002). On an average of three extractions the proportion of the oil present in the film is 68.50%. The determination of the peroxide index and the acid number gives an image of the state of degradation of the oil. The low values of thèse two indices show that our sample has not undergone any oxidative and hydrolytic deterioration during storage. iodine and saponification indices indicate their preponderance oflong chain C18 fatty acids with a higher rate of initiation. The value observed in the yellow of the color lovibon confirms our yellow color of the extraction, the observed value which is of the order of 80.7 confirme our color of the oil that is yellow of ours ample during our extraction.

Table 4. Composition in% of the fatty acids of the film

Components	Name	K	RT	%
Palmitic acid	C16	1090	12.241	28.150
Palmitoleic acid	C161A9	1659	13.010	1.376
Oleic acid	C181A9	1290	18.880	26.562
Linoleic acid	C181A9.12	1120	20,860	24,571
linoleic a acid	C183A9.12	1510	23,050	5,991
Stearic acid	C18	1640	17.823	4.089
Cicric acid	C19	1014	5.614	4.089

The table shows the absence of the acid tha is considereed undesirable because of its pathological effect on the cardiac muscle.7 The value of the high rate of palmitic acid could open a way for use in the industry as an example, manufacture of biscuits indeed this oil is remarkable for its high content of acid linoleic acid sought for various industrial applications.

CONCLUSION

This botanical plant *Pistacia lentiscus* L. presented on the Algerian tell for essentially medicinal purposes constitutes in the light of these results a plant material quite interesting which one must depend the study through the qualitative and quantitative analysis of the important constituents of the unsaponifiable fraction this evaluation to corne soon in our newspaper.

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Statement of Conflict of Interest

There is no conflict of interest

Authors' Contributions

We did collective work distributed equally between the tasks assigned to each member of the team between paratical writing and discussion of the results.

REFERENCES

1. Quezel. P et SANTAA (1963) nouvelle flore d'Algerie et des regions désertiques Ed CNRS pp 31-40. IBN BITAR les medicaments, explication du livre DIACIKO Maison du Magreb BEYROUT.
2. M LABRA, F. Grassi, M BARDINI plant science, 2003 pp 1129-36.
3. LECHERC H. 1994 precis de phytothérapie Ed Masson pp 257.
4. Recueil de normes Française des corps gras oleagincuses produits derives (1984) édit AFNOR.
5. ADAMS RP, 2001 identification of essentials composants by gras chromatography/quadripole mars spectroscopy USA pp 454.
6. OLLE, M 2002 Analyse des corps gras technique d'ingénieur pp 3325 – 1-15.
7. KRISHNA, MURTY, RAJA/AKSH MIS, Malika T et al (1983) journal of Food sciences pp 20-32.
8. BELLAKHDAR (1978) Medicine traditionnelle et toxicologie Ouest Sahariennes.
9. FLANDERSA, ABDELKARIM SM (1985) Journal AOCS Vol 62 n° 7 pp 1134-1136.
10. KALERKIND A (1982) Manuel des corps gras Vol 1 et 2 Lavoisier Ed, Paris ed Toc et doc.
11. LEGRAND P 2008 interêt nutritionnel des principaux acides gras des Lipides Sciences des aliments, 28 34-43.
12. R.A FLATH, J.M TAKAHASKI Journal agoic Food chemistry 1978, 26, pp 834-8.
13. M.F RAMADAN, IT MORSEL Food chemistry 2000, 82 pp 209-27.

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ABSTRACT

ORAL PRESENTATIONS

Antihemolytic Activity of Ethyl Acetate and Butanolic Extracts of *Helichrysum stoechas* (L.) Moench

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ABSTRACT: Oxidative stress is involved in apoptosis and cellular aging but also hemolytic anemia. The latter is an indicator of free radical damage to the red blood cell membrane, which antioxidants can help prevent. This study aimed to examine the antihemolytic activities of ethyl acetate and butanolic extracts from the aerial part of *Helichrysum stoechas* (L.) Moench against AAPH-induced hemolysis. The antihemolytic activity of the two extracts showed that pretreatment of human erythrocytes with various doses significantly reduced AAPH-induced hemolysis in a dose-dependent manner. The recorded HT_{50} value reached 185.58 ± 7.45 min and 138.50 ± 1.57 min for ethyl acetate and butanolic extracts at a concentration of 40 µg/ml, versus 52.30 ± 0.31 min for the control. These values are better than those obtained with Trolox (115.47 ± 0.41 min), used as a reference. Our results demonstrate that *Helichrysum stoechas* (L.) Moench attenuates AAPH-induced hemolysis and can be used to prevent and treat hemolytic anemias.

Keywords: *Helichrysum stoechas*, Hemolysis, AAPH, Phenolic compound, Antioxidant

Phenolic Content and Antioxidant Activity of Algerian Caper Leaves (*Capparis Spinosa* L.)

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ABSTRACT: *Capparis spinosa*, commonly known as the caper, is native to the Mediterranean region and is part of the *Capparaceae* family. It is used as a medicinal plant because it contains many biologically active phytochemicals, such as phenols, flavonoids, tannins, triterpenoids and saponins. This study was carried out to determine the phenolic content and the antioxidant activity of methanolic extract of *Capparis spinosa* leaves using the DPPH free radical, metal chelating, linoleic acid peroxidation and reducing power assays. The total polyphenol, flavonoid and tannin content of the methanolic extract was found to be 102.87 ± 6.62 µg/mg gallic acid equivalent, 53.66 ± 2.72 µg/mg quercetin equivalent and 55.88 ± 4.20 µg/mg tannic acid equivalent, respectively. The methanolic extract showed a good free radical scavenging activity with IC_{50} value of 55.57 ± 3.66 µg/ml. In addition, the extract showed a good concentration-dependent chelating activity with IC_{50} of 45.72 ± 3.28. Moreover, the extract at 50 µg/ml inhibited strongly (80%) linoleic acid peroxidation. Whereas, methanolic extract exerted a strong concentration-dependent reducing power. These results revealed the high phenolic contents and the antioxidant potential of *Capparis spinosa*. Thus, our findings provide evidence that *Capparis spinosa* is a potential source of natural antioxidants.

Keywords: Antioxidant activity, *Capparis spinosa,* Methanolic extract, Phenolic compound, Caper leaves

Physiological and Biochemical Characterization of *Trichoderma* species and Their Antifungal Activities against *Botrytis cinerea* and *Alternaria solani*

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ABSTRACT: Some species of *Trichoderma* have been successfully used in commercial biological pesticides against fungal pathogens, e.g., *Botrytis cinerea* and *Alternaria solani*, two economically important airborne pathogens of tomatoes (*Solanum lycopersicum*). The objectives of the present study were to provide physiological (pH and temperature) and biochemical (catalase (Cat), pectinase production (PP), proteolytic activity (PA), amylolytic activity (AA), β-1,3-glucanase activity (GA), indole-3-acetic acid (IAA), hydrocyanic acid (HCN), atmospheric nitrogen fixation (ANF) and phosphate solubilization (PS)) characterization of three *Trichoderma* species (Tr1, Tr2 and Tr3) isolated from the rhizosphere, and to assess the ability of each species to inhibit *B. cinerea*and *A. solani* development via dual confrontation assay. The mycelial growth of *Trichoderma* spp. at 19, 30 and 45°C revealed that the optimum temperature for best growth was 30°C, and the values ranged between 6.63 (Tr3) and 7.63 cm (Tr1) after 8 days of incubation. Similarly, it was found that the maximum mycelial growth was recorded at pH 9, with 7.10 (Tr2) and 12.27 cm (Tr3) after 8 days of incubation. The results revealed that all *Trichoderma* species are able to produce Cat, AIA (except Tr2) and HCN. The mycelial growth of *B. cinerea* and *A. solani* decreased in the presence of Tr1 which varied respectively from 2.58 to 2.68 cm after 7 days of incubation (untreated control = 5.34 cm for *B. cinerea*; 5.88 cm for *A. solani*).

Keywords: *Trichoderma* spp., *Botrytis cinerea*, *Alternaria solani*, Plant-growth-promoting fungi, Antagonism

Sustainable Management of Soil and Water Resources in Arid Regions by the Application of Date Palm Derived Biochar*

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ABSTRACT: Arid and semi-arid regions are more vulnerable to the land degradation owing to water scarcity and lower soil organic carbon contents. Therefore, sustainable management of soil and water resources in such areas is critical in enhancing crop productivity and soil health. Biochar has recently came up as an ideal candidate to improve soil conditions and water conservation in arid and semi-arid areas due to large surface area, higher porosity, abundant functional groups, and high cation exchange capacity. Thus, the efficiency of date palm waste-derived biochar to improve soil hydro-physical properties and subsequent increase in tomato growth was studied under lab-scale and greenhouse trials. Biochar was produced by at 300°C, 500°C, and 700°C using date palm waste and separated into various size fractions (<0.5 mm, 0.5–1 mm, and 1–2 mm). Columns experiments were conducted by amending the calcareous sandy soils at 1%, 2.5%, and 5% of the produced biochar along with a control (without biochar). The results of column trials suggested that biochar applications resulted in decreased saturated hydraulic conductivity while increased cumulative evaporation. Lower pyrolysis temperature (300°C and 500°C) resulted in higher cumulative evaporation, while higher pyrolysis temperature (700°C) resulted in reduced cumulative evaporation. Likewise, smaller particle size resulted in higher cumulative evaporation. Greenhouse trials demonstrated that biochar application resulted in 5.48%–8.11% improvement in soil moisture content than control and substantially increased tomato growth. Thus, biochar application enhanced soil health and water conservation in sandy soil, consequently increasing tomato plant growth.

Keywords: Water conservation; Cumulative infiltration; Sustainable soil management

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Impact of Unconventional Feed Additives on the Growth of Digestive Organs and Egg Formation in Adler Silver Hens*

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ABSTRACT: The results of the investigation reveal the potential advantages of integrating unconventional feed supplements into poultry farming techniques. Rigorous experiments demonstrated that these supplements, derived from peat and feathers, positively influenced the physiological and morphofunctional characteristics of the digestive and reproductive systems in Adler Silver breed chickens. The identified outcomes from the application of these distinctive feed supplements disclosed several notable benefits. Particularly, there was a distinct improvement in the proper development and efficient functioning of the ovary and oviduct. This finding carries broad implications, suggesting a promising approach for enhancing egg production in Adler Silver breed laying hens. An especially interesting discovery involved the expedited physiological maturation observed in the juvenile subjects within the experimental groups when contrasted with the control group. This occurrence directly manifested in noticeable anatomical modifications, particularly growth in the dimensions of the ovary and oviduct. These changes strongly indicate the possibility of increased egg output within the Adler Silver breed. This research provides compelling evidence to emphasize the feasibility of incorporating unconventional feed supplements originating from feathers and peat into poultry nutritional approaches. The improvements observed in reproductive characteristics not only indicate the potential for higher egg production but also contribute to a more profound comprehension of the complex interaction between nutrition and physiological development in poultry. These results create opportunities for additional investigation and refinement of feed compositions, potentially revolutionizing poultry farming practices and elevating overall production efficiency.

Keywords: *Chicken*, Digestive organs, Feather meal, Oviduct, Peat meal

Insights into Citrus Viroids: Molecular Characterization, Prevalence, and New Host Plant Unveiling in Punjab, Pakistan

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ABSTRACT: Citrus plants are susceptible to various viroid infections that weaken their vigor and reduce productivity. Viroids are the smallest, single-stranded RNAs without a protein coat infectious agents that attack the citrus plant and become a major concern for yield reduction. To assess the prevalence and distribution of Viroid species, the present study was conducted based on specific viroids symptoms such as gumming, wood pitting, bark shelling, bark cracking discoloration, browning of the phloem tissues, and leaf bending. A total of 130 citrus leaf samples of various citrus cultivars, including Feutrell's Early, Kinnow mandarin, sweet orange, sweet lime, grapefruits, lemon, tangerines, and tangelos, were collected from the seven districts (Sahiwal, Sargodha, Khanewal, Rahim Yar Khan, Multan, Layyah, and Toba Tek Singh) of Punjab, Pakistan. RT-PCR techniques were applied with new design-specific viroids primers (CVd-V AF1/AR1, HSVd AF1/AF1, CVd III AF1/AR1, CBLVd AF1/AR1) for the detection of Viroid species at the molecular level. RT-PCR amplifications confirmed that CVd-V with 293 bp was detected in 62 of 130 (47.69%), HSVd with 301 bp was detected in 32 of 130 (24.61%), CVd-III with 291 bp was detected in 46 of 130 (35.38%), and CBLd with 324 bp was detected in 19 of 130 (14.61%) samples. The viroid infection was confirmed by biological indexing on indicator host Etrog citron. Sequencing analysis confirmed that Palestinia sweet lime, Roy Ruby, Olinda Valencia, Kaghzi lime, and Dancy were identified as new citrus hosts of CVd-V for the first time in Pakistan. Furthermore, the 'Palestine sweet lime' was identified as the new host of CVd-III in Pakistan for the very first time during this study. The new host of CVd-V and CVd-III shows 98–100% nucleotide sequence homology with those reported previously from other countries, while the isolates reported from Pakistan show 100% sequence homology. These findings suggest a dire need to develop possible strategies to diagnose and stop the further spread of viroids in citrus orchards for sustainable citrus production.

Keywords: Viroids species, RT-PCR, ssRNA, Biological indexing, Sequence analysis

Application of Modern Technologies and Methods in Ecological Grape Cultivation in The Central Region of The Republic of Moldova*

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ABSTRACT: The development of the grape and wine industry, specifically the introduction of ecological farming in the Republic of Moldova, holds great potential at both the national and international levels. Conditions are being established to promote ecological agriculture, drawing on key methods and technologies from the international community. The Republic of Moldova boasts extensive agricultural lands with diverse microclimatic conditions, enabling the testing and successful implementation of new methods for producing organic products, such as grapes, for various purposes, including fresh consumption and/or the production of different types of wines. In the face of ongoing climate change, it is imperative to formulate recommendations and practices tailored to specific climatic conditions, soil types, and target agricultural practices. It is noteworthy that research in this field is insufficient, and there is an untapped research potential for the industry's development. In the case of perennial plantings, particularly vineyards, the possibilities are not limited to the production of planting material and growing grapes. Currently, there is an urgent need to leverage all available opportunities in precision viticulture as the foundation for cultivating grapes ecologically, incorporating all modern methods and technologies. Our research took place in the central grape-wine-growing region of the Republic of Moldova at the Roman Shtefirtsa farm, which practices ecological viticulture. The farm has undergone an audit and received certification according to a scheme recognized as equivalent to the provisions of regulation (EC) - Ecocert Organic Standard. We studied the Viorica grape variety of Moldavian selection.

Keywords: Grapes, Viorica, Slope, Ecology, Technologies, Productivity

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Importance of Nano-Sized Feed Additives in Animal Nutrition

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ABSTRACT: "Nano", which derives from the Latin word nanus and means dwarf, refers to a very small unit of measurement equal to one billionth of a metre. Nanotechnology, which deals with the manipulation of matter at the atomic and molecular level, has an application area in animal husbandry as well as in many fields. Nano-sized feed additives, which have come to the forefront in the livestock sector in recent years, have become an innovative application used to increase the nutritional value of feeds and optimise animal health and performance. Since these additives are nano-sized particles with increased specific surface area, they can have a positive effect on a number of factors such as digestibility, nutrient absorption, immune system, growth and development. Minerals in the form of nanoparticles used as feed additives can increase bioavailability by passing through the intestinal wall to body cells faster compared to larger particles. The nano level of the substance not only increases the productivity of animals, but also brings the potential to improve the functionality of feed molecules. Nano feed additives increase the digestion and absorption of feed, allowing animals to benefit from feed more effectively. In this article, current studies on nano-sized feed additives that offer potential advantages in animal nutrition are discussed.

Keywords: Nanotechnology, Animal Nutrition, Feed Additives

Bioactivity and Chemical Quality of *Ammodaucus leucotrichus* Essential Oils from Algeria

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ABSTRACT: The immunomodulatory properties of natural agents have received much attention in recentdecades. Some plant-derived agents are known to be immunomodulators that can affect both innate and adaptive immunity. This study aimed to evaluate the immunomodulatory properties of essential oils of Ammodaucus leuchotricus in silico.one plant species from Algeria's Saharan region. Gas chromatography-mass spectrometry (GC-MS) was used in the current work to examine essential oils of *A. leuchotricus* in order to identify significant functional groups and phytochemical constituents. With an IC50 of 966.48 9.95 g/ml, GC-MS analysis of the essential oils of *A. leuchotricus* revealed the presence of 62 phytochemical components and shown impressive anti-inflammatory activity. These 62 substances were then examined for their bioactivity using in silico molecular docking techniques. The findings showed that one of the discovered phytochemical compounds may have trypsin inhibiting action.

Keywords: GC_MS, Anti-inflammatory, In silico, Molecular docking, Trypsin, İmmunomodulatory

The Effect of Plant Growth-Promoting Bacteria on the Development of *Vicia faba* L.

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ABSTRACT: Plants have always been in a symbiotic relationship with soil microbes (bacteria and fungus) during their growth and development. The symbiotic free-living soil microorganisms inhabiting the rhizosphere of many plant species and have diverse beneficial effects on the host plant through different mechanisms such as nitrogen fixation and nodulation are generally referred to as Plant Growth Promoting Bacteria (PGPB). In this study; the effect of *Pseudomonas chlororaphis* (PGB-26) and *Pseudomonas chlororaphis* (PGB-26) + *Enterebocter cloaceae* (PGB-28) combination of bacterial strains with biofertilizer potential on the development of *Vicia faba* plants was investigated. The experiment was set up with 3 replications and sdH_2O was used as a negative control. According to the results obtained, it was observed that PGB-26 bacteria increased the total wet weight by 22.72%, and the PGB-26 + PGB-28 combination increased by 19.31% compared to the control. As a result, the applicability of these two applications should be investigated by testing them under field conditions.

Keywords: Biofertilizer, PGPB, *Vicia faba*

Ethnobotanical Survey of Plant Used for the Treatment of Sexual Hormonal Disorder in two South Localities in Algeria

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ABSTRACT: For centuries, plant resources have played an important role in human life. Some have nourished him, others have cured him of his deseases. Through time and experience, we have acquired knowledge about plants, discovered the medicinal ones among them, and developed practices and skills to preserve our health. Medicinal plants still have an important place in humanity's therapeutic arsenal. They are an important source of bioactive molecules that are generally part of secondary metabolites, which are molecules essential to plant life and their interaction with the environment, and are also important sources for pharmaceutical products. In this context, an ethnobotanical survey was carried out among 100 people in the South of Algeria between November 2022 and March 2023, has recorded the use of 55 plants for treatment of the reproduction disorder both in males and females. The maily used species were *Origanum majorana* and *Glycyrrhiza glabra*. Leaves and flowers were the most parts used with 44,9% and 15,09 respectively, decoction was the prefered method to prepare remedies. This study constitutes a source and scientific basis for chemists and biologists in algeria in the development of new drugs.

Keywords: Ethnobotanical survey, Medicinal plants, Infertility, South algeria

Antibacterial Activity of *Ruta Montana* L. and *Artemisia* sp

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ABSTRACT: As part of the development of plant resources, we are interested in the chemical study of essential oils of *Ruta montana* L. and *Artemisia* sp which they are widely used as tea or external use to treat several diseases such as diseases of the digestive system. In this study we evaluated the antibacterial activity of these medicinal plants. The essential oils were obtained by hydrodistillation of the aerial parts "stems, leaves and flowers" from *R. montana* and *Artemisia sp.* The antibacterial activity was tested by using the agar diffusion test. Gram positive and negative pathogenic bacteria: *Staphylococcus aureus* ATCC 25923, *Schegalla sonnei* and *Escherichia coli, Pseudomonas aeruginosa* ATCC27853 and *klebsiella pneumoniae* ATCC 700603 were used to evaluate this activity. The results are expressed by measuring the diameter of inhibition of the different concentrations of the samples. The essential oil of *R. montana* and *Artemisia sp* showed significant effect against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 and weak activity against *Schegalla* sp, whereas they have no effect on *klebsiella pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853.

Keywords: *Ruta montana* L., *Artemisia* sp, Antibacterial activity, Essential oil

Characterization of Walnut Seed Coat Bioactive Components by GC-MS

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ABSTRACT: Walnut (*Juglans regia* L.), which has a wide usage area in the world, is a very important tree species both ecologically and economically. Its importance is increasing due to its positive effect on human health through the bioactive components it contains. Walnut seed coat (WSC), which is another part of the walnut that is not known enough, unlike the parts of the walnut that are widely used in various sectors such as leaves, fruit and shell, is the main material of this study. Utilizing this material, which is burned or determined as waste during the process, will prevent both resource waste and ecological pollution. In addition, it will pioneer innovations in the chemical industry and biomedical fields. Considering this situation, in the study conducted, bioactive components in WSC were determined. First of all, WSC was extracted in hexane, which is an important solvent. Then, the components of H-WSC were detected using the gas chromatography mass spectrometry (GC-MS) method. These components; It was identified as β-sitosterol (32.91%), ethyl iso-allocholate (52.06%), 3-(octadecyloxy) propyl (9E)-9-octadecenoate (8.41%) and santa camphor (4.45%).

Keywords: Walnut seed coat, Gas chromatography-mass spectrometry, Phytosterol, Terpenoid, Steroid

Determination of the Nutritional Value of Chickpea Seed (*Cicer arietinum*)

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ABSTRACT: The use of plant-based proteins as food ingredients has grown. Among the various types of legume in the world, chickpea is the third most abundant crop, with a high protein content. The aim of the present work is to determine the different fractions of chickpea. Chickpea seeds are processed into fine flour. The flour is delipidated with hexane for 1 h to improve extraction of the protein fraction. The resulting product is air-dried for 48 h and then ground. For the extraction of water-soluble proteins, the treated flour is placed in distilled water and agitated for 20 h on an impeller at room temperature, then centrifuged at 18000 g at 4°C for 20 min. The pellet is subjected to 5 successive extractions for 2h. The optical density of each extract is measured λ=280 nm using a UV-Visible spectrophotometer, to verify extraction of the total water-soluble protein fraction. The total protein content is determined by kjeldhal method. The water-soluble protein content was calculated using the Bradford method. The results show that the total and water-soluble protein content of chickpea is 21.85% and 3.27% respectively, while the water-insoluble protein content is 18.58%. These results confirm the high protein content of chickpea seed.

Keywords: Chickpea, Protein, Kjeldhal method, Extraction

Optimising Shoot Proliferation in *Kaempferia parviflora* Wall. Ex Baker through Cytokinin-Auxin Synergies*

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ABSTRACT: *Kaempferia parviflora* Wall. Ex Baker (KP) is a valuable rhizomatous crop for its high methoxyflavone content. Conventional propagation of KP using rhizome is time consuming due to long crop cycle (9 months), thus plant micropropagation is utlised as a powerful tool for rapid clonal propagation. This study aimed to assess whether using combinations of cytokinins and auxins might yield better proliferation in KP compared to using standard 6-benzyladenine (BA) alone. Shoot tip explants were cultured on Murashige and Skoog (MS) media, fortified with BA (2 – 8 mg/L) and Indole-3-acetic acid (IAA) (0.2 – 0.8 mg/L). Explants cultured on 8 mg/L BA + 0.8 mg/L IAA, gave the highest mean shoot number (7.3 shoots/explant) and shoot length (5.9 cm/shoot) compared to BA (8 mg/L) (5.73 shoots/explant; 3.18 cm shoot length) and significantly increased the total number of plantlets obtained (527 plantlets) compared to BA alone (215 plantlets) per unit of explant after six sub-culture cycles. The regenerants were successfully acclimatised in the field with high survival (90 – 100%). Genetic fidelity assessment via Conserved DNA-derived polymorphism (CDDP) showed very low polymorphism (5.75%; 35 out of 609 total scorable bands) in the regenerants, whereas ploidy stability was confirmed *via* flow cytometry analysis. Based on the current study, BA-IAA combination was advantageous in enhancing KP plantlet production without compromising the genetic stability under a controlled sub-culture regime.

Keywords: BA, Genetic fidelity, IAA, CDDP, Polymorphism, Sub-culture

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Berberis vulgaris L. Tea and Optimization of Brewing Conditions

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ABSTRACT: Uncovering new and alternative antioxidant intake methods, especially from natural sources, is a current subject of nutrition for human health. Wild fruits are known as the leading natural antioxidant sources, and one is barberry. It is described as a deciduous shrub plant with yellow flowers and red fruits. The roots, stems, leaves and fruits of barberry contain ascorbic acid, vitamin K, triterpenoids, more than ten phenolic compounds and more than 30 alkaloid types. This fruit has anticancer, anti-inflammatory, antimicrobial, antioxidant, and antidiabetic properties. Fruits that are not consumed daily, such as barberry, can be consumed in different forms and their consumption should be widespread. In this study, instant barberry tea was produced using dried barberry fruits, and its properties were investigated depending on the brewing conditions. For this purpose, the brewing conditions of Berberis vulgaris L. tea were optimized. Within the scope of the study, an appropriate experimental design was created using the Box-Behnken method, using three different independent variables (temperature, time and sample amount). DPPH and total phenolic compound analysis were performed on the samples. Finally, barberry tea brewing conditions were optimized using the response surface method.

Keywords: *Berberis vulgaris*, Fruit tea, Barbery, Barbery tea

Changes in The Microbial Flora of The Digestive Tract of Rainbow Trout Fed with Mealworm Larvae

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ABSTRACT: Replacement of protein materials in aquatic feed is one of the ways to ensure sustainable growth in the aquaculture sector, which has opened a new world of insects in this field. In addition to increasing the amount of fish protein, this replacement also affects the amount and type of bacterial flora in the fish's digestive system, and these changes can increase its shelf life and health. Yellow mealworm (Tenebrio Molitor) (TM) larval meal is one of seven confirmed insect species used in aquaculture and a frequently investigated candidate for fish diets. In this research, a feeding experiment was carried out with 240 pieces of rainbow trout weighing 5±25 grams, which were fed for 60 days with a diet containing 15, 30 and 45% of meal worm larvae powder, and after sampling the microbial flora of this plant. This plant was tested in the digestive system of fish and the sequence of 16 sRNA genes was performed on the isolated bacteria. One of these bacteria, which was isolated and identified for the first time from the digestive system of fish fed with meal worm larva powder, was *Microbacterium maritypicum.* Recent research has shown that the consumption of Tenebrio Molitor larvae powder in the diet of rainbow trout has resulted in favorable fish performance. It has also increased beneficial bacteria and probiotics in the digestive tract of fish fed with meal worm larva powder, which has led to better fish health and increased survival rate.

Keyword: Rainbow trout, *Tenebrio molitor*, Gut micro flora

Relationship Study among Soils Physico-Chemical Properties and Mycoflora Densities for Vineyard Fields in The Centre of Tunisia

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ABSTRACT: The soil is the main reservoir of microorganisms. It is inhabited by a wide range of beneficial microorganisms such as bacteria and fungi. The aim of this investigation was to assess the relationship between physicochemical soils properties (soil pH (pH), electrical conductivity (EC), water content (WC), organic matter (OM), organic carbon (OC), total nitrogen (N) and carbon and nitrogen ratio (C/N), available phosphorus (P_2O_5), available total limestone (CT), available potassium (K_2O), nitrate (NO_3^-), sand (S), clay (C) and silt (S)) and spatial distribution of fungal and bacterial densities. Thirty-two fields were chosen for this study for growing seasons 2021/2022, located in Regueb (Sidi Bouzid, Tunisia). Ten antagonist species [five fungi species (*Trichoderma harzianum*, *T. viride*, *Cladosporium* sp., *Metarhizium* sp., *Beauveria* sp.) and five bacteria isolates (J.Rh1, J.Rh4, J.R,h7, J.Rh12 and J.Rh14)] were tested for their mycelial growth inhibition via dual confrontation method against *Plasmopara viticola*; the causal agent of downy mildew of grapevine. All tested antagonists for *in vitro* confrontation were used for biochemical characterization (catalase (Cat), pectinase production (PP), proteolytic activity (PA), amylolytic activity (AA), β-1,3-glucanase activity (GA), hydrocyanic acid (HCN), atmospheric nitrogen fixation (ANF) and phosphate solubilization (PS)). The results revealed that four factors exhibited a significant positive correlation with fungal density; OM, OC, WC and N. However, the bacterial population increased with some physic-chemical properties abundance such as OM, OC, WC, C/N and C. In *in vitro* assay, J.Rh1 seemed to be the most effective bioagent against *P. viticola* with mycelial inhibition rate above 90%. The mycelial growth of phytopathogen decreased in the presence of *T. harzianum*, *T. viride,* and J.Rh4 (>61%). The results revealed that tested species and isolates presented a biochemical characterization.

Keywords: Mycoflora, Soil proprieties, *Vitis vinifera*, *Plasmopara viticola*, Plant-growth-promoting

Caractirezation of Biscuits with Banana Peels Flour

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ABSTRACT: Banana are a standout amongst the most tropical organic products of the planet. Banana peel accounts about 40% of overall weight of the fresh fruit, Banana peels have different medical advantages to amazing dietary status, and it treats the intestinal sore, looseness of the bowels, diarrhea, ulcerative colitis, nephritis, gout, heart infection, hypertension and diabetes. In this work we used Algerien banana peels powder in biscuit making with a 10 ,15,50% of banana peels quantity. The results showed that 71.5% of tasters found that PA biscuit with 10% was extremely pleasant. The biscuit obtained have optimal characteristics with a beautiful appearance including a good appearance, regular shape and a smooth crust. The interior is also pleasant, with a very supple crease, little elastic, non-sticky and easy tearing. The physico-chemical parameters of the peel were evaluated in order to determine the water content, the acidity and the ash content whose results are (3,22, ± 2,64) ;(0,209±0,074) and (12±0,04) respectively, this last result explains the richness of the peel in mineral elements such as sodium and potassium. These good characteristics of the biscuits are the result of a good balance between elasticity and extensibility. The banana peels biscuit have kept a good quality (organoleptic and microbiological) after 7 days of storage at 6° C in plastic packaging.

Keywords*:* Banana peels, Biscuit, Bio-Product, Biovalorisation, Nutrition

Antioxidant and α-Amylase Inhibitory Activities of Wild and Nabali Muhassan Olive Leaves Extracts from Jordan

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ABSTRACT: The present study was designed to evaluate the antioxidant and α-amylase inhibitory activities of aqueous, ethanol and ethyl acetate extracts from Nabali and wild olive leaves grown in Jordan. The extracts were procured through ultrasonic-assisted extraction. Three experimental models were employed for the antioxidant activity evaluation of each extract (DPPH radical scavenging activity, chelating power and reducing power activities). The enzymatic inhibitory activity of α-amylase was evaluated by CNP-G3 assay. Moreover, total phenolics, flavonoids and flavonols contents of the olive leaves extracts were quantified. The ethanolic wild leaves extract showed the highest total phenolics content (113.97 mg GAE/g), followed by the ethyl acetate extract of Nabali leaves (102.2 mg GAE/g). Flavonoid and flavonol contents were significantly ($P \leq 0.05$) the highest in ethyl acetate extract of wild leaves (123.07 mg RE/g and 91.3 mg RE/g, respectively). The ethanolic wild leaves extract and ethyl acetate extract of Nabali leaves showed the highest DPPH scavenging activity with IC_{50} value of 192.1 µg/ml. The total antioxidant activity was found to be the highest in ethanolic wild leaves and ethyl acetate of Nabali leaves extracts (202.1 and 202.3 µg ascorbic acid equivalent for 1mg extract, respectively). The ethanolic, ethyl acetate wild leaves extracts and ethyl acetate of Nabali extract at concentration of 100 µg/ml showed the highest chelating activity of ferrous ions (52.4, 50.5 and 47.2 %, respectively). The ethanolic extracts of wild and Nabali leaves showed the highest inhibitory activity against α-amylase from the porcine pancreas by 65.1% and 62.3%, respectively at concentrations of 10 mg/ml. All extracts showed remarkable antioxidant activities determined with different methods in a dose dependent manner and the effects depend strongly on the solvent used for the extraction.

Keywords: *Olea europaea*, Antioxidant activity, DPPH, CNP-G3, Phenolics

Efficacy of meta Topolin in Inducing Direct Shoot Regeneration in *Curcuma zedoaria* (White Turmeric)

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ABSTRACT: *Curcuma zedoaria,* also known as white turmeric, is a medicinal herb belonging to the Zingiberaceae family. The plant is an integral part of traditional medicine and is also used as condiment and dye. Cultivation through conventional rhizome propagation is hindered by slow germination, long dormancy period and susceptibility to diseases. The aim of this study was to establish an enhanced protocol for micropropagation of *C. zedoaria*. Current protocol for multiple shoot production uses 6-Benzyladenine (BA) but reports in other crops have utilized meta Topolin with higher efficiency. Hence, MS media supplemented with varying concentration of BA (as control) and meta Topolin were tested. The highest number of shoots were observed in MS media supplemented with 1 mg/l meta Topolin and yielded an average of 620 shoots from a single explant at the end of 24 weeks. However, BA only produced an average of 64 shoots. Furthermore, meta Topolin also facilitated the formation of roots, thereby a separate media for in vitro root formation was not required. The genetic stability of the plants was confirmed using ISSR markers. Therefore, the protocol described in this study can be used to produce genetically uniform *C. zedoaria* plants at a large scale.

Keywords: *Curcuma zedoaria*, Zingiberaceae, BA, Meta topolin, Micropropagation, Genetic stability

In Vitro Characterization of Probiotic Properties of *Lactococccus lactis* NL4 Strain Isolated from Yoghurt*

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ABSTRACT: The aim of this study was to characterize some functional and probiotic properties of *Lactococcus lactis* NL4 strain isolated from yoghurt. For this purpose, some important probiotic properties of NL4 strain were analysed in vitro. The strain showed a good tolerance to low pH, high bile salt, pancteatin, lysozyme and phenol. In the presence of pepsin at pH 2.0, *L. lactis* strain showed 66% survival at 0. hour and 4% survival at 1. hour, but did not show viability at 3. hour. The NL4 strain showed an autoaggregation of 39% and a coaggregation of 16% with *E. coli* at the end of 5 h incubation. Apart from these, the cell-free supernatant of NL4 strain formed a good zone diameter against *Bacillus subtilis* RK-483, a poor zone diameter against *Staphylococcus aureus* RK-484, but no zone against other pathogens in the study. The strain was resistant to gentamicin and rifampin and sensitive to vancomycin and 10 other antibiotics. It was also determined that NL4 strain was able to produce phytase enzyme. When all the results of the study were evaluated, it was concluded that the strain has probiotic properties and can be evaluated commercially. In addition, further in vivo studies should be carried out to fully understand its mechanism of action and determine its safety and probiotic effects.

Keywords: Lactic acid bacteria, *Lactococcus lactis*, Probiotic properties, Phytase

Food Fermentation: Local Food Product with High Nutritional Value, Fermented in Underground Silos Matmor (Fraguigue, Algeria) Intended for Human Consumption

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ABSTRACT: Wheat grain is a staple food that contains numerous compounds beneficial to nutrition and health. Fermentation is of importance to improving nutritional attributes of cereal grains for human consumption. This study aims at characterizing fermented wheat “El Hammoum” with biochemical, physicochemical, mineral, and phytochemical. The algerienne fermented prodect of “El Hammoum” contains considerable amount of proteins, is a good source of lipids, fibre, polyphenols and decreases total in gliadin, gluten protein. Moreover, mineral analysis demonstrated a higher content of k and Zn in fermented wheat “El Hammoum”, and interesting amounts of Fe. The fermented wheat “El Hammoum” was considered a food with medicinal properties in the prevention and treatment of many intestinal pathological and physiological complications. In addition, these fermented wheat fit into a gluten-free cereal group unlike wheat unfermented. The significant variations found between fermented wheat and unfermented wheat for the different components studied asserts the influence of the fermentation by improving the value nutritional of wheat. In conclusion value of the fermented wheat “El Hammoum” seeds are retained, and the amounts of some active substances increase significantly.

Keywords: Matmora, Fermented wheat, Unfermented wheat, Biochimie properties

Identification of Bioactive Compounds in Leaf Extracts of *Olea Europaea* Subsp. *Laperrinei* Originated from the Algerian Desert

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ABSTRACT: *Olea europaea* L. notably, the olive leaf currently has aroused the interest of researchers in ethnopharmacology due to their beneficial effects on human health, including antimicrobial properties and antioxidant potential due to their phenolic content, the laperrine's olive is a member of the olive complex and is endemic from the Saharan mountain. The purpose of this work was to evaluate the chemical composition, total phenolic, and flavonoids contents of both aqueous and methanolic extracts of *Olea europaea* subsp. *laperrinei* areal parts, in order to evaluate their environmental stress effect. Chromatography liquid with High-performance (HPLC) was used to identify and quantify the constituents of sub-specie, the total phenolic and flavonoids content were determined using Folin-Ciocalteu and spectrophotometric method respectively. HPLC analysis showed that Oleuropein is the main compound in all extracts, Furthermore, All the extracts obtained showed high total phenolic and flavonoid contents and the highest values were obtained using methanol as solvents than water.

Keywords: *Olea europaea* L. subsp. *Laperrinei,* Olive leaf, Chemical composition, Total phenolic, Flavonoids contents

The Effect of Rosemary Extract in the Diet of Roosters on Sperm Quality

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ABSTRACT: Rosemary plant (*Officinalis rosmarinus*) belongs to the mint family (Lamiacea), rosemary is a plant with antibacterial and anti-inflammatory properties, containing useful compounds such as essential oil, flavonoids and organic acids. The antioxidant property of this plant is well known. It contains important antioxidant compounds such as rosmarinic, carnosol and carnosic acid. It contains about 1 to 2.5% essential oil, borneol, limonene, camphor, 1 and 8 cineole, camphor and alpha pinene are the main components of rosemary essential oil. Phenolic acids such as carnosic acid, rosmarinic acid, caffeic acid and chlorogenic acid are among the other compounds found in this plant, the antioxidants in semen include two enzymatic and non-enzymatic groups so that they neutralize the activity of free radicals and It protects the sperm from it. Protects against the harmful effects of reactive oxygen species (ROS). Lipids are one of the important components of the sperm membrane and play an important role in sperm fertilization. In fact, the phospholipids of bird sperm membranes contain a large amount of unsaturated fatty acids with several double bonds, which make sperms very sensitive to lipid peroxidation. Lipid peroxidation is positively correlated with male infertility, which can ultimately make the animal infertile. The results of various studies show that the use of antioxidant properties of rosemary extract in the diet reduces oxidative stress indicators and improves sperm concentration, viability and motility. In general, natural antioxidants such as rosemary can have positive effects on the quality of bird sperm.

Keywords: Rosemary, Rooter, Reproduction, Antioxidant

The Influence of Agricultural Supports on the Capital Structure of Enterprises Engaged in Forage Crop Cultivation

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ABSTRACT: Capital is one of the most critical factors in agricultural production, representing the entirety of funds and assets required for the establishment and continuous operation of enterprises. Insufficiency of capital in enterprises can adversely affect their success. Therefore, each necessary form of capital should be present in specific proportions. Additionally, while enterprises may vary based on type and production methods, it is generally desirable for efficiently operating enterprises to have half of their active capital as farm capital, and the other half as operational capital. Hence, it is imperative to analyze the capital structure of agricultural enterprises. In this study, the capital structure of enterprises producing forage crops in Erzurum province was examined, and the impact of agricultural subsidies on this evolution and development was attempted to be determined. Data from 306 enterprises engaged in forage crop production in Erzurum province were evaluated. Enterprises receiving and not receiving agricultural subsidies were compared in terms of their capital structures. It was determined that enterprises receiving agricultural subsidies were closer to achieving the desired capital distribution. Furthermore, it was observed that supported enterprises had higher levels of equity, and the subsidies augmented the working capital.

Keywords: Agriculture supports, Forage crop cultivation, Capital

Biosynthesis of Silver Nanoparticles from *Madhuca longifolia* and its Influence on *In vitro* Regeneration of *Evolvulus alsinoides* L.

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ABSTRACT: This study was performed to develop an improved direct regeneration protocol from nodal explants of *E. alsinoides* using biosynthesized silver nanoparticles. Silver nanoparticles were synthesized using leaf extract of *Madhuca longifolia.* The optimized condition for the biosynthesis with high yield of silver nanoparticles (AgNPs) was obtained by using a reaction mixture with the volume ratio of 1:9 (5% plant extract: 1 mM $AgNO_3$), pH 7, at 35ºC with 24 hours of incubation. Presence of MLAgNPs was confirmed by the apperanceof UV-absorption spectra peak at 420 nm, functional groups (Alcohol, Alkane, Alkene, Amine) involved in bioreduction of silver ions were confirmed with FTIR spectra at 3416, 2920, 1629, 1046. Spherical shaped nanoparticle (10 to 20 nm) was determined *via* SEM analysis. Inclusion of MLAgNPs at 20 mg/l into MS medium containing BA (1.0 mg/l) resulted in maximum shoot induction after three weeks of culture with 9 shoots/explant, whereas in control it was only 5 shoots/explant. Rooting was successfully achieved in MS medium containing IAA (1.0 mg/l) with 20 mg/l of MLAgNPs with 8 roots per shoot and an average root length of 1.8 cm, whereas in control it was 4 roots per shoot. The results presented herein have highlighted the use of MLAgNPs for enhancement of in vitro multiplication of *E. alsinoides*.

Keywords: Biosynthesis, Silver nanoparticles, UV – Vis Spectroscopy, FTIR, SEM, Regeneration

Optimization of *In vitro* Germination of Tagnanan Tall Coconut (*Cocos nucifera* L.) Pollen for Higher Viability and Vigor*

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ABSTRACT: Assessing pollen quality prior to controlled pollination is a crucial step in coconut hybrid production. Estimation of pollen viability and vigor is essential in optimizing fruit set and maximizing yield. To date, *in vitro* germination prevails as the method to determine pollen viability. Although various media existed in the literature for pollen viability testing, the currently used media in Malaysia which comprises of sucrose, agar and distilled water, is unable to realize the pollen potential of Tagnanan Tall (TAGT) coconut variety as germination is low (<10%). In addition, a clear pollen tube is often not visible making estimation of pollen germination subjective. This study incorporated 0 – 0.2% boric acid into the germination media and optimized its concentration. Notably, the inclusion of boric acid revealed that a 2-hour incubation period was sufficient for optimal pollen germination and subsequent pollen tube growth compared to the currently utilized duration (4 hours), facilitating efficient pollen quality assessment. This study proposes an improved method for pollen germination by using a media which consists of 10% sucrose, 0.3% agar, 0.005 – 0.01% boric acid for 2 hours, resulting in a more reliable pollen potential assessment and allowed the estimation of germination percentage to increase from 6.5% to 34% with significantly higher vigor.

Keywords: Coconut, Tagnanan tall, Pollen germination, Boric acid, Incubation temperature, Incubation duration

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Experimental and Numerical Study of Conductive Drying Kinetics of Carob Leaves

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ABSTRACT: The drying of carob leaves is an important operation that increases the shelf-life and reduces the bulk and the weight of the product. This study was carried in a conductive drying in order to study and understand the drying kinetic and obtain the characteristic drying curve in different temperatures (35, 45, 55 °C). It was concluded that an increase in the drying temperature reduces the drying time. A mathematical model was used to interpret the experimental data; it was found that model was properly able to describe the drying curves. The diffusivity coefficient increases with drying and it varies from 9.15×10^{-12} and 3.28×10^{-11}. Arrhenius equation, permitted the calculation of the activation energy that was found to be 73.36 kJ/mol.

Keywords: Activation energy, Conductive drying, Diffusivity coefficient, Mathematical modeling, Mint leaves

POSTER PRESENTATIONS

Comparative Study of the Effect of Extracts of Essential Oils from two Aromatic Plants on Adults of *Thrips tabaci* (Thysanoptera: Thripidae)

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ABSTRACT: This present work aims to evaluate the insecticidal activity by contact-inhalation of essentials oils extracted from the *Laurus nobilis* and *Salvia rosmarinus* plants against the Onion *Thrips tabaci* (Thysanoptera: Thripidae) by determining the LD50. These plants were selected for there importance of use in traditional medicine. We used the hydrodistillation extraction method, four concenctrations of oil were tested in-vitro; 1, 2,4 and 8 ųl/ml, after dilution of distilled water. With four replicates for each dose, the count of thrips mortalities is carried out 6 hours, 24 hours, 48 hours and 72 hours after treatment. The results obtained show that the essential oil of *laurus nobilis* is effective which causes 96% mortality of thrips adults and 34.24% mortality with *Salvia rosmarinus* and a LD50 of 1.45ųl/ml, 9.73 ųl/ml for *Laurus nobilis* and *Salvia rosmarinus* respectively. As a result, the essentiel oil of *Laurus nobilis* it has great efficiency and the analysis of variance gave a highly significant result. Biopesticides of plant origin can offer an alternative to the "all chemical" that has prevailed in recent decades.

Keywords: Insecticidal, *Thrips tabaci*, *Laurus nobilis*, Contact-Inhalation, Extracted

Alternative Methods of Thrips Control: Effectiveness of Extracts of a Plant *Origanum vulgare* and their Insecticidal Activity

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ABSTRACT: This present work aims to evaluate the insecticidal activity by contact-inhalation of essential oil extracted from the *Origanum vulgare* plant against the Onion *Thrips tabaci* (Thysanoptera: Thripidae) by determining the LD50. This plant was selected for its importance of use in traditional medicine. We used the hydrodistillation extraction method, four concenctrations of oil were tested in-vitro; 1, 2,4 and 8 ųl/ml, after dilution of distilled water. With four replicates for each dose, the count of thrips mortalities is carried out 6 hours, 24 hours, 48 hours and 72 hours after treatment. The results obtained show that this essential oil is effective which causes 63.33% mortality of thrips adults with a LD50 of 5.49 ųl/ml, and the analysis of variance gave a highly significant result. Biopesticides of plant origin can offer an alternative to the "all chemical" that has prevailed in recent decades.

Keywords: Insecticidal, *Thrips tabaci*, *Origanum vulgare*, Contact-Inhalation, Extracted

Evaluation of The Beneficial Effects of *Prunus persica* L. Leaf Extract on Plasma Vitamin C Levels in Rats with Metabolic Syndrome

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ABSTRACT: The historical use of medicinal plants across cultures is well-documented, with some, like Prunus persica L. (peach tree), serving as rich sources of vitamin C. Incorporating these plants into diets or traditional medicine practices helps maintain optimal vitamin C levels. Considering the association between metabolic syndrome and cardiovascular risks, measuring plasma vitamin C becomes crucial. This assessment aids in determining the effectiveness of medicinal plants or herbal supplements in restoring vitamin C balance and managing metabolic syndrome. Monitoring vitamin C levels allows healthcare professionals to gauge the efficacy of these natural remedies, contributing to overall health. The investigation assessed the impact of Prunus persica L. leaf extract on Plasma Vitamin C Levels in these groups over a 6-week period, utilizing the method of Roe and Kuether (1943) with Dinitrophenylhydrazine-Thiourea-Copper. The results indicated a noteworthy increase in Vitamin C levels in the Extract-Treated Group (Group 4), along with a slight decrease in Vitamin C levels observed in both the Diabetic Group (Group 2) and the Metformin-Treated Group (Group 3) compared to their respective control groups. These findings suggest an enhanced antioxidant status in rats treated with the extract, underscoring its potential as an antioxidant and its role in preventing oxidative stress.

Keywords: Medicinal plants, *Prunus persica* L., Vitamin C, Metabolic syndrome

Measurement of Nature's Eggs vs Breeding of the Barbary Partridge (*Alectoris barbara*, bonnaterre, 1792) in the North Algeria

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ABSTRACT: This article presents a summary of the indirect evolution of the biometric parameters of Barbary Partridge eggs: weight, length and width. This analysis is carried out on the basis of measurements carried out by our predecessors and successors, at the level of the Protected Area of the Zéralda-Algeria Hunting Reserve and in Ouarsanis. Data from 2002 to 2016 include measurements taken on a Total sample, ΣNik = 434 eggs measured in situ. These measurements are compared to a sample n = 120 eggs randomly drawn from the Zéralda Hunting Center. The biometric characteristics seem to stabilize over ten years of selection in captivity with a slight difference in breeding, while those controlled in the wild remain stable. We conclude the existence of low variability and the eggs generally seem slightly heavier in breeding compared to natural population.

Keywords: *Barbary partridge*, Egg, Biometric traits, Captive breeding, Hunting

Spatial Distribution of The Population of The Barbary Partridge (*Alectoris barbara*) in The Northwest Region of Algeria

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ABSTRACT: *Alectoris* species are the most popular and most hunted wild game in the world. With us the Barbary partridge (*Alectoris Barbara*) is no exception. The first signal marked in recent years is declining in their density which judges the importance of maintaining and restoring this population in their habitats. Our research on the Barbary partridge on the ground located on the semi-arid bioclimatic level of the region of Chlef. After regular follow-up, we obtained a density of 14.21couples / 100ha, the average egg-laying size and 11.77 eggs / nest. During our research, we have demonstrated that we need natural population's management. This can only be achieved by carrying out a sampling plan.

Keywords: Barbary partridge, Game, Population, Chlef, Density

Potential Antiartritic Effect of Thymoquinone against Collagen II-İnduced Arthritis

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ABSTRACT: Thymoquinone (TQ) is the principal compound present in the volatile oil derived from Nigella sativa seeds, known for its diverse pharmacological properties. This study aims to assess the antiarthritic effects of TQ in male rats with Collagen II-induced arthritis. In this research, arthritis was induced in male rats through collagen II immunization. These rats received oral TQ treatment for a span of 40 days. Paw thickness measurements were taken every 4 days using a digital caliper, and a histopathological examination of the joints was conducted. The results revealed that arthritis onset occurred on the 14th day, characterized by a significant increase in paw thickness and severe joint damage in arthritic rats, which suffered severe destruction of cartilage and bone. The synovial membrane is inflamed leading to the formation of pannus with inflammatory cells infiltration. However, TQ treatment resulted in a substantial improvement in joint histology and a delay in the onset of the disease. These findings suggest that thymoquinone holds promise as a potential remedy for arthritis, as it is associated with reduced paw swelling, enhanced joint histology, and a postponed disease onset.

Keywords: Antiarthritic, Arthritis, Collagen, Swelling, Thymoquinone

Exploring the Antioxidant Effects Associated with Hepatocurative Potential of Thymoquinone

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ABSTRACT: The primary objective of this study is to evaluate the in vivo antioxidant effect of thymoquinone (TQ) in conjunction with its curative potential against hepatotoxicity induced by carbon tetrachloride (CCl_4). Hepatotoxicity was induced in male rats through the intraperitoneal administration of a 1:1 (V/V) mixture of CCl_4 and olive oil at a dose of 3 ml/kg. The curative treatment involved the application of TQ for a period of 7 days, utilizing two different doses. The antioxidant effect of TQ hepatocurative treatment was assessed by measuring the activities of superoxide dismutase (SOD) and catalase (CAT), along with the levels of reduced glutathione (GSH) in both plasma and liver homogenate samples. The findings of this study revealed that hepatotoxicity induced by CCl4 resulted in a significant decrease ($p \leq 0.01$) in the activities of SOD and CAT, as well as a reduction in GSH levels, observed in both plasma and liver homogenate samples. However, the curative treatment involving TQ, administered at doses of 2.5 mg/kg and 5 mg/kg, led to a dose-dependent increase in the antioxidant status in both plasma and liver homogenate. In conclusion, these results demonstrate that thymoquinone possesses antioxidant properties when employed as a curative treatment for CCl_4-induced hepatotoxicity.

Keywords: Antioxidant, Ccl_4, Curative, Hepatotoxicity, Thymoquinone

Antioxidant Activity of Cinnamon Powder

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ABSTRACT: Cinnamon is the bark extracted from the small tree called cinnamon, mainly planted in countries in South Asia or the Middle East such as India or China. With its very particular smell and taste which adds deliciousness to our recipes, we almost forget that it is above all an excellent health ally. According to a study in the American Journal of Clinical Nutrition published in 2006, cinnamon is ranked among the 50 most antioxidant and anti-inflammatory foods, calms heartburn, cinnamon has antibacterial, anti-virus, anti parasitic and antiseptic. All these characteristics reduce infections of the intestinal flora. Its virtues help our body fight against digestion problems and reduce nausea. The aim of our work is to evaluate the effect of cinnamon powder on antioxidant activity. The antioxidant activity of cinnamon powder was carried out according to the DPPH method or according to the protocol of (Krings and Berger, 2001). Our results of the analyzes carried out on the cinnamon powder used are similar to those found in the bibliographic reviews. These results show that the average antioxidant activity of grenadine powder is (284.44 ± 0.39 µg/ml). We will conclude that the results found prove the quality of our cinnamon powder and will allow us to continue our scientific and advanced research to develop other, more effective techniques.

Keywords: Cinnamon powder, Antioxidant activity, DDPH, Diet

Evaluation of The Antioxidant Activity of Phenolic Extracts of *Corchorus olitorius* (Moloukhya)

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ABSTRACT: Antioxidant compounds are the subject of a great deal of research because, in addition to their use as preservatives in foods tuffs replacing synthetic antioxidants, they are involved in the treatment of numerous pathologies. In the context of the discovery of new antioxidants from natural sources, we have focused in this work on the study of phenolic compounds and the evaluation of the antioxidant properties of extracts from the medicinal plant *Corchorus olitorius* (Moloukhya). The first part of the study concerns the extraction and quantification of total phenols by the Folin-ciocalteu reagent and of flavonoids by aluminum trichloride. The second part concerns the study of the antioxidant activity of phenolic extracts using four techniques: (a) DPPH- radical scavenging, (b) iron reduction, (c) inhibition of β-carotene oxidation and (d) quantification of total antioxidant capacity. The results show a high polyphenol content of 28.9090 ± 0.7966 (mg PE/ g DW) and a flavonoid content expressed as catechin equivalent of 0.4280 ± 0.0004m (mg CE / g DW). Antioxidant activity methods show that the plant extract studied has significant antioxidant properties. The phenolic extract has a high DPPH- radical scavenging capacity (IC50 = 0.4298 ± 0.0051 mg/ml) comparable to that of ascorbic acid 0.3405 ± 0.0018 mg/ml, β-carotene bleaching inhibitory power 96.004 %, an iron reducing capacity of 2.155 and a total antioxidant capacity of 0.34205 ± 0.03425 mg EAA/ g. All four tests were positively correlated with total polyphenol content (R2 = 0.884 - 0.997). In conclusion, the study revealed that Corchorus olitorius, a plant widely used in traditional medicine, does indeed have antioxidant power.

Keywords: Polyphenols, Flavonoids, Antioxidant activities, *Corchorus olitorius*

The Role of Bone Marrow-Derived Mesenchymal Stem Cells as a Therapeutic Measure in Autoimmune Hepatitis İnduced by ConA

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ABSTRACT: An autoimmune disease that is becoming more common worldwide is autoimmune hepatitis. The therapy choices for AIH are still limited, and the common medications' unfavorable side effects commonly result in patients with low quality of life. A well-known method widely used for inducing autoimmune hepatitis (AIH) in mice that replicates the pathogenic changes that take place in humans is concanavalin A (ConA). Pluripotent stem cells with a low immunogenicity mesenchymal stem cells (MSCs) are easily collected. MSCs-based therapy is emerging as a viable strategy for treating liver illnesses based on its benefits. As a form of mesenchymal stem cell, bone marrow-derived stem cells (BM-MSCs) have demonstrated tremendous promise in the treatment of numerous disorders. The liver function and level of inflammation were then assessed by measuring the serum levels of ALT and AST the pathologic modification of liver tissue. Serum levels of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and H&E staining showed that the use of BM-MSCs might reduce the severity of hepatitis caused by ConA. While BM-MSCs could reduce the percentage of ConA-induced macrophages in the liver tissue, the proportions of CD68 cell macrophages were elevated by ConA injection. Although injection of 2×106MSCs efficiently reduced ConA-induced hepatitis after 12 days, this had no significant effect after 18 days. Therefore, maybe twice as many BM-MSCs or second doses were required to alleviate ConA-induced hepatitis. Our results revealed that even if there is a considerable short-term improvement, a single injection might not achieve a steady long-term therapeutic benefit.

Antibacterial Effect of *Opuntia ficus İndica* Cladode Extract

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ABSTRACT: In this study, conducted on the extraction of total phenolic compounds from the *Opuntia ficus indica* (OFI) cladodes with (CC) and without (CWC) cuticle from Bir-Essalam region (Béjaia), we focused on the effectiveness of two extraction methods, namely conventional extraction (CE) and ultrasound assisted extraction (UAE), in terms of total phenolic compound (TPC) content, total flavonoids (TF), antioxidant and antibacterial activities. Quantitative assay revealed the richness of *Opuntia* cladodes in TPC and TF. The extraction yields of TPC, TF and antioxidant activities are better for CC than those of CWC. The extraction results show that the highest yields of TPC (14.14 ± 0.35 mg EAG/g DM) and antioxidant activity (inhibition rate of 55.05 ± 6.13 %) are obtained by the UAE extract for the cladode with cuticle, whereas the yield in TF is better with the EC for the same cladode. The UAE extract of cladode with cuticle showed improvements in the zones of inhibition of the tested bacteria compared to the EC extract, this is in adequation with the infrared analysis result where the CC extract showed a larger spectrum than that of CWC. The *in vitro* antimicrobial potential of hydro-ethanolic extracts by the disc method and the microplates method on the 12 tested (Gram + and -) bacterial strains show the potential of crude extracts of OFI cladodes as antibacterial agents.

Keywords: *Opuntia ficus indica*, Conventional extraction, Ultrasound-assisted extraction, Antioxidant activity, Antibacterial activity

The Effect of Thyme Extract on Cryopreservation and Quality of Ram Sperm

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ABSTRACT: Thyme, a plant with potent antioxidant properties against free radicals, exhibits stronger antioxidant effects compared to some other antioxidants such as vitamin E, fruits, and vegetables. This plant contains compounds like thymol, gamma-terpinene, carvacrol, and also citole, which, by inducing the hydroxylase enzyme, increases testosterone levels and enhances sperm concentration. Antioxidants are substances that help maintain the health and proper function of cells by reducing the activity of free radicals. During sperm production, sperm loses significant amounts of its cytoplasm, and since most enzymes are in the cell cytoplasm, sperm has limited antioxidant capacity. On the other hand, the concentration of antioxidant factors in seminal fluid decreases during sperm processing and dilution. By adding extracts containing antioxidants, efforts are made to introduce the necessary antioxidant compounds into sperm. Various antioxidants have been used to reduce oxidative stress effects and, consequently, improve sperm quality. The results of various studies indicate that the use of the antioxidant properties of thyme extract, with its own antioxidant effects, reduces oxidative stress indices and improves sperm concentration, viability, and motility. In general, natural antioxidants like thyme can have positive effects on the short-term preservation of ram sperm.

Keywords: Thyme, Sperm, Ram, Antioxidant, Cryopreservation

Impact of Psychological Stress and Diet on the Reduction of Antioxidant Capacity and Development of Inflammatory Bowel Diseases (IBD): An Analytical Case-Control Approach

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ABSTRACT: Inflammatory Bowel Diseases (IBD) is chronic inflammatory conditions affecting the digestive tract's wall. They primarily encompass three entities: Crohn's Disease (CD), Ulcerative Colitis (UC), and Indeterminate Colitis (IC). Among the environmental factors involved in the genesis of IBD, stress and diet are the most significant. The main objective of this study is to understand the impact of stress on the development of IBD. We conducted a comparative case-control analytical study involving 30 IBD patients and 30 controls recruited from Tlemcen Hospital (Algeria) over a three-month period. The obtained results revealed that the average age of the cases was 51.40, while the average age of the controls was 51.33, with a clear male predominance. The mean ORAC in cases was 0.8350 and 1.7960 in controls; this difference was highly significant with a *p-value* < 0.0001. We conclude that our initial hypothesis was well-confirmed, clearly demonstrating that psychological stress and dietary factors have an influence on the onset and progression of IBD. This influence can be explained by a decrease in antioxidant capacity during inflammation, contributing to intestinal inflammation and the genesis and/or maintenance of tissue lesions in both UC and CD.

Keywords: Inflammatory Bowel Diseases (IBD), Environmental factors, (ORAC), Diet

FULL TEXT

ORAL PRESENTATIONS

Texture and Color Properties of Argan Oil Oleogels Prepared with Carnauba or Candelilla Waxes and Their Blends

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ABSTRACT: Oleogelation is a promising method for using oils in a solid form as a replacement for solid fats in food products. Since the oils and oleogelators used in the oleogelation are the main factors that affect the properties of the oleogels, determining the properties of the oleogels prepared with different oils and oleogelators is very important. In this study, the texture and color properties of argan oil oleogels prepared with carnauba and candelilla waxes and their blends were determined. The oleogelator used in the oleogelation had a significant effect on textural properties (P<0.01). The use of carnauba wax in oleogelation caused an increase in hardness, adhesiveness, springiness, and gumminess. The highest hardness and springiness were determined in the oleogel prepared with an oleogelator containing 25% candelilla and 75% carnauba waxes. The color properties of the oleogels (L*, a*, b*) were also significantly affected by the oleogelator used (P<0.01). While the highest L* and b* values were determined in the oleogel prepared with candelilla wax, the lowest values were detected in the oleogel containing carnauba wax. These results reveal the effects of the oleogelator blends on the texture and color properties of argan oil oleogels prepared with carnauba and/or candelilla waxes.

Keywords: *Oleogel*, Carnauba, Candelilla, Argan oil, Texture, Color

INTRODUCTION

Oleogelation is the process of converting liquid oils into a solid oil-like structure with the help of oleogelators. Oleogels are complex microstructured systems in which liquid oils are held in a three-dimensional gel network by oleogelators. In the oleogels, the properties of oils are preserved, and trans-fat formation does not occur. The oils and oleogelators used in oleogelation are the main factors that determine the properties of the formed oleogel. Depending on the use of oleogelators, oleogels can be prepared with one oleogelator (monocomponent) or with two or more oleogelators (multicomponent). While monocomponent oleogels may be insufficient to mimic the properties of solid fats in some cases, it has been reported that multi-component oleogels can show better performance due to the synergistic combinations between oleogelators (Okuro et al., 2020). Multicomponent oleogels can be categorized into three groups depending on the role of each component acting as an oleogelator. The first group contains two or more oleogelators, which can form gels independently or together. The second group is oleogels prepared with oleogelators, which can not form gels alone but can form gels with a synergistic effect when used together. The last group contains an oleogelator and a non-gelling component (Li et al., 2022).

Argan oil is a vegetable oil obtained from the kernel of the fruits of the argan tree (*Argania spinosa*). Argan oil, characterized by the high content of oleic (46-48%) and linoleic (31-35%) acids, is rich in polyphenols and tocopherols that show significant antioxidant activity (Khallouki et al., 2003; Charrouf and Guillaume, 2008; Cabrera-Vique et al., 2012; Guillaume and Charrouf, 2013; Guillaume et al., 2019). It has been suggested that argan oil may be beneficial in preventing cardiovascular diseases and cancer due to its high content of specific antioxidants and mono- and polyunsaturated fatty acids (Cherki et al., 2006; Cabrera-Vique et al., 2012; El Abbasi et al., 2014; Guillaume et al., 2019). Drissi et al. (2004) found that regular argan oil consumption reduced LDL cholesterol, thus they reported that argan oil could be considered as a natural supplement to reduce cardiovascular risk. In the literature, there are limited studies on argan oil, and these generally focus on its production method, its effect on health, and its applications in cosmetics. However, no studies have been found on the use of argan oil in oleogel formation. In this study, the texture and color properties of argan oil oleogels prepared with carnauba and candelilla waxes and their mixtures were determined.

MATERIAL AND METOD

Edible argan oil used in the research was obtained from Jibal Azyar firm in Agadir, Morocco, and carnauba and candelilla waxes were obtained from a company that sells food additives. Argan oleogels were prepared to contain 10% oleogelator (Figure 1). The combinations of oleogelators used in the oleogelation are given in Table 1. Candelilla and/or carnauba waxes and argan oil were weighed and mixed, kept in a water bath at 90°C until wholly melted, then taken from the water bath and vortexed. After the samples cooled to room temperature, stored in the refrigerator at 4°C for 24 hours (Choi et al., 2020). The texture and color properties of argan oleogels prepared with carnauba and/or candelilla waxes were determined. All analyses were performed at room temperature.

Table 1. The combinations of oleogelators used in the oleogelation

Treatment	Candelilla wax (%)	Carnauba wax (%)
T1	100	0
T2	75	25
T3	50	50
T4	25	75
T5	0	100

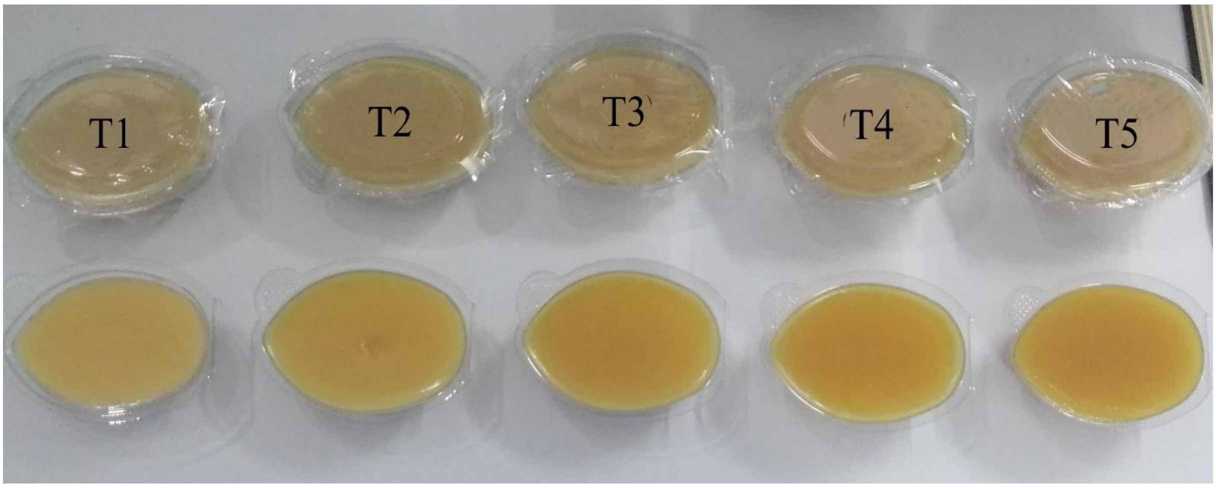

Figure 1. Oleogels obtained from argan oil with carnauba and/or candelilla waxes

Texture Profile Analysis

Texture profile analysis (TPA) of the samples was performed using a texture analyzer (CT3, Brookfield Engineering Laboratories, USA). Argan oil oleogels were analyzed at room temperature with two compression cycles using a 12.7 mm cylindrical probe (TA 10, Brookfield Engineering Laboratories, USA). In the analysis, the test speed was set to 1 mm/s, compression distance to 10 mm, and recovery time to 5 s. From the obtained force-time curves, hardness, adhesiveness, springiness, cohesiveness, and gumminess values were calculated.

Color Properties

Color intensities of the argan oil oleogels were detected according to the criteria given by CIE (Commission Internationale de l'Eclairage) based on three-dimensional (L*, a*, and b*) color measurements using a colorimeter (CR-400, Minolta Co). L* defines the color lightness (ranging from 0 for black to 100 for white), a* indicates the degree of color between red and green (negative values indicate green color and positive values indicate red color), and b* indicates color degree between yellow and blue (negative values indicate blue colors and positive values indicate yellow colors).

Statistical analysis

The study was conducted according to the completely randomized design. Two measurements were performed in color analyses. However, six measurements were performed in the TPA analysis. The analysis of variance was applied to the obtained data (one-way ANOVA), and differences between means were compared by Duncan’s multiple comparison test at the 95 % confidence level (IBM SPSS Statistics 20). All data were given as mean values ± standard error in the tables.

RESULTS AND DISCUSSION

Texture

The results of the textural properties determined in argan oil oleogels formed with different ratios of carnauba and/or candelilla waxes are given in Table 2. The oleogelator combination used in oleogelation significantly affected all textural parameters (P<0.01). While the lowest hardness value was determined as 0.73±0.05 N in the oleogel formed using 100% candelilla wax, the highest hardness value was determined as 1.64±0.05 N in the oleogel containing a mixture of 75% carnauba wax and 25% candelilla wax (T4). On the other hand, there was no statistical difference between the hardness values of T2, T3, and T5 group oleogels. It is observed that the use of carnauba wax in argan oil oleogel causes the formation of a harder oleogel. The adhesiveness of oleogel prepared with only candelilla wax was lower than others. However, it was determined that this group (T1) had the highest cohesiveness. There were no statistical differences between T2, T3, T4, and T5 oleogels for both cohesiveness and adhesiveness. In terms of springiness, it was observed that T4 oleogel had the highest value with 9.99±0.50 mm. While the lowest gumminess was determined in the T1 group, the highest values were detected in the T4 and T5 groups.

Table 2. Textural properties of argan oil oleogels prepared with carnauba and/or candelilla waxes

	T1	T2	T3	T4	T5	Significance
Hardness (N)	0.73±0.05^{a}	1.51±0.03^{b}	1.42±0.03^{b}	1.64±0.05^{c}	1.47±0.03^{b}	**
Adhesiveness (mJ)	1.83±0.37^{a}	4.07±0.26^{b}	4.23±0.19^{b}	3.82±0.24^{b}	3.73±0.17^{b}	**
Cohesiveness	0.33±0.03^{b}	0.20±0.01^{a}	0.20±0.01^{a}	0.24±0.01^{a}	0.24±0.01^{a}	**
Springiness (mm)	8.01±0.39^{a}	7.89±0.36^{a}	8.62±0.27^{ab}	9.99±0.50^{c}	9.64±0.15^{bc}	**
Gumminess (N)	0.23±0.01^{a}	0.31±0.02^{b}	0.28±0.02^{b}	0.39±0.02^{c}	0.36±0.01^{c}	**

$^{a\text{-}c}$: Means marked with different letters in the same line are statistically different from each other (P<0.05); T1, Argan oil oleogel with %100 candelilla wax; T2, Argan oil oleogel with %75 candelilla + % 25 carnauba waxes; T3, Argan oil oleogel with %50 candelilla + % 50 carnauba waxes; T4, Argan oil oleogel with %25 candelilla + % 75 carnauba waxes; T5, Argan oil oleogel with %100 carnauba wax; ** P<0.01

In a study, the hardness value of grape seed oil oleogels prepared using different ratios of candelilla wax and glyceryl monostearate increased as the amount of candelilla wax increased (Choi et al., 2020). Kim et al. (2022) determined the hardness values in canola oil oleogels prepared with candelilla wax and glyceryl monostearate. In this study, it was reported that there was an increase in hardness with the use of candelilla wax, but the highest hardness was determined in the oleogel prepared with a mixture of 60% candelilla wax and 40% glyceryl monostearate. In another study, the hardness of corn oil oleogels prepared using a mixture of

1-docosanol and carnauba wax in different proportions was determined. It was reported that the highest hardness among the oleogels was determined in the oleogel using carnauba wax: 1-docosanol in the ratio of 1:4, followed by the oleogel formed with carnauba wax: 1-docosanol in the ratio of 4:1 (Xia et al., 2022).

Color

L*, a*, and b* values of argan oil oleogels formed with carnauba and/or candelilla waxes are given in Table 3. Different oleogelator combinations significantly affected the color properties of argan oil oleogel (P<0.05). It was determined that as the carnauba wax amount increased in the oleogels, the L* value decreased. While the highest a* value was determined in the T5 group, the b* value decreased with the use of carnauba wax, and it was 11.95±0.08 in the oleogel prepared with 100% candelilla wax (T1) and 8.77±0.71 in the oleogel containing 100% carnauba wax (T5). It was seen that the different combinations of carnauba and candelilla waxes have a significant effect on the color changes. In a study, the color values of oleogels obtained from coconut oil using different ratios of lycopene and stearic acid were examined. In the study, the use of different oleogelator combinations caused changes in the color values of the oleogels was reported (Dhulipalla et al., 2023). Adrah et al. (2021) determined the color properties of oleogels obtained with canola oil and carnauba wax. The L* value of the oleogels using 10% carnauba wax was determined as 37.17 ± 3.89, a* value as 13.46 ± 0.28 and b* value as 24.73 ± 1.50. It is possible to say that, in addition to the oleogelators, the color properties of the oils used in the oleogelation can also affect the color properties of the oleogels.

Table 3. Color properties of argan oil oleogels formed with carnauba and/or candelilla waxes

	T1	T2	T3	T4	T5	Significance
L*	45.51±0.14^{d}	40.27±0.32^{c}	39.72±0.14^{c}	34.34±0.86^{b}	32.32±0.05^{a}	**
a*	-2.01±0.02^{a}	-1.85±0.15^{a}	-2.04±0.02^{a}	-1.63±0.15^{a}	-0.89±0.18^{b}	*
b*	11.95±0.08^{d}	10.16±0.16^{c}	9.78±0.12^{bc}	8.41±0.22^{a}	8.77±0.71^{ab}	**

$^{a-d}$: Means marked with different letters in the same line are statistically different from each other (P<0.05); T1, Argan oil oleogel with %100 candelilla wax; T2, Argan oil oleogel with %75 candelilla + % 25 carnauba waxes; T3, Argan oil oleogel with %50 candelilla + % 50 carnauba waxes; T4, Argan oil oleogel with %25 candelilla + % 75 carnauba waxes; T5, Argan oil oleogel with %100 carnauba wax; ** P<0.01; * P<0.05

CONCLUSION

In the research, it was observed that different oleogelators and their combinations significantly affected the texture and color properties of argan oil oleogels. Using carnauba wax in oleogelation increased the hardness, adhesiveness, and springiness of argan oil oleogels, and it caused higher L* and b* values and less a* value. It was also observed that the combined use of carnauba and candelilla waxes, which can form oleogels alone, had synergistic effects on the textural and color properties of oleogels. Additionally, the use of argan oil in oleogelation in this study can be considered an innovative approach in terms of food technology.

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Statement of Conflict of Interest

The authors declare that they do not have any conflict of interest.

Authors' Contributions

All authors contributed to the conceptualization, investigation, analysis, and writing.

REFERENCES

Adrah, K., Adegoke, S.C., Nowlin, K., Tahergorabi, R., 2021. Study of oleogel as a frying medium for deep-fried chicken. J Food Meas Charact, 16, 1114–1123. https://doi.org/10.1007/s11694-021-01237-6

Cabrera-Vique, C., Marfil, R., Gimenez, R., Martinez-Augustin, O., 2012. Bioactive compounds and nutritional significance of virgin argan oil – an edible oil with potential as a functional food. Nutr. Rev., 70: (5), 266–279. https://doi.org/10.1111/j.1753-4887.2012.00478.x

Charrouf, Z., Guillaume, D., 2008. Argan oil:Occurrence, composition and impact on human health. Eur J Lipid Sci Technol., 110, 632–636. https://doi.org/10.1002/ejlt.200700220

Cherki, M., Berrouui, H., Drissi, A., Adlouni, A., Khalil A., 2006. Argan oil: Which benefits on cardiovascular diseases? Pharmacol. Res., 54, 1–5. https://doi.org/10.1016/j.phrs.2006.02.004

Choi, K-O., Hwang, H-S., Jeong, S., Kim, S., Lee, S., 2020. The thermal, rheological, and structural characterization of grapeseed oil oleogels structured with binary blends of oleogelator. J. Food Sci., 85: (10), 3432-3441. https://doi.org/10.1111/1750-3841.15442

Dhulipalla, H., Syed, I., Munshi, M., Mandapati, R. N., 2023. Development and Characterization of Coconut Oil Oleogel with Lycopene and Stearic Acid. J Oleo Sci., 72: (8), 733-743. https://doi.org/10.5650/jos.ess22398

Drissi A., Girona J., Cherki M., Godas G., Derouiche A., Emessal M., Saile R., Kettani A., Sola R. Masanal. M., Adlouni A., 2004. Evidence of hypolipemiant andantioxidant properties of argan oil derived from the argan tree (Arganiaspinosa). Clin Nutr., 23,1159-1166. https://doi.org/10.1016/j.clnu.2004.03.003

El abbassi A., Khalid N., Zbakh H., Ahmad A., 2014. Physicochemical Characteristics, Nutritional Properties, and Health Benefits of Argan Oil: A Review. Crit Rev Food Sci., 54: (11), 1401-1414. https://doi.org/10.1080/10408398.2011.638424

Guillaume, D., Charrouf, Z., 2013. Argan oil for nutritional and skin care applications. H&PC Today, 8, 28–30.

Guillaume, D., Pioch, D., Charrouf, Z. (2019). Argan [*Argania spinosa (L.) Skeels*] Oil. In: Ramadan, M. (eds) Fruit Oils: Chemistry and Functionality. Springer, Cham. https://doi.org/10.1007/978-3-030-12473-1_16

Khallouki, F., Younos, C., Soulimani, R., Oster, T., Charrouf, Z., Spiegelhalder, B., Bartsch, H., Owen, R.W., 2003. Consumption of argan oil (Morocco) with its unique profile of fatty acids, tocopherols, squalene, sterols and phenolic compounds should confer valuable cancer chemopreventive effects. Eur. J. Cancer Prev., 12, 67–75. https://www.jstor.org/stable/45050860

Kim, M., Hwang, H-S., Jeong, S., Lee, S., 2022. Utilization of oleogels with binary oleogelator blends for filling creams low in saturated fat. LWT, 155, 112972. https://doi.org/10.1016/j.lwt.2021.112972

Li, L., Liu, G., Bogojevic, O., Pedersen, J. N., Guo, Z., 2022. Edible oleogels as solid fat alternatives: Composition and oleogelation mechanism implications. Compr. Rev. Food Sci. Food Saf., 1-28. https://doi.org/10.1111/1541-4337.12928

Okuro, P.K., Martins, A.J., Vicente, A.A., Cunha, R.L., 2020. Perspective on oleogelator mixtures, structure design and behaviour towards digestibility of oleogels. Curr. Opin. Food Sci., 35, 27–35. https://doi.org/10.1016/j.cofs.2020.01.001

Xia, T., Wei, Z., Xue, C., 2022. Impact of composite gelators on physicochemical properties of oleogels and astaxanthin delivery of oleogel-based nanoemulsions. LWT, 153, 112454. https://doi.org/10.1016/j.lwt.2021.112454.

Thermal, Textural and Color Properties of Olive Pomace Oil Oleogels Prepared with Different Waxes

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ABSTRACT: Oleogelation is the process of converting oils into a solid oil-like structure with the help of oleogelators. In oleogels, the properties of oils are preserved, and trans-fat formation does not occur. In this study, the thermal, textural, and color properties of olive pomace oil oleogels prepared with carnauba or candelilla waxes were determined. The melting peak temperatures and enthalpy values of olive pomace oil oleogels were significantly affected by the use of different oleogelators ($P<0.05$). The mean peak temperatures of oleogels prepared with carnauba or candelilla waxes were determined as 75.83±0.22°C and 73.65±0.08°C, respectively. While the mean melting enthalpy of the oleogel formed with carnauba wax was determined as 14.75±0.01 J/g, the mean melting enthalpy of the oleogel prepared with candelilla wax was detected as 4.40±0.27 J/g. In addition, the hardness, springiness, and gumminess of the oleogels were significantly affected by the oleogelators ($P<0.05$) and were higher in olive pomace oil oleogels formed with carnauba wax. The color properties (L*, a*, b*) of the oleogels were also significantly affected by the use of carnauba or candelilla waxes ($P<0.05$). The L* and b* values were higher in the oleogels prepared with candelilla wax than in the oleogels formed with carnauba wax.

Keywords: Olive pomace oil oleogel, Carnauba, Candelilla, DSC, Texture, Color

INTRODUCTION

Fats in the composition of food products have significant effects on taste, texture, and appearance. However, the fats used in the production of foods have some health risks because they contain high amounts of saturated fatty acids. In this context, studies are being carried out on obtaining liquid oils in a solid form and using them instead of solid fats in food products. Liquid oils can be solidified by methods such as hydrogenation, interesterification, and fractionation and can be used in the production of various food products. However, oleogelation technique has also been a promising method for solidifying liquid oils in recent years. The oleogelation process allows oils to be obtained in a solid-like structure using oleogelators. In oleogelation, the properties of oils are preserved, and unlike other solidification methods, trans fat is not formed (Pehlivanoğlu et al., 2018; Li et al., 2022). For these reasons, the use of oleogel to mimic solid fats in the production of healthier food products is increasing daily.

Pomace oil is obtained by drying the pomace formed during olive oil production and then subjecting it to solvent extraction (Bozkurt et al., 2017). In the Turkish Food Codex, three different pomace oils are defined: crude pomace oil, pomace oil, and refined pomace oil (Anonymous, 2017). While refined pomace oil is preferred as a frying oil, pomace oil can be used as an edible oil. Pomace oil is defined as oil consisting of a mixture of refined pomace oil and natural olive oils suitable for direct consumption, with a free fatty acidity of not more than 1.0 grams per 100 grams in terms of oleic acid. Olive pomace oil has a composition similar to olive oil in terms of oleic acid content (55-83%) and is a more economical oil than olive oil. Studies on olive pomace oil have focused mostly on the production process and quality characteristics (Sánchez-Gutiérrez et al., 2015; Özdikicierler et al., 2016; Bozkurt et al., 2017; Yanık, 2017; Yorulmaz, 2018; Moya et al., 2018; Ketenoglu et al., 2018; Chanioti and Tzia, 2018, 2019; Giuffre et al., 2020; Jabeur et al., 2020; Çelekli et al., 2021). Despite this increasing interest in the production process of olive pomace oil, its quality characteristics, and its relationship with health, no study has been found in the literature on the use of olive pomace oil in oleogelation. In this study, oleogels were formed from olive pomace oil using different oleogelators, and the thermal, textural, and color properties of these oleogels were determined.

MATERIAL AND METOD

The olive pomace oil and the carnauba and candelilla waxes used in the research were obtained from commercial companies. Olive pomace oil oleogels were prepared to contain 10% wax (Figure 1). Candelilla and carnauba waxes and olive pomace oil were weighed and mixed, kept in a water bath at 90°C until wholly melted, then taken from the water bath and vortexed. After the samples cooled to room temperature, stored in the refrigerator at 4°C for 24 hours (Choi et al., 2020). Thermal, textural, and color properties of olive pomace oil oleogels prepared with carnauba or candelilla waxes were determined. All analyses were performed at room temperature.

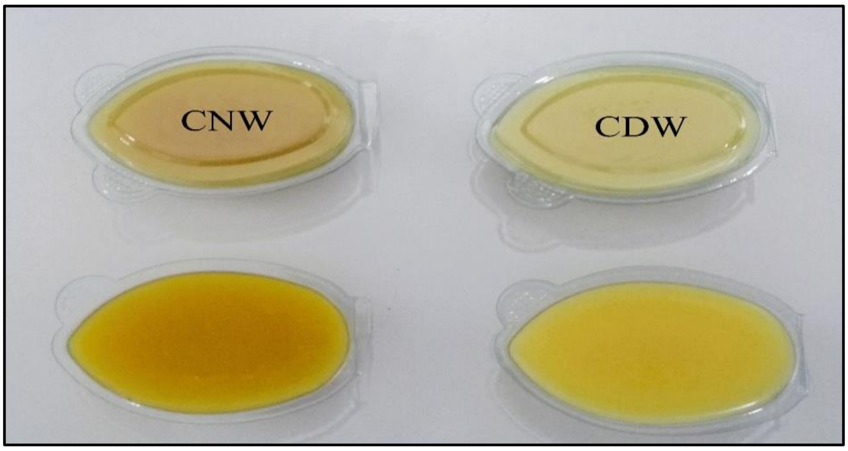

Figure 1. Olive pomace oil oleogels prepared with carnauba and candelilla waxes.

Thermal Properties

The thermal properties of the samples were determined by Differential Scanning Calorimetry (DSC-60; Shimadzu Corporation, Japan). Samples were taken from the prepared olive pomace oil oleogels, and their melting peak temperatures and enthalpies were determined. For this purpose, samples were weighed approximately 3-4 mg into aluminum sample pans and heated from room temperature to 120°C with a heating rate of 5°C/min using an empty pan as a reference. Nitrogen gas was used as the ambient atmosphere in the measurements, and the flow rate was set to 50 ml/min. Peak temperatures and enthalpies were determined from the melting peaks observed from the thermograms obtained as a result of the measurements.

Texture Properties

Texture profile analysis (TPA) of the samples was performed using a texture analyzer (CT3, Brookfield Engineering Laboratories, USA). Olive pomace oil oleogels were analyzed at room temperature with two compression cycles using a 12.7 mm cylindrical probe (TA 10, Brookfield Engineering Laboratories, USA). In the analysis, the test speed was set to 1 mm/s, compression distance to 10 mm, and recovery time to 5 s. From the obtained force-time curves, hardness, adhesiveness, springiness, cohesiveness, and gumminess values were calculated.

Color properties

Color intensities of the olive pomace oil oleogels were detected according to the criteria given by CIE (Commission Internationale de l'Eclairage) based on three-dimensional (L*, a*, and b*) color measurements using a colorimeter (CR-400, Minolta Co). L* defines the color lightness (ranging from 0 for black to 100 for white), a* indicates the degree of color between red and green (negative values indicate green color and positive values indicate red color), and b* indicates color degree between yellow and blue (negative values indicate blue colors and positive values indicate yellow colors).

Statistical analysis

The study was conducted according to the completely randomized design. Two measurements were performed in thermal and color analyses. However, six measurements were performed in the TPA analysis. The analysis of variance was applied to the obtained data (one-way ANOVA), and differences between means were compared by Duncan's multiple comparison test at the 95 % confidence level (IBM SPSS Statistics 20). All data were given as mean values ± standard error in the tables.

RESULTS AND DISCUSSION

Thermal Properties

The thermal properties of olive pomace oil oleogels prepared with carnauba or candelilla waxes are given in Table 1. The use of different oleogelators in the preparation of oleogel significantly affected the melting peak temperature and melting enthalpy (P<0.05). Higher melting peak temperature and enthalpy values were found in oleogels prepared with carnauba wax. While the mean melting peak temperature of the oleogel prepared with candelilla wax was determined as 73.65±0.08 °C, the mean melting peak temperature of the olive pomace oil oleogel formed with carnauba wax was detected as 75.83±0.22 °C. The melting enthalpies of oleogels prepared with carnauba and candelilla waxes were determined as 14.75±0.01 J/g and 4.40±0.27 J/g, respectively.

Table 1. Thermal properties of olive pomace oil oleogels prepared with carnauba or candelilla waxes

	CNW	CDW	Significance
Onset (°C)	65.14±0.66	64.81±0.52	ns
Peak (°C)	$75.83{\pm}0.22^{b}$	$73.65{\pm}0.08^{a}$	*
Endset (°C)	$81.78{\pm}0.09^{b}$	$78.59{\pm}0.02^{a}$	**
Heat (J/g)	$14.75{\pm}0.01^{b}$	$4.40{\pm}0.27^{a}$	**

$^{a-b}$: Means marked with different letters in the same line are statistically different from each other (P<0.05); CDW, Olive pomace oil oleogel with candelilla wax; CNW, Olive pomace oil oleogel with carnauba wax; ** P<0.01; * P<0.05; ns P>0.05

Ghazani et al. (2022) determined the thermal properties of oleogels prepared with olive oil and mixtures of different waxes. In the study, the melting profiles of olive oil oleogels formed with beeswax or candelilla waxes and their combinations were evaluated, and it was determined that the melting point decreased with the use of candelilla wax. The authors stated that this may be due to the diversity of different components of candelilla wax, such as free fatty acids, free fatty alcohols, and hydrocarbons, compared to other wax types that contain mainly esters. In a study conducted by Öğütçü and Yılmaz (2014), the thermal properties of oleogels prepared from olive oil with carnauba wax were determined. In the study, the melting peak temperature and the enthalpy value of olive oil oleogels containing 10% carnauba wax were 76.00±0.13 °C and 14.81±1.56 J/g, respectively. Kim and Oh (2022) reported that the melting peaks of oleogels prepared with candelilla or carnauba waxes varied between 32-50°C and 60-80°C, respectively. It has been stated that the melting enthalpy of the oleogel prepared with carnauba wax is higher than other oleogels, and this is due to the higher resistance of this oleogel to temperature changes.

Texture

Textural properties of olive pomace oil oleogels prepared with carnauba or candelilla waxes are given in Table 2. The hardness, springiness, and gumminess values of the oleogels were significantly affected by the use of different waxes (P<0.05). However, adhesiveness and cohesiveness values did not show a statistical difference. Using carnauba wax resulted in olive pomace oil oleogels with a higher hardness, springiness, and gumminess. The hardness values of oleogels formed with carnauba and candelilla waxes were found 1.73±0.02 N and 1.37±0.08 N, respectively. While the springiness of the oleogel formed with candelilla wax was determined as 8.68±0.17 mm, the oleogel prepared with carnauba wax was 9.91±0.22 mm.

Öğütçü and Yılmaz (2014) determined the textural properties of oleogels prepared using different oleogelators and reported that oleogels formed with extra virgin olive oil using 10% carnauba wax had the highest hardness and adhesiveness. In another study, the hardness of oleogels prepared with different waxes was determined, and the hardness of oleogels formed with carnauba and candelilla waxes was determined as 1.21±0.07 N and 2.65±0.01 N, respectively (Kim and Oh, 2022). Li et al. (2022) formed oleogels from sunflower oil with different oleogelators and determined the hardness of these oleogels. Among the oleogelators used in the study, carnauba wax caused the highest hardness.

Table 2. Textural properties of olive pomace oil oleogels prepared with carnauba or candelilla waxes

	CNW	CDW	Significance
Hardness (N)	1.73±0.02^{b}	1.37±0.08^{a}	**
Adhesiveness (mJ)	4.65±0.28^{a}	3.90±0.28^{a}	ns
Cohesiveness	0.25±0.01^{a}	0.25±0.02^{a}	ns
Springiness (mm)	9.91±0.22^{b}	8.68±0.17^{a}	**
Gumminess (N)	0.43±0.02^{b}	0.34±0.02^{a}	*

$^{a-b}$: Means marked with different letters in the same line are statistically different from each other (P<0.05); CDW, Olive pomace oil oleogel with candelilla wax; CNW, Olive pomace oil oleogel with carnauba wax; ** P<0.01; * P<0.05; ns P>0.05

Color

The color properties of olive pomace oil oleogels are given in Table 3. L*, a*, and b* values were significantly affected by the use of different oleogelators (P<0.05). The L* values of oleogels formed with carnauba and candelilla waxes were determined as 34.71±0.03 and 48.40±0.00, respectively. It was observed that oleogels formed with candelilla wax had a higher L* value. The a* value of the olive pomace oil oleogel prepared with carnauba wax was higher, and the b* value was lower than the oleogel formed with candelilla wax. Öğütçü and Yılmaz (2014) determined the L* value of olive oil oleogels prepared with carnauba wax as 54.46±0.34 and the b* value as 25.22±0.33.

Table 3. Color properties of olive pomace oil oleogels

	CNW	CDW	Significance
L*	34.71±0.03^{a}	48.40±0.00^{b}	**
a*	-2.24±0.01^{b}	-5.02±0.07^{a}	**
b*	10.33±0.05^{a}	11.88±0.15^{b}	*

[a-b]: Means marked with different letters in the same line are statistically different from each other (P<0.05); CDW, Olive pomace oil oleogel with candelilla wax; CNW, Olive pomace oil oleogel with carnauba wax; ** P<0.01; * P<0.05

CONCLUSION

It was observed that the thermal, textural, and color properties of olive pomace oil oleogel changed significantly with the use of different oleogelators. Using carnauba wax in oleogelation provided darker but relatively less yellowness oleogels. In addition, the hardness, adhesiveness, and springiness of the oleogels increased with the use of carnauba wax. These results showed that the oleogelator used in oleogelation affects the properties of olive pomace oil oleogel, and the oleogelator should be chosen according to the intended use of olive pomace oil oleogel. In this study, the use of olive pomace oil in oleogelation can also be considered an innovative approach in terms of food technology.

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Statement of Conflict of Interest

The authors declare that they do not have any conflict of interest.

Authors' Contributions

All authors contributed to the conceptualization, investigation, analysis, and writing.

REFERENCES

Anonymous, 2017. Türk Gıda Kodeksi Zeytinyağı ve Pirina Yağı Tebliği, Sayı : 30183, Ankara

Bozkurt, S., Yazıcı, U.M., Bozbey, S., Yorulmaz, A., 2017. Mikrodalga ısıtmanın pirina yağının oksidatif stabilite ve yağ aside bileşimine etkisi. ADÜ Ziraat Derg., 14(2), 51-55. https://doi.org/10.25308/aduziraat.315284

Chanioti, S., Tzia, C., 2018. Extraction of phenolic compounds from olive pomace by using natural deep eutectic solvents and innovative extraction techniques. Innov Food Sci Emerg Technol., 48, 228–239. https://doi.org/10.1016/j.ifset.2018.07.001

Chanioti, S., Tzia, C., 2019. Evaluation of ultrasound assisted and conventional methods for production of olive pomace oil enriched in sterols and squalene. LWT, 99,209-2016. https://doi.org/10.1016/j.lwt.2018.09.068

Choi, K-O., Hwang, H-S., Jeong, S., Kim, S., Lee, S., 2020. The thermal, rheological, and structural characterization of grapeseed oil oleogels structured with binary blends of oleogelator. J. Food Sci., 85: (10), 3432-3441. https://doi.org/10.1111/1750-3841.15442

Çelekli, A., Gün, D., Bozkurt, H., 2021. Bleaching of olive pomace oil with Spirulina platensis as an eco-friendly process. Algal Res., 54, 102210. https://doi.org/10.1016/j.algal.2021.102210

Ghazani, S. M., Dobson, S., Marangoni, A. G., 2022. Hardness, plasticity, and oil binding capacity of binary mixtures of natural waxes in olive oil. Curr. Res. Food Sci., 5, 998-1008. https://doi.org/10.1016/j.crfs.2022.06.002

Giuffre, A.M., Capocasale, M., Macri, R., Caracciolo, M., Zappia, C., Poiana, M., 2020. Volatile profiles of extra virgin olive oil, olive pomace oil, soybean oil and palm oil in different heating conditions. LWT, 117, 108631. https://doi.org/10.1016/j.lwt.2019.108631

Jabeur, H., Marrakchi, F., Drira, M., Ghodhbani, I., Bouaziz, M., 2020. 2-glyceryl monopalmitate in virgin and pomace olive oil during refining processes. Food Biosci., 38, 100774. https://doi.org/10.1016/j.fbio.2020.100774

Ketenoglu, O. Ozkan, K.S., Yorulmaz, A., Tekin, A., 2018. Molecular distillation of olive pomace oil ─ Multiobjective optimization for tocopherol and squalene. LWT, 91, 198–202. https://doi.org/10.1016/j.lwt.2018.01.051

Kim, D., Oh, I., 2022. The Characteristic of Insect Oil for a Potential Component of Oleogel and Its Application as a Solid Fat Replacer in Cookies. Gels, 8, 355. https://doi.org/10.3390/gels8060355

Li, J., Guo, R., Wang, M., Bi, Y., Zhang, H., Xu, X., 2022. Development and Characterization of Compound Oleogels Based on Monoglycerides and Edible Waxes. Food Sci. Technol., 2, 302-314. https://doi.org/10.1021/acsfoodscitech.1c00390

Li, L., Liu, G., Bogojevic, O., Pedersen, J.N., Guo, Z., 2022. Edible oleogels as solid fat alternatives: Composition and oleogelation mechanism implications. Compr. Rev. Food Sci. Food Saf., 1-28. https://doi.org/10.1111/1541-4337.12928

Moya, M., Alcala, S., Ocana, M.T., Vidal, A., Espinola, F., 2018. Oil mill coadjuvants: Aggregation due to moisture and action on olive-pomace oils. J Food Eng., 236, 51-59. https://doi.org/10.1016/j.jfoodeng.2018.05.013

Öğütçü, M., Yılmaz, E., 2014. Oleogels of virgin olive oil with carnauba wax and monoglyceride as spreadable products. Grasas Aceites, 65: 3, e040. http://dx.doi.org/10.3989/gya.0349141

Özdikicierler, O., Yemişçioğlu, F., Gümüşkesen, A.S., 2016. Effects of process parameters on 3-MCPD and glycidyl ester formation during steam distillation of olive oil and olive pomace oil. Eur. Food Res. Technol., 242, 805–813. https://doi.org/10.1007/s00217-015-2587-7

Pehlivanoğlu, H., Demirci, M., Toker, O.S., Konar, N., Karasu, S., Sagdic, O., 2018. Oleogels, a promising structured oil for decreasing saturated fatty acid concentrations: Production and food-based applications. Crit Rev Food Sci., 58(8), 1330-1341. https://doi.org/10.1080/10408398.2016.1256866

Sanchez-Gutierrez, C.A., Casas, M., Lucero, M.J., Ruiz-Mendez, M.V., 2015. Physico-chemical and rheological characterization of olive-pomace oils. Eur J Lipid Sci Technol., 117, 87–91. https://doi.org/10.1002/ejlt.201300490

Yanık, D.K., 2017. Alternative to traditional olive pomace oil extraction systems: Microwave-assisted solvent extraction of oil from wet olive pomace. LWT, 77, 45-51. https://doi.org/10.1016/j.lwt.2016.11.020

Yorulmaz, A., 2018. Influence of industrial refining on some characteristics of olive pomace oil. Riv. Ital. delle Sostanze Grasse., 95(3), 167-172.

Determination of Feed Value and Usability of Olive Tree (*Olea europaea L.*) Leaves with Some Roughages

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ABSTRACT: Approximately 70-80% of the production costs in animal breeding are feeding and feed costs. Since using quality and cheap feeds in ruminant animal breeding increases the profitability of the business, feed producers and animal breeders have focused on finding alternative feed sources. Alternative roughage sources play an important role in reducing feed costs by substituting feeds in the same nutrient group. In this study, it was aimed to determine the nutritional value of olive tree leaves and to determine their use with some roughages. In the study, olive tree leaves were substituted into alfalfa hay, oat straw and dry meadow grass at 0%, 25%, 50%, 75% and 100% rates. When the data obtained at the end of 24 hours were analyzed, it was observed that the increase in olive leaves in alfalfa decreased gas, metabolic energy, net energy and organic matter digestion compared to 100% alfalfa group ($P<0.01$). The same was observed in dry fescue-olive leaf and oat straw-olive leaf mixtures when compared to 100% FFF and 100% YS groups ($P<0.05$). In conclusion, it is suggested that the substitution of olive tree leaves to the roughages used in ruminant animal nutrition decreases the energy and digestibility values obtained from the feed, but in areas where feed is scarce, olive leaves left over after pruning or olive harvest can be used in animal nutrition.

Keywords; Olive leaves, Gas production, Feed value, Alternative feed

INTRODUCTION

Ruminants play a key role in sustainable agriculture by helping to transform plant resources, which are roughages and agricultural by-products that are not consumed by humans, into quality foods such as meat and milk that humans can consume daily thanks to the microorganisms in their digestive tract (Hristov et al. 2013; Pulina et al. 2017). Turkey, which ranks first in Europe with 18,630,728 livestock units (LAR) in 2023, cannot maintain the same position in terms of yield per animal (Sarıçiçek and Kılıç 2002; TÜİK, 2023). The reason for this is that the need for cheap and sufficient amount of quality roughage, which is available all over the world, cannot be provided regularly (Anonymous, 2018). In recent years, the increasing human population, climate change and drought have led to a decrease in agricultural land and the misuse of agricultural land, and as a result, the areas for the production of forage crops have decreased (Özkan et al. 2020; Yavuz et al., 2020). In Turkey, quality roughage is provided from forage crops cultivated in field agriculture and meadows and pastures. However, it is reported that the yield and quality of pastures have decreased due to excessive, untimely grazing and late or non-performance of maintenance works, and pastures have become areas open to severe erosion (Yolcu and Tan, 2008; Sürmen et al., 2008). For these reasons, forage crops and meadow-pastures produced in the field cannot meet the nutrient needs of ruminant animals sufficiently. The unmet feed deficit of ruminants is provided by roughages with low nutritional value (straw, stalks, husks, etc.) or by concentrated mixed feed resources. However, the high inputs of concentrated compound feed resources increase the prices of animal products. Feed and feeding costs constitute almost 60-70% of the expenses incurred during the production of food obtained from animals. For a sustainable animal production, it is possible to provide quality roughage at cheaper prices (Alçiçek et al., 2010; Kuşvuran et al., 2011; Bıçakçı and Açıkbaş, 2018). For this reason, the need for more affordable alternative feed resources that positively affect the health and digestive efficiency of animals comes to the fore (Özkan and Şahin Demirbag, 2016). Olive tree (Olea europaea L.), whose homeland is the Mediterranean region, is cultivated naturally in our country and according to the data of TUIK, (2020), it is reported that there are 187,163 thousand olive trees (TUIK, 2020). It is reported that 25 kg of olive leaves are shed from each adult olive tree during the harvest and pruning period, and producers collect olive leaves and burn them in an area (Molina-Alcaide and YanezRuiz 2008). Considering the climatic and ecological conditions of Turkey, in semi-arid and arid regions, the least forage production is seen in the summer season, and the increasing greenhouse gases in recent years have caused an increase in arid areas and periods due to their effect on global warming and climate changes. This leads to a restriction of natural feeding, i.e. grazing, for livestock. However, trees, shrubs and small trees grown in the "silvopastoral system" have deep root systems that allow them to maintain the nutritional quality of their leaves during these dry periods and are an indispensable savior in areas where grazing is scarce and they are of great importance for the nutrition of annual ruminants (Papachristou and Nastis, 1996; Paterson, 1998). From this point of view, it was aimed to investigate the effect of substitution of olive leaves (0, 25, 50, 75, 100 %) at different rates (0, 25, 50, 75, 100 %) to roughages (oat straw, alfalfa hay and dry meadow grass) used in animal feeding on rumen parameters.

MATERIALS AND METHODS

Olive tree leaves required for the study were obtained from the leaves fallen under at least 10 trees after olive harvest in Muğla province. Oat straw, dry meadow grass and alfalfa hay, which were the roughage materials of the study, were obtained from a private farm in Erzurum province. The feed materials were dried in the laboratory at 105°C in an oven for 24 hours to be used in the analysis. After drying, the feed materials were ground with a 1 mm sieve mill. Olive leaves were substituted into the roughages at the rates of 0, 25, 50, 75, 100 %. Chemical composition analyses of the prepared feed materials were performed in 3 replicates. Dry matter (DM), crude ash (CA), crude protein (CP), eter extract (EE) contents were determined according to AOAC (1990). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were determined according to Van Soest et al. (1991). The nutrient contents of the analyzed feed materials are given in Table 1.

Table 1. Chemical composition of roughages

Roughage mixtures	**DM (%)**	**CA (%)**	**CP (%)**	**EE (%)**	**NDF (%)**	**ADF (%)**
Olive tree leaf	89,39	4,97	10,98	5,14	49,87	35,18
Oat straw	91,75	6,91	5,49	1,11	59,88	35,17
Dry meadow grass	94,45	7,72	7,42	2,16	62,06	31,08
Alfalfa hay	93,79	9,18	17,03	2,22	61,07	38,69
O25+OS75	91,38	6,43	2,12	6,86	57,38	35,17
O50+OS50	90,64	5,94	3,13	8,24	54,88	35,18
O75+OS25	89,91	5,46	4,13	9,61	52,37	35,18
O25+DMG75	93,11	7,03	2,91	8,31	59,01	32,11
O50+DMG50	91,80	6,35	3,65	9,20	55,97	33,13
O75+DMG25	90,48	5,66	4,40	10,09	52,92	34,16
O25+AAH75	92,23	6,90	3,01	15,53	57,51	37,91
O50+AAH50	91,45	7,02	3,69	14,14	55,42	37,04
O75+AAH25	90,31	6,04	3,93	12,29	52,17	36,08

OS: oat straw, DMG: dry meadow grass, AAH: alfalfa hay, O: Olive tree leaf.

The prepared mixtures were weighed 0.2 g into syringes in triplicate. Gas and methane production were carried out according to the in vitro gas production technique reported by Menke et al. (1979). The rumen fluid used in the study was taken from the rumen of 3 healthy Simmental crossbred animals that had received slaughter approval from the ethics committee of a private slaughterhouse and had completed rumen development, as reported by Kılıç and Abdiwali (2016). The rumen fluid was taken to Atatürk University, Faculty of Agriculture, Department of Animal Husbandry, Feed Analysis Laboratory in a screw cap bottle with a thermos with a lid containing water at approximately 39°C. The rumen fluid was filtered through a four-layer cheesecloth by providing anaerobic environment under CO2 gas and used for in vitro gas production technique. At the end of 24 hours of incubation, methane levels of the gas released were determined using an infrared methane analyzer (Goel et al., 2008). ME, NEL and OMS values of the experimental groups formed by adding olive tree leaves at different rates were calculated with the following equations proposed by Menke and Steingass (1988). HP, HY, HK contents used in the equations were used as %.

ME(Mj/kg KM) = (2,2)+(0,1357* GP)+(0,057* CP)+(0,002839* EE*EE)

NE_L(Mj/kg KM) =(0,101*GP)+(0,051*CP)+(0,112*EE)

OMS = 15,38 + 0,8453 x GP + 0,0595 x CP + 0,0695 x CA

Statistical analysis

The data obtained to determine the effect of olive tree leaves added to roughages at different rates on fermentation parameters were subjected to analysis of variance (ANOVA) in Tukey multiple comparison test in SPSS 11.0 package program (SPSS, 2011).

RESULTS AND DISCUSSION

Fermentation parameters of olive tree leaves added to roughages at different rates are given in Table 2 and significantly affected in vitro gas production, methane production values, metabolic energy (ME), net energy lactation (NEL) and in vitro organic matter digestion (IVOMSD) ($p<0.01$).

Table 2. Fermentation parameters of olive tree leaves added to roughages at different rates

Roughage mixtures	Gas (ml)	CH_4 (ml)	CH_4 (%)	ME (Mj/kg KM)	NE_L (Mj/kg KM)	IVOMD (%)
Olive tree leaf	30,02^{g}	5,35^{e}	17,85^{ab}	6,98^{i}	3,88^{h}	49,73^{i}
Oat straw	39,55^{b}	7,33^{a}	18,55^{a}	7,88^{d}	4,54^{cd}	57,01^{d}
Dry meadow grass	33,72^{de}	5,27^{e}	15,62^{e}	7,21^{ghi}	4,06^{fg}	53,22^{fg}
Alfalfa hay	42,86^{a}	7,56^{a}	17,64^{ab}	9,00^{a}	5,31^{a}	66,61^{a}
O25+OS75	37,62^{c}	6,80^{b}	18,09^{ab}	7,71^{de}	4,42^{d}	55,59^{de}
O50+OS50	34,79^{d}	6,34^{c}	18,24^{a}	7,42^{fg}	4,21^{ef}	53,37^{fg}
O75+OS25	33,15^{de}	5,83^{d}	17,59^{ab}	7,29^{fgh}	4,11^{efg}	52,21^{gh}
O25+DMG75	32,85^{ef}	5,24^{e}	15,94^{de}	7,16^{ghi}	4,02^{gh}	52,40^{gh}
O50+DMG50	32,53^{ef}	5,31^{e}	16,31^{cde}	7,18^{ghi}	4,03^{gh}	52,06^{gh}
O75+DMG25	31,27^{fg}	5,32^{e}	17,01^{bcd}	7,07^{hi}	3,96^{gh}	50,89^{hi}
O25+AAH75	40,13^{b}	7,24^{a}	18,04^{ab}	8,56^{b}	4,99^{b}	62,02^{b}
O50+AAH50	37,55^{c}	6,36^{c}	16,93^{bcd}	8,14^{c}	4,70^{c}	59,18^{c}
O75+AAH25	33,41^{de}	5,82^{d}	17,44^{abc}	7,48^{ef}	4,24^{e}	54,03^{ef}
SHO	0,49	0,98	0,33	0,07	0,05	0,44
p	0,000	0,000	0,000	0,000	0,000	0,000

SHO: Standard error of the mean, p: Significance level, ME: Metabolic energy, NEL: Net energy lactation, IVOMD: In vitro organic matter digestion degree, OS: oat straw, DMG: dry meadow grass, AAH: alfalfa hay, O: Olive tree leaf.

In vitro gas production technique, as a result of 24 hours fermentation of roughage mixtures; gas production between 30,02 ml and 42,86 ml, methane production between 5,24 ml and 7,56 ml, %methane values between 15,62 and 18,55 metabolic energy values between 6,98 Mj/kg KM and 9,00 Mj/kg KM net energy lactation between 3,88 Mj/kg KM and 5,31 Mj/kg KM in vitro organic matter digestion degree was determined between 49,73% and 66,61% and found to be statistically significant ($p<0,01$). It is reported that the differences between the gas, methane, ME, NEL, OMD values of feed mixtures may be due to the fermentation of rumen microorganisms, the amount of degradable matter in the feed and secondary metabolites (Olomonchi et al., 2022). Boğa et al. (2020) stated that ADF and NDF in the structure of feed or feed mixtures are related to gas production and decreasing these values will increase gas production. When we look at the 24-hour gas production values in the study, we observe a decrease in gas, methane and energy values of feeds due to the substitution of olive tree leaves at different rates to the control groups of oat straw, alfalfa hay and dry meadow grass. It is reported that this is due to the high crude oil and ADF content in the structure of olive tree leaves. It is stated that the % methane gas values of the feeds used in ruminant rations can be classified as low antimethanogenic (>11% and ≤14%), medium antimethanogenic (>6% and <11%) and high anti-methanogenic (>0% and <6%) according to the method determined by Lopez et al. (2010) and that the preparation of TMR by considering these classifications of feeds given to animals can increase the efficiency of energy use in ruminants and reduce methane gas which causes global warming. In this study, no anti-methanogenic effect was observed according to the classification reported by Lopez et al. (2010) when we looked at the methane (%) of the experimental groups. Animals meet the energy (ME, NEL) required for their survival and productivity needs from the feeds or feed mixtures they consume (Schingoethe, 2017). According to the control groups, it is seen that the energy (ME, NEL) values decreased due to the increase in the addition rate of olive tree leaves, and it is seen that if it is given to the animal, the amount of feed consumption will increase due to the decrease in energy values.

CONCLUSION

In *in vitro* gas production technique, it is stated that the gases released as a result of 24-hour fermentation play an important role in determining the energy content of feeds, but methane gas, one of these gases, causes both energy loss in animals and global warming. It was determined that olive tree leaves, which were substituted into roughages at different ratios, did not show anti-methanogenic effect and statistically affected in vitro fermentation parameters ($p<0.01$). In conclusion, it is suggested that substitution of olive tree leaves into roughages used in ruminant animal nutrition decreases the energy and digestibility values obtained from feed, but in areas where feed is scarce, olive leaves left over after pruning or olive harvest can be used as a substitute feed in animal nutrition.

REFERENCES

Alçiçek, A., Kılıç, A., Ayhan, V., & Özdoğan, M. (2010). Türkiye'de kaba yem üretimi ve sorunları. *Türkiye Ziraat Mühendisliği VII. Teknik Kongresi*, *2*, 11-15.

AOAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, Vol. II, 15th ed. Sec.985.29. The Association: Arlington, VA.

Bıçakçı, E., & Açıkbaş, S. (2018). Bitlis ilindeki kaba yem üretim potansiyelinin hayvan varlığına göre yeterliliğinin belirlenmesi. *Bitlis Eren Üniversitesi Fen Bilimleri Dergisi*, *7*(1), 180-185.

Boga, M., Kurt, O., Ozkan, C. O., Atalay, A. İ., & Kamalak, A. (2020). Evaluation of some commercial dairy rations in terms of chemical composition, methane production, net energy and organic matter digestibility. *Progress In Nutrition*, *22*(1), 199-203.

Goel, G., Makkar, H. P. S., & Becker, K. (2008). Changes in microbial community structure, methanogenesis and rumen fermentation in response to saponin-rich fractions from different plant materials. *Journal of applied microbiology*, *105*(3), 770-777.

Hristov, A. N., Oh, J., Firkins, J. L., Dijkstra, J., Kebreab, E., Waghorn, G., ... & Tricarico, J. M. (2013). Special topics—Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. *Journal of animal science*, *91*(11), 5045-5069.

Kılıç, Ü., & Abdıwalı, M. A. (2016). Alternatif kaba yem kaynağı olarak şarapçılık endüstrisi üzüm atıklarının in vitro gerçek sindirilebilirlikleri ve nispi yem değerlerinin belirlenmesi. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, *22*(6).

Kuşvuran, A., & Veyis Tansı, R. İ. N. (2011). Türkiye'de ve Batı Karadeniz Bölgesi'nde çayır-mera alanları, hayvan varlığı ve yem bitkileri tarımının bugünkü durumu. *Journal of Agricultural Faculty of Gaziosmanpaşa University (JAFAG)*, *2011*(2), 21-32.

López, S., Makkar, H. P., & Soliva, C. R. (2010). Screening plants and plant products for methane inhibitors. *In vitro screening of plant resources for extra-nutritional attributes in ruminants: nuclear and related methodologies*, 191-231.

Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D., & Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. *The Journal of Agricultural Science*, *93*(1), 217-222.

Menke, K.H. and H. Steingass, 1988. Estimation of the assessment of nutritional quality of feeds: A energetic feed value obtained from chemical review. Anim. Feed Sci. Technol., 72: 261-281. analysis and gas production using rumen fluid.

Molina-Alcaide, E., & Yáñez-Ruiz, D. R. (2008). Potential use of olive by-products in ruminant feeding: A review. *Animal Feed Science and Technology*, *147*(1-3), 247-264.

Olomonchı, Euloge Akambı Ogoukayode; Garipoğlu, Ali Vaiz; Ocak, Nuh; And Kamalak, Adem (2022) "Nutritional values and in vitro fermentation parameters of some fodder species found intwo rangeland areas in the Republic of Benin," *Turkish Journal of Veterinary & Animal Sciences*: Vol. 46: No. 1.

Özkan, Ç. Ö., Cengiz, T., Yanık, M., Evlice, S., Selçuk, B., Ceren, B., & Kamalak, A. (2020). Ruminant Hayvan Beslemede Kullanılan Bazı Kaba Ve Kesif Yemlerin İn Vitro Gaz Üretiminin, Metan Üretiminin, Sindirim Derecesinin Ve Mikrobiyal Protein Üretiminin Belirlenmesi. *Black Sea Journal of Agriculture*, *3*(1), 56-60.

Özkan, U., & Şahin Demirbağ, N. (2016). Türkiyede kaliteli kaba yem kaynaklarının mevcut durumu. *Türk Bilimsel Derlemeler Dergisi*, *9*(1), 23-27.

Papachristou, T. G., & Nastis, A. S. (1996). Influence of deciduous broadleaved woody species in goat nutrition during the dry season in northern Greece. *Small Ruminant Research*, *20*(1), 15-22.

Paterson, S. (1998). Evidence for balancing selection at the major histocompatibility complex in a free-living ruminant. *Journal of Heredity*, *89*(4), 289-294.

Pulina, G., Francesconi, A. H. D., Stefanon, B., Sevi, A., Calamari, L., Lacetera, N., ... & Ronchi, B. (2017). Sustainable ruminant production to help feed the planet. *Italian Journal of Animal Science*, *16*(1), 140-171.

Sarıçiçek, B. Z., & Kılıç, Ü. (2002). Üzüm cibresinin in situ rumen parçalanabilirliğinin belirlenmesi. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, *33*(3).

Schingoethe, D. J. (2017). A 100-Year Review: Total mixed ration feeding of dairy cows. *Journal of dairy science*, *100*(12), 10143-10150.

Sürmen, M., Yavuz, T., Çankaya, N., Töngel, M. Ö. (2008). Karadeniz Bölgesinde hayvan besleme alışkanlıkları üzerine bir araştırma. International Journal of Agricultural and Natural Sciences, 1(1), 49-53.

TÜİK, (2023). Türkiye Hayvansal Üretim İstatistikleri, Türkiye İstatistik Kurumu, http://www.tuik.gov.tr

Van Soest, P. V., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of dairy science*, *74*(10), 3583-3597.

Yavuz, T., Kır, H., Gül, V. (2020). Türkiye'de Kaba Yem Üretim Potansiyelinin Değerlendirilmesi: Kırşehir İli Örneği. Türkiye Tarımsal Araştırmalar Dergisi, 7(3), 345-352.

Yolcu, H., Tan, M. (2008). Ülkemiz yem bitkileri tarımına genel bir bakış. Tarim Bilimleri Dergisi, 14(3), 303-312

The Effect of Pomegranate Peel on Performance and Egg Quality in Laying Hen

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ABSTRACT: In this study, the effects of adding different levels of pomegranate peel to laying hen diets on performance and egg quality were examined. 120 Lohman LSL laying hens, 64 weeks old, were divided into five groups. There were 24 animals in each group and consisted of 6 subgroups. The first group was the control group and was fed with basal feed, while the other groups were fed with feeds with 150, 300, 450 and 600 mg/kg pomegranate peel added to the basal feed, respectively. Animals were given feed and water ad-libitum during the 8-week experiment. Performance values and egg quality parameters were determined by measurements made every two weeks.

At the end of the experiment, it was determined that feed consumption was highest in the 300 and 450 mg/kg PP groups. The highest egg weight was determined in the 300 mg/kg PP group, and the best feed conversion ratio was determined in the 300 and 600 mg/kg PP groups. It was observed that egg production increased significantly ($P<0.01$) in groups other than the control and 150 mg/kg PP group. Shell ratio, shell thickness and Hough Unit were not affected by the treatments. In 300 mg/kg PP group, albumen and yolk ratios were found to be significantly higher, but shell breaking strength was also high. As a result, it is thought that adding 450 mg/kg PP to laying hen diets will bring positive results, as it increases egg production and improves feed conversion ratio.

Keywords: *Laying Hen*, Performance, Pomegranate Peel

INTRODUCTION

Feed additives are widely used in order to obtain more weight gain, higher yield and better product quality. Many feed additives have been used for this purpose and successful results have been obtained. There are several features that should be taken into consideration when using feed additives, the most important of which are that they are economical and do not adversely affect animal and human health.

Pomegranate (*Punica granatum L.*), a tropical climate fruit, grows abundantly in our country and ranks 3rd in the world in terms of production. Approximately 30% of the pomegranate, which will produce around 728,112 tons (TUIK, 2023) in 2022, is exported, while the remaining part is used for domestic consumption. Pomegranate, whose total weight consists of approximately 48% of the peel (Zarei et al., 2011), shows high levels of antioxidant and antitumor activity thanks to the phenolic substances it contains (Akbulut et al., 2010). Although pomegranate peel is extremely rich in terms of hydrolyzable phenolics, it contains primarily ellagitanen and its isomers, as well as smaller amounts of punicalin (4,6-galla-gylglucose), gallic acid, ellagic acid and ellagic acid glycosides (hexoside, pentoside, rhmnoside etc.). (Tokuşoğlu, 2013). It has been suggested that pomegranate peel or pulp, which becomes inert after processing, can be used as an alternative feed source (Sarıca, 2011).

The unused part of the seed and bark contains bioactive components rich in polyphenols (the peel contains 44.0% and the seed is 02.6% phenolics), vitamins, and polyunsaturated fatty acids (Singh et al., 2002; Seeram et al., 2005). It was reported that the addition of 4% of pomegranate peel, which is rich in bioactive components, to broiler diets using waste oil reduced the peroxide value in meat (Sadabadi et al., 2021; 2022). In the current study, the effect of adding pomegranate peel, which is an extremely valuable and economical waste, to laying hen rations at different levels was investigated on performance and egg quality.

MATERIAL AND METHOD

The study consists of 120 Lohman LSL laying hens at 64 weeks of age, divided into 5 groups, each containing 24 animals (subdivided into 6 subgroups). The first group serves as the control group and is fed with basal feed, while the other four groups are fed with feeds containing different concentrations of pomegranate peel (150, 300, 450 and 600 mg/kg) added to the basal feed. The trial lasts for 8 weeks, during which the animals have ad-libitum access to both feed and water. Additionally, the research protocol has been approved and adheres to the Animal Ethics Committee Guidelines of Atatürk University (No: 2016/78). The feeds used in the experiment were obtained from a commercial feed company. After the pomegranates were peeled and cleaned, the resulting peels were dried at room temperature and then ground.

Determination of General Performance Values

Feed consumption and egg production, egg weight and damaged egg rate were determined by measurements made every two weeks. In addition, egg quality parameters such as shape index, shell thickness, breaking strength, white ratio, shell ratio, yolk ratio and Haugh unit were determined in eggs collected from each subgroup.

The differences between the groups were determined by analysis of variance (ANOVA) using the SPSS package program (SPSS 20.0). Duncan multiple comparison test were used to compare the treatment averages.

RESULTS AND DISCUSSION

The results of the performance values of laying hens to which different levels of pomegranate peel were added to their diets are presented in Table 1.

When Table 1 was examined, it was determined that the feed consumption value was highest in the groups in which 300 and 450 mg/kg pomegranate peel was added to the diet. The highest egg production and best feed conversion values were detected in the groups supplemented with 300, 450 and 600 mg/kg pomegranate peel, while the highest egg weight was observed in the group supplemented with 300 mg/kg pomegranate peel.

In the current study, it was observed that egg production increased and feed conversion ratio improved in parallel with the increase in pomegranate peel level. Abbas et al. (2017), added pomegranate peel to quail diets at different levels (0, 2.5, 5 and 7.5%) and reported that feed intake, egg weight and egg production increased with increasing pomegranate peel level and feed conversion was significantly improved. The present researchers attributed this positive result to the flavor and digestive stimulating effects of the additives. Yassein et al. (2015), reported that adding 10 and 15 g pomegranate peel powder/kg to quail diets improved feed conversion by reducing feed consumption. Positive effects of herbal feed additives on gastrointestinal enzymatic activity and increased nutrient absorption and digestibility were reported by Banerjee et al. (2013). Gharagozloo et al. (2023), reported that the addition of pomegranate seeds to the diet of laying hens improved egg production due to the antioxidant effect of pomegranate. Bölükbaşı et al. (2023), suggested that adding 1.5 ml/kg pomegranate seed oil to the laying hen diet significantly increased egg production.

Table 1. Effect of pemagranate peel on performance in laying hens

Groups	Feed Intake (g)	Egg Weight (g)	Egg Production (%)	Feed Conversion Ratio (g:g)
Control	116.66b	66.96b	73.38b	2.41a
150 mg/kg PP	122.36ab	67.26b	76.27b	2.42a
300 mg/kg PP	128.58a	72.02a	86.31a	2.10b
450 mg/kg PP	130.28a	68.21b	87.59a	2.21b
600 mg/kg PP	122.03ab	69.08ab	84.46a	2.11b
SE	1.49	0.54	1.21	0.045
P	0.025	0.021	0.001	0.050

[a, b]**:** The column average is significantly different ($p<0.05$) **SE:** standard error.

The results of egg quality criteria in laying hens to which different levels of pomegranate peel were added to their diets are presented in Table 2. When Table 2 was examined, it was determined that egg shell ratio, egg shell thickness and Hough Unit were not affected by the treatments. It was determined that adding 150 mg/kg pomegranate peel to the diet significantly ($P<0.05$) reduced the albumen rate and increased the egg yolk content. The highest damaged egg rate and the lowest shape index were seen in the group in which 300 mg/kg pomegranate peel was added to the diet.

It was determined that the addition of 150 mg/kg pomegranate peel to the diet increased the shell breaking strength significantly (P<0.01) compared to other groups. It was determined that the lowest shell breaking resistance was in the control group and the 300 mg/kg PP group. Yassein et al. (2015), found that the addition of 10 and 15 g pomegranate peel powder/kg to quail diets had no effect on egg quality criteria. Some studies have shown that adding pomegranate seed pulp to the laying hen diet (Saki et al., 2014) and adding pomegranate juice to drinking water (Gültepe et al., 2022) have no effect on egg breaking strength.

Table 2. Effect of pomagranate peel on egg quality in laying hens

Groups	Albumen (%)	Yolk (%)	Shell (%)	Damaged Egg Ratio (%)	Shell Thickness (µm)	Shape Index	Hough Unit	Shell breaking Strength (Kg/cm^2)
Control	55,23a	32,20b	12,58	0.20b	0.446	72.91a	87.37	2.08d
150 mg/kg PP	54,55b	32,62a	12,82	0.25b	0.447	73.19a	87.97	2.84a
300 mg/kg PP	55,13a	32,44ab	12,42	0.74a	0.438	70.89b	85.63	2.05d
450 mg/kg PP	55,56a	32,15b	12,29	0.08b	0.437	73.43a	87.10	2.32c
600 mg/kg PP	55,24a	32,35b	12,41	0.20b	0.449	71.90ab	82.74	2.60b
SE	0.28	0.15	0.091	0.06	0.005	0.29	0.72	0.07
P	0.025	0.018	0.344	0.005	0.944	0.041	0.099	0.001

a, b: The column average is significantly different (p<0.05)

SE: standard error

CONCLUSION

In conclusion, our findings indicate that incorporating 450 mg/kg of PP into laying hen diets yields positive outcomes, evidenced by significantly elevated albumen and yolk ratios, along with enhanced shell breaking strength. This supplementation not only increases egg production but also improves the feed conversion ratio, suggesting its potential as a beneficial additive for optimizing poultry nutrition and performance.

Statement of Conflict of Interest

The authors declared that they have no conflicts of interest to this work

Authors' Contributions

ŞCB designed and analyzed the research, BD studies arranged. ŞCB and BD worked on the preparation of pictures and tables. All authors contributed to the writing of the article and took part in the process of publication of the article and read and approved it.

REFERENCES

Abbas, R.J., Al-Salhie, K.C.K., Al-Hummod, S.K.M. 2017. The effect of using different levels of pomegranate (*Punica granatum*) peel powder on productive and physiological performance of japanese quail (Coturnix coturnix japonica). Livest Res Rural Dev, 29, 1-7.

Akbulut, G., Yıldız, Atilla., Yalınca, R. 2010. Nar: Bilimi ve Potansiyel Sağlık Etkileri, Kafkas Üniv Fen Bil Enst Derg, 3(2):53-64.

Banerjee, S., Mukhopadhayay, S.K., Haldar, S., Ganguly, S., Pradhan, S., Patra, N.C., Niyogi, D. and Isore, D.P. 2013. Effect of Phytogenic Growth Promoter on Broiler Birds. Journal of Pharmacognosy and Phytochemistry, 1:183–188.

Bölükbaşı, Ş.C., Dumlu, B., and Yağanoğlu, A.M. 2023. Improved biological value of eggs due to the addition of pomegranate seed oil to laying-hen diets, Arch. Anim. Breed., 66, 121-129, https://doi.org/10.5194/aab-66-121-2023

Gharagozloo, A., Kheiri, F., Nasr, J., & Faghani, M. 2023. Egg production, egg quality, blood lipids, and ovarian related indices in laying hens fed pomegranate by-products. Journal of the Hellenic Veterinary Medical Society, 74:(3), 6227-6232. https://doi.org/10.12681/jhvms.31293

Gultepe, E.E., Iqbal, A., Çetingül, İ.S., Uyarlar, C., Özçınar, Ü., Bayram, İ. 2022. Effects of pomegranate (*Punica granatum L.*) juice as a shortterm water supplement during the peak production cycle in laying hens. Ankara Üniversitesi Veteriner Fakültesi Dergisi.

Sadabadi, M.G., Ebrahimnezhad, Y., Sis, N.M., Teli, A.S., Ghalehkandi, J.G. and Veldkamp, T. 2021. Supplementation of pomegranate processing waste and waste soybean cooking oil as an alternative feed resource with vitamin E in broiler nutrition nutrition: effects on productive performance, meat quality and meat fatty acid composition, Arch. Anim. Nutr., 75, 355–375, https://doi.org/10.1080/1745039X.2021.1965414

Sadabadi, M.G., Ebrahimnezhad, Y., Sis, N.M., Teli, A.S., Ghalehkandi, J.G. and Veldkamp, T. 2022. Effects of supplementation of pomegranate processing by-products and waste cooking oils as alternative feed resources in broiler nutrition, Sci. Rep., 12, 21216, https://doi.org/10.1038/s41598-022-25761-7

Saki, A.A., Rabet, M., Zamani, P., Yousefi, A. 2014. The effects of different levels of pomegranate seed pulp with multi-enzyme on performance, egg quality and serum antioxidant in laying hens. Iranian J Appl Anim Sci 4:803-808.

Sarıca, Ş. 2011. Nar suyu yan ürünlerinin hayvan beslemede kullanım olanakları. Gaziosmanpaşa Üniversitesi, Ziraat Fakültesi Dergisi 28:(2), 97-101.

Seeram, N.P., Adams, L.S., Henning, S.M., Niu, Y., Zhang, Y., Nair, M.G. and Heber, D. 2005. In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice, J. Nutr. Biochem., 16, 360-367.

Singh, R.P., Chidambara Murthy, K.N. and Jayaprakasha, G.K. 2002. Studies on the antioxidant activity of pomegranate (Punica granatum) peel and seed extracts using in vitro models, J. Agr. Food Chem., 50, 81–86.

Tokuşoğlu, Ö. 2013. Her Yönüyle NAR. Man.Gıda, Tarım, Hayvancılık. Gıda, Tarım ve Hayvancılık Müdürlüğü Dergisi. Yıl:3, Sayı:6, Ocak-Şubat-Mart 2013. Sh:28-30.

Türkiye İstatistik Kurumu (TÜIK): Meyveler içecek ve baharat bitkileri, https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-Istatistikleri-2022-45504 (last access: 20 November 2023), 2022 (in Turkish).

Yassein, D.M.M., Abdallah, E.A., Ismail, I.I and Faddle, A.A. 2015. Effect of Dietary Supplementation of Pomegranate Peel Powder and Butylated hydroxy Toluene on some Productive, Physiological and Immunological Parameters of Japanese Quail. Egyptian Journal of Animal Production 52 (Suppl. Issue):105-113.

Zarei, M., Azizi, M., and Bashir-Sadr, Z. 2011. Evaluation of physicochemical characteristics of pomegranate (Punica granatum L.) fruit during ripening, Fruits, 66, 121-129.

Effects of Boric Acid Application on Some Quality Parameters and Yield Values of Karaerik Grape Variety Grown under Greenhouse

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ABSTRACT: In this study, the effects of boric acid in solution applied to soil and plant leaves on some fruit quality parameters and yield values of grapevines were investigated. In the research, 5-year-old Kararerik grape variety grown on its own roots under greenhouse; Boric acid (BA) was applied from soil, leaves and soil+leaf. In the study carried out for two years, applications were made twice for each vegetation period, including the flowering and veraison periods. The research was carried out in coarse textured (sandy loam) greenhouse soil, which provides a suitable environment for the development of the Karaerik grape variety. For each application period, 15lt/application from soil was applied to all plants in the 1st group, 5lt/application from the leaves was applied to all plants in the 2nd group, and 5lt/application from soil+leaf was applied to all plants in the 3rd group (2.5lt soil+2.5lt leaf). In the study, yield values as well as quality parameters such as berry width, berry length, berry weight, cluster width, cluster length, average cluster weight and number of clusters in the vines treated with boric acid were determined. As a result of the study, it was determined that depending on the boric acid applications, soil, leaf and soil+leaf applications increased the average yield value of the vines by 15%, 17% and 14%, respectively, compared to the control group.

Keywords: Boric acid, Grapevine, Greenhouse soils, Karaerik

INTRODUCTION

Grape (*Vitis vinifera* L.) is one of the most important commercial fruit products of subtropical regions in the world and is grown successfully in tropical and temperate regions. The production potential and quality of grapes, which is one of the most important fruit species in our country with 4,175,000 tons of grape production in 467,093 ha area, depends on various factors such as climate, cultural practices in the vineyard, mineral nutrition, irrigation, etc. (Küpe, 2021; TUIK, 2021). One of the most basic ways to increase the income from viticulture is to increase the grape yield obtained from unit area (Çakır and Gürsöz, 2002). As in other plant production branches, fertilization is one of the methods frequently used by producers to increase yield and quality in viticulture (Özçelik and Özer, 2006; Akın ve Sarıkaya, 2012; Akçay, 2013). The dose, content, application method and period of the fertilizer to be applied to the vineyards directly affect the grape yield and quality (Akgül et al., 2007; Tudor et al., 2013; Lasa et al., 2012). Although vines require less micronutrients than macronutrients, adequate intake of micronutrients is important for grape yield and quality. Insufficient intake of Fe, B, Zn and Cu, which are micronutrients, causes some physiological disorders (Çelik, 2011; Kasap, 2012). Boron is the most frequently deficient microelement among these elements (Gupta, 1993). Boron deficiency causes significant reductions in berry set, yield and quality in grapevines (Christensen et al., 2006). Boron in the form of boric acid $B(OH)_3$ or borate B(OH) in the soil is taken up by plants in the form of boric acid (Aybaba, 2010). The factors affecting the uptake of B by plants from the soil are the plant-available B content of the soil, soil pH, the type of exchangeable ions in the soil, the amount and type of minerals in the soil, and the organic matter content of the soil (Keren and Bingham, 1985; Goldberg, 1997). Boron (B) plays a very important role in the development and maintenance of structural properties in grapevine. This element was determined to be absolutely essential for higher plants about 84 years ago and entered the literature as one of the essential elements for plant nutrition (Waqar et al., 2012). Boron is a mineral that causes significant problems in grapevine when it is deficient or excessive. It is reported that boron fertilization in grapevines has positive effects on the formation of plant tissues, pollen germination, fruit yield and development (Ebadive et al., 2001; Peacock and Christensen, 2005; Rolshausen and Gubler, 2005). In boron deficiency; the internodes in the shoot are narrowed and the leaves at the shoot tip are discolored from the leaf margin to the inner parts and drying and shrinking of the leaves are observed. In excessive boron deficiency, the dried leaves fall off (Esetlili ve Anaç, 2010). There is a decrease in the berry set of the clusters (Atalay, 1982). Fortunati (2006) found that boron deficiency caused yellowing of the leaves, abnormal fruit set and the development of grape berries of different sizes in the cluster.

In this study, a total of 4 applications of Boric acid (BA) were made to 5-year-old vines at the beginning of the flowering period and at veraison periods during the 2-year study. In the research, Boric acid was applied to the Karaerik grape variety grown under greenhouse conditions in 3 different ways: soil, leaf and soil + leaf. As a result of the study, the effects of boric acid application on grape berries and clusters, as well as on yield values, were determined.

MATERIAL AND METHOD

In the research, 5-year-old vines of Karaerik grape varieties, which are widely grown in the Erzincan region, were used. Vineyards grown in unheated greenhouse conditions were watered once a month during the vegetation period. A total of 20 plants were used in the study. In the experiment, 5 plants each were used in the control group, soil, leaf and soil + leaf Borik asit (H_3BO_3) applications. For each application period, 15lt/application from soil was applied to all plants in the 1st group, 5lt/application from the leaves was applied to all plants in the 2nd group, and 5lt/application from soil+leaf was applied to all plants in the 3rd group (2.5lt soil+2.5lt leaf). In the study, 25 lt stock solution was prepared with distilled water using 10 g boric acid. In soil application (1st application) 3 lt per plant (1.2 mg/plant B), in foliar application (2nd application) 1 lt per plant (0.4 mg/plant B), in soil + leaf (3rd application) 1 lt per plant (0.2 mg/plant B soil + 0.2 mg/plant B leaf) were used. Boric acid application was made to the plant root area and leaves twice a year, at the beginning of the flowering period and during veraison.

The research was carried out for 2 years, 2021 and 2022, and at the end of the study, fruit pomological characteristics such as berry width, berry length, berry weight, cluster width, cluster length, number of clusters and average cluster weight, as well as yield parameters, were determined. For each application, the clusters on the vine were counted one by one, during the harvest period. Berries that could represent the cluster were selected from the middle region of the cluster. The width and length of all clusters and berries on a vine were determined with the help of calipers, and their weight values were determined with a precision scale. Berry weight values were determined in grams by weighing 10 berries taken from the clusters of vines on a 0.1g sensitive scale (Gürsöz, 1993). Yield values were determined as a total by measuring the individual weights of all clusters on a vine during the harvest period (Kupe and Kose, 2015).

According to USDA (1999), the soils used in the research are in the sandy loam class. Sub-samples to be used for basic analyzes were prepared by sieving through 2 mm sieves from soil samples that were duly taken from the greenhouse and air-dried under laboratory conditions. Soil texture was determined by Bouyoucos hydrometer method (Gee and Bauder, 1986), particle density were determined by pycnometer method (Blake and Hartge, 1986), organic matter content by Smith Weldon method (Nelson and Sommers, 1982), soil reaction (pH) by glass electrode pH meter (McLean, 1982), lime content by Scheibler calcimeter (Nelson, 1982), electrical conductivity (EC) value with electrical conductivity instrument (Rhoades, 1982), Ca^{++}, Mg^{++}, Na^{+} and K^{+} contents of the soils were determined by (Kacar, 2008) method. Statistical analysis was performed by ANOVA, and differences between means were tested using Duncan's multiple range test.

RESULTS AND DISCUSSION

Basic soil analysis results are presented in Table 1. In this study, the texture class of the soils was determined as coarse textured in the sandy clay loam texture class (60% sand, 26% silt, 14% clay). Soil organic matter content (3.40%) is in the well class; pH level was found to be 7.58 and neutral, the EC level of the working soils is 1,23 dS/m without salt, the $CaCO_3$ level was determined as 5.18% in the medium calcareous (Ülgen and Yurtsever, 1995), class. According to the available phosphorus contents, the class of the soils was determined as medium (Ülgen and Yurtsever, 1995), Ca++, Mg++, Na+, K+ contents were determined as 8.20, 5.40, 1.24, 2.32 me/100gr, respectively. Particle density were determined as 2.68 (g/cm3) (Table 1).

Table 1. Results of some basic physical and chemical analyzes of the researched soils

Soil Properties	
Sand (%)	60
Silt (%)	26

Clay (%)	14
Texture class	Sandy loam
Particle density (g/cm^3)	2.68
Organic matter (%)	3.40
pH	7.58
EC (dS/m)	1.23
$CaCO_3$ (%)	5.18
Ca, Mg, Na, K (me/100gr)	8.20; 5.40; 1.24; 2.32

EC: Electrical conductivity

Boron is taken from the soil by passive absorption by the roots, and soil pH, amount of organic matter, moisture and temperature are also effective in this uptake (Goldbach, 1997; Günes, 2003). According to current information, it has been determined that boron uptake by plants and its transport to different organs are closely related to the water uptake of the plant, and boron transport varies among plant species (Marschner, 1995).

Table 2. Yield and pomological features of Karaerik

Yield and pomological features	**Karaerik Variety**			
	Control	**Soil**	**Leaf**	**Soil+Leaf**
Yield (gr)	8295b	9540a	9690a	9452a
Berry Width (cm)	1.83b	2.3a	2.2a	2.1a
Berry Size (cm)	2.25c	2.70ab	2.87a	2.58b
Berry Weight (gr)	6.32c	7.59b	8.87aab	7.81
Cluster Width (cm)	13.7a	15.06a	14.9a	14.6a
Cluster Size (cm)	19.5b	21.20a	21.06a	19.77b
Number of Clusters (item)	21.33a	24.66a	21.33a	21.66a
Cluster Weight (gr)	389.5b	476.6a	485.4a	430ab

When yield and pomological development parameters were examined, it was determined that there was a statistical difference between the application and control groups ($p<0.05$). When the yield parameter of the vines was examined, the highest value (9.690 g) was determined in the vines to which Boric acid was applied foliar (2nd group), while the lowest value (8.295 g) was determined in the control group. While the average yield value in the soil application (1st group) was 9540 g, the average yield in the soil + leaf (3rd group) application was found to be 9452 g. Depending on the soil, leaf and soil + leaf Boric acid applications, it was determined that there was an increase of 15%, 17% and 14%, respectively, compared to the control group (Table 2). It is not possible to achieve the targeted yield in vineyards established on soils that do not contain sufficient levels of boron. As a matter of fact, Kasap (2012), reported that B deficiency in the vineyard soil can be eliminated by fertilizing the soil if it is detected while the vines are in dormancy, and if this situation is detected during the vegetation period, foliar fertilization instead of soil fertilization will give faster and more effective results. Khanduja and Balasubramanyam (1976), in their study examining the effect of boron on grape quality, applied 0.1-2.0% boric acid as a spray. As a result of the study, they determined that boric acid was effective in the formation of flower arches, increase in yield and sugar content. In a similar study, it was determined that BA applications caused a regular increase in cluster weight in Bez El-Anza grape variety (Mostafa et al., 2006). Also, Ally et al., (2015) and Er et al., (2011) reported in their study that boron applications had positive effects on yield. When the changes in pomological properties depending on BA applications were examined in the research; It was determined that the highest berry width was 2.3 cm in the 1st application (soil), the highest berry length was 2.87 cm in the 2nd application (leaf), and the highest berry weight value was 8.87 g in the 2nd application (leaf). Depending on the applications, it was determined that grain width, berry length and berry weight values increased by 25.7%, 27.5% and 40.3%, respectively, compared to the control group (Table 2).

Bavaresco et al., (1989) examined the effect of boron application to grape leaves on boron deficiency in the soil. They determined that boron applications had an effect on increasing yield and improving fruit set in Garganega grape variety. Singh and Rethy (1996), applied boric acid as a foliar spray to 6-year-old grapevines twice in March. In the study, the effect of boric acid on yield and quality was examined. It was determined that the application of 0.1% boric acid increased quality and yield. In addition, they obtained the highest cluster and berry number values from the lower boron application dose of 0.05%.

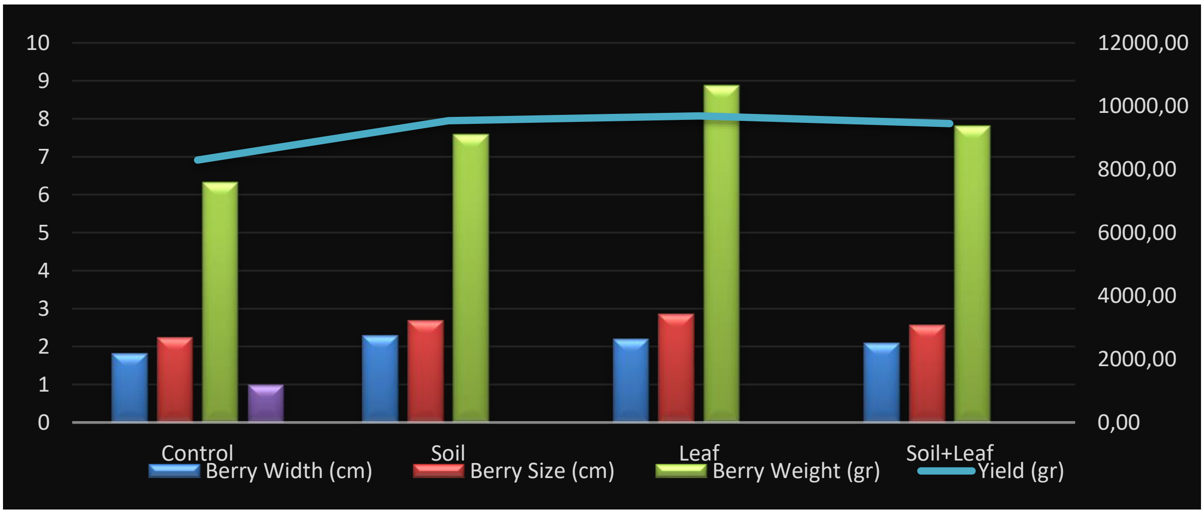

Figure 1. The relationship between yield and berry characteristics depending on Boric acid applications in Karaerik grape variety.

The change caused by boric acid applied to soil, leaves and soil + leaves in Karaerik grape variety on yield parameters as well as berry properties is presented in Figure 1. When Figure 1 is examined, it is seen that there is an increase in yield values in parallel with the increase in berry width, berry length and berry weight depending on the applications.

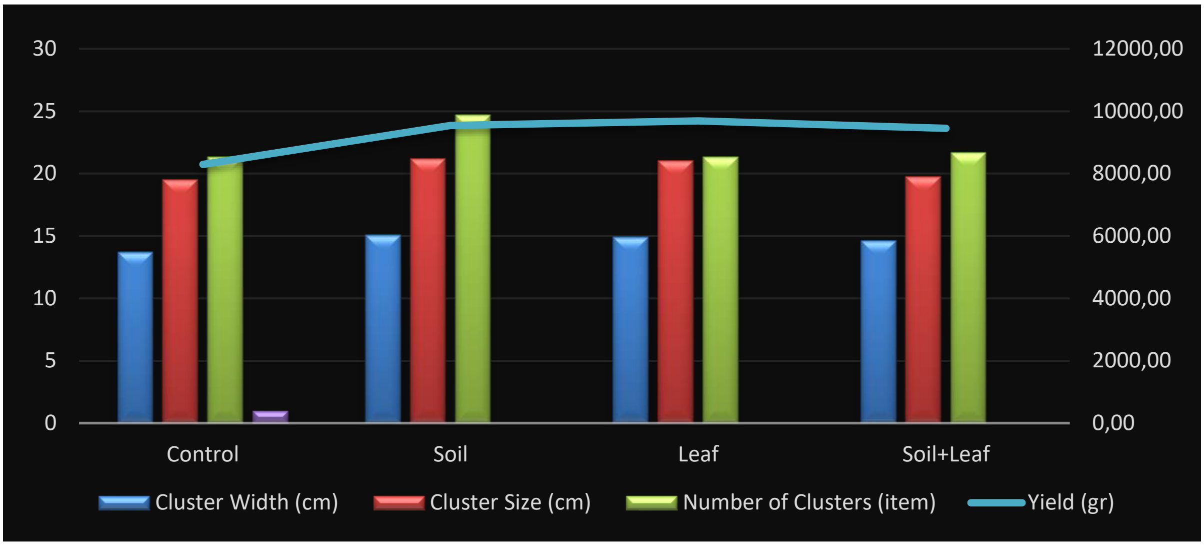

Figure 2. The relationship between yield and cluster characteristics depending on Boric acid applications in Karaerik grape variety.

In the Karaerik grape variety applied with boric acid; It was determined that there was an increase in cluster width, cluster length and cluster number values compared to the control group, and in parallel with this increase, the average yield value per vine also increased (Figure 2). Research conducted on many grape varieties belonging to the *V. vinifera* L. species shows that foliar application of B to vines will increase grape yield and quality (Longbottom et al., 2010; Er et al., 2011). Depending on the boric acid applications, it was observed that the values of cluster width, cluster length and number of clusters reached the highest value in the soil application, while the highest cluster weight value was determined in the leaf application. Depending on the applications, it was determined that the cluster width, cluster size, cluster number and cluster weight values increased by 9.92%, 8.71%, 15.61% and 22.30%, respectively, compared to the control group (Table 2). As a matter of fact, Ally et al., (2015) reported in their study that the boron they applied foliarly at a concentration of 7% to the Superior grape variety, when the berries reached 6-8 mm and during the veraison period, had positive effects on the yield by significantly increasing the cluster weight. Similarly, Akın and Çoban (2016), reported that cluster weight increased significantly in their research where they applied 1000 mg l-1 BA along with cluster thinning at different rates to the vines of the Alphonse Lavallée grape variety, one week before flowering and during the berry setting period.

In this study, it was determined that Boric acid applications made from soil and leaves had positive effects on the yield of Karaerik grape variety grown under greenhouse conditions.

In parallel, there was a positive effect on the fruit pomological characteristics on grain width, grain length, grain weight, cluster width, cluster length, number of clusters and cluster weight.

CONCLUSION

In this study, the effects of boric acid applications to soil, leaf and soil+leaf at different periods on yield and some quality parameters of Karaerik grape variety consumed as table grape were investigated.

In the application made twice during a vegetation period, before flowering and during veraison, it was observed that all Boric acid applications were effective in terms of average yield value per vine, but the highest results were obtained from foliar application. It has also been determined that boric acid applications have effects on cluster and berry development. It is known that boron deficiency is common in the Northeastern Anatolia Region of Turkey and this may affect production and vine quality. When the results of this study are evaluated, it is predicted that the determination of boron deficiency in soil and plants in the cultivation of Karaerik grape variety in open and greenhouse conditions will contribute to more economical viticulture in the region due to the increase in yield and quality with foliar and soil boron application.

REFERENCES

Akçay, K., 2013. Sultani çekirdeksiz üzüm çeşidinde farklı seviyede yaprak alma ve yaprak gübresi uygulamalarının üzüm verimi ve kalitesine etkileri. Graduate School of Natural and Applied Sciences, master Thesis, Konya (in Turkish)

Akgül, A., Kara, S., Çoban, H., 2007. Yapraktan çinko (Zn) uygulamalarının sultan çekirdeksiz (*vitis vinifera* L.) üzüm çeğidinde üzüm verimi ile bazı kalite özelliklerine etkisi. Celal Bayar Üniversitesi Fen Bilimleri Dergisi, 3(2): 183–190.

Akın, A. Sarıkaya, A., 2012. Hasandede üzüm çeşidinde salkım ucu kesme ve hümik asit uygulamalarının üzüm verimi ve kalitesine etkileri. Sakarya Üniversitesi Fen Edebiyat Dergisi, 2012(1): 267-274.

Akın, A., Çoban, H., 2016. The effects on yield and yield components of different level cluster tip reduction and foliar boric acid applications on alphonse lavallee grape cultivar. International Journal of Agricultural and Biosystems Engineering, 10(5): 262-267.

Ally, M.A., Thanaa, M.E., Harhash, M.M.M., Rehab, M.A., 2015. Effect of foliar potassium, boron treatments and girdling on growth, productivity and leaves chemical composition of table grape "superior cv. "covering with plastic sheets. Middle East Journal of Agriculture Research, 4 (2): 170-180.

Atalay, I.Z., 1982. Bağlarda beslenme bozukluklarının teşhisi. Ege Üniversitesi Ziraat Fakültesi Toprak Bölümü, Ders Notları. (in Turkish)

Aybaba, Ş., 2010. Gemlik zeytin çeşidinde dal eğme ile birlikte yapraktan bor ve çinko uygulamalarının meyve verimi ve kalite üzerine etkisi. Graduate School of Natural and Applied Sciences, master Thesis, Adana. (in Turkish)

Bavaresco, L., Fregonı, M., Fraschjnı, P., 1989. Investigations on iron uptake and reduction by excised roots of different grapevine rootstocks and a *V. vinifera* cultivar. 5th Intern. Symp. Iron Nutrition and Interaction in Plants.

Blake, G.R., Hartge, K.H., 1986. Bulk density. In A. Klute (ed.), Methods of Soil Analysis. Part 1. 2nd ed. Agronomy Monographs. 9. Soil Science Society of America, Madison, 363-382.

Christensen, L., Beede, R., Peacock, W., 2006. Fall foliar sprays prevent boron-deficiency symptoms in grapes. California Agriculture, 60(2): 100-103.

Çakır, A., Gürsöz, S., 2002. GAP alanında yetiğtirilen bazı sofralık ve şaraplık üzüm çeşitlerinde farklı sulama düzeylerinin besin maddesi alımına etkileri. Türkiye Bağcılık ve şarapçılık Sempozyumu, Nevşehir (Kapadokya) Türkiye.

Çelik, S., 2011. Bağcılık (Ampeloloji), Cilt 1 (3. Baskı), Anadolu Matbaa San. ve Tic. Ltd. şti., Tekirdağ. (in Turkish)

Ebadive, A., Atashkar, D., Babalar, M., 2001. Effect of boron on pollination and fertilization in seedless grapevine cvs. white seedless and askary. Iran Journal of Agricultural Science, 32: 457-465.

Er, F., Akın, A., Kara, M., 2011. The Effect of different ways and dosages of boron application on black dimrit (*Vitis vinifera* L.) grape's yield and quality. Bulgarian Journal of Agricultural Science, 17 (4): 544-550.

Esetlili, B.Ç., Anaç, D. 2010. Bağ yetiştiriciliğinde gübreleme. Önemli kültür bitkilerinin gübrelemesi. İzmir, 1-11. (in Turkish)

Fortunati, P., 2006. Foliarel OK for control of boron deficiency. Vignevini, 33:54–56.

Gee, G.W., Bauder, J.W., 1986. Particle-size analysis. In: Klute, A. (Ed.), Methods of soil analysis. Part 1. Physical and mineralogical methods. 2nd edition. American Society of Agronomy. Soil Science Society of America, Madison, pp. 383-411.

Goldbach, H.E., 1997. A critical review on current hypotheses concerningthe role of boron in higher plants: suggestions for further research andmethodological requirements. J. Trace Micr. Techn. 15: 51-91.

Goldberg, S., 1997. Reactions of boron with soils plant and soil, Kluwer Academic Publishers. USDA, 193: 35-48.

Gupta, U.C., 1993. Deficiency and toxicity symptoms of boron in plants. Boron and its role in crop production. CRC Press, Boca Raton, FL, pp.147-56.

Günes, A., 2003. Asmaların (Vitis spp.) bor toksisitesi ve tuzluluğa karşı toleransının belirlenmesine yönelik olarak bor, sodyum ve klor alımlarının karşılaştırılması. Journal of Agricultural Sciences, 9(4).

Gürsöz, S., 1993. GAP alanına giren güneydoğu anadolu bağcılığı ve özellikle şanlıurfa ilinde yetiştirilen üzüm çeşitlerinin ampelografik nitelikleri ile verim ve kalite unsurlarının belirlenmesi üzerinde bir araştırma. Graduate School of Natural and Applied Sciences, doctorate Thesis, Adana. (in Turkish)

Kacar, B., İnal, A., 2008. Bitki analizleri, Fen Bilimleri, Nobel Yayın No: 1241, 892. (in Turkish)

Kasap, Y., 2012. Bağcılık ve gübreleme. Ravza Yayınları, İstanbul. (in Turkish)

Keren, R., Bingham, F.T., 1985. Boron in water, soils, and plants. In Advances in soil science Springer, New York, pp. 229-276.

Khanduja, S.D., Balasubramanyam, V.R., Agnihotri, O.P., (1976). Effect of boron on fruit quality, nucleic acid and protein content of leaves in grapevine. Indian Journal of Horticulture, 216-219.

Küpe, M., 2021. Türkiye bağcılığında son yıllardaki değişimler ve mevcut durum. Tohumdan Sofraya Sürdürülebilirlik Tarımsal Kaynak Tüketimi İçin Bazı Öneriler. İksat Yayınevi. Ankara, pp. 147-174. (in Turkish)

Küpe, M., Köse, C., 2015. Determining suitable pruning level after winter frost damage in Karaerik grape cultivar. Ziraat Fakültesi Dergisi, Atatürk Üniversitesi, 46(1): 21-28.

Lasa, B., Menendez, S., Sagastizabal, K., Cervantes, M.E.C., Irıgoyen, I., Muro, J., Aparicio-Tejo, P.M., Arız, I., 2012. Foliar application of urea to sauvignon blanc and merlot vines: doses and time of application. Plant Growth Regul, 67: 73-81.

Longbottom, M.L., Dry, P.R. Sedgley, M., 2010. Effects of sodium molybdate foliar sprays on molybdenum concentration in the vegetative and reproductive structures and on yield components of Vitis vinifera cv. Merlot. Australian Journal of Grape and Wine Research, 16, 477-490.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Second Edition. Academic Press New York.

McLean, E.O., 1982. Soil pH and Lime Requirement. In: Page, A. (Ed.), Methods of Soil Analysis Part 2. Chemical and Microbiological Properties. 2nd Edition. Agronomy, 9: 199- 224.

Mostafa, E.A.M., El-Shamma, M.S., Hagagg, L.F., 2006. Correction of b deficiency in grapevines of bez-elanaza cultivar. American-Eurasian Journal of Agricultural and Environmental Sciences, 1: 301-305.

Nelson, D.W., Sommers, L.E., 1982. Total carbon, organic carbon, and organic matter. In: Page, A.(Ed.), Methods of soil analysis. part 2. Chemical and Microbiological Properties. Madison, 2nd edition. Agronomy, Wisconsin USA, 1159 (9): 539-579.

Nelson, R.E., 1982. Carbonate and gypsum. Methods of soil analysis. Part 2. Chemical and microbiological properties. 2nd edition. Agronomy, 181-197.

Özçelik, A., Özer, O.O., 2006. Çiftçilere yapılan kimyevi gübre desteği ve tarımsal faaliyette kullanılan mazot için destekleme ödemelerinin değerlendirilmesi. Tarım Bilimleri Dergisi, 13(1): 1-8.

Peacock, W., Christensen, L., 2005. Drip irrigation can effectively apply boron to San Joaquin Valley vineyards. California Agriculture, 59(3): 188-191.

Rhoades, J.D., 1982. Soluble salts. In: Page, A. (Ed.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd Edition. Agronomy, Madison, isconsin USA, 1159 (9): 167-178.

Rolshausen, P.E., Gubler, W.D., 2005. Use of boron for the control of Eutypa dieback of grapevines. Plant Disease, 89(7): 734-738.

Singh, B., Rethy, P., 1996. Effect of certain micronutrients and NAA on the yield of kagzi lime (Citrus aurantifolia Swingle). Advances Hort and Forestry, 5: 43-49.

Tudor, E., Grigore, A., Dumitru, M., Sirbu, C., Cioroianu, T., 2013. Influence of four foliar fertilizers on the quality and quantity of the production of cabernet sauvignon grapes in the context of iron chlorosis. Lucrari Ştiintifice, 56(2), 159-164.

TUİK, 2021. Türkiye İstatistik Kurumu (TUİK), https://tuik.gov.tr, (Date: 23.04.2023).

USDA, 1999. United States Department Of Agriculture. Soil Taxonomy, A Basic System of Soil Classification for Making and Interpreting Soil Surveys. Second Edition.

Ülgen, N., Yurtsever, N., 1995. Türkiye Gübre ve Gübreleme Rehberi (4. Baskı). Başbakanlık Köy Hizmetleri Genel Müdürlüğü Toprak ve Gübre Araştırma Enstitüsü Müdürlüğü Yayınları. Genel Yayın, Teknik Yayınlar. Ankara 209: pp 66-230. (in Turkish)

Waqar, A., Munir, H.Z., Sukhdev, S.M., Abid, N., Saifullah H., 2012. Boron deficiency in soils and crops. A review. In: Crop plant, Goyal, A. (ed.), InTech, 77–114.

Effects of Mulch Use on Soil Physical Properties, Plant Growth and Yield Parameters in Strawberry Cultivation under Erzurum Conditions

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ABSTRACT: In this study, plant growth and yield parameters of Monterey and Albion strawberry cultivars were investigated under Erzurum central conditions (altitude: 1850m) in open plots prepared without mulch and on standard raised bed covered with black pilastic mulch. At the end of the 2-year study conducted under field conditions in 2021-2022 and 2022-2023, aggregate stability, water permeability, bulk density values, porosity and organic matter contents of under mulch and non-mulched soil were determined. According to the research findings, there was a statistical difference between the average values of the varieties in terms of total yield per plant in both years. The highest yield value was obtained from the Monterey strawberry variety (265 g/plant) grown on mulch in the 2nd year of the study, while the lowest yield value was obtained from the Monterey strawberry variety (140 g/plant) grown on non-mulched soil in the 1st year of the study. When plant development parameters were examined, the number of fruits per plant, average fruit weight, fruit width, fruit length, leaf width, leaf length and petiole length of the Monterey and Albion varieties using mulch were found to be higher than strawberries grown in the non-mulched field. As a result, it has been determined that growing Monterey and Albion strawberry varieties under Erzurum conditions in a mulched system will be economically more advantageous than growing in the open without mulching.

Keywords: *Strawberry*, Erzurum, Mulching, Soil physical properties

INTRODUCTION

Strawberry is the most important species in the berry group after grapes. Strawberries belong to the genus Fragaria in the Rosaceae family of the Rosales order, with up to 12 species growing naturally in Europe, Asia, South and North America. Strawberry has important advantages due to its easy reproduction, short fruiting period, suitability for seasonal cultivation, export and domestic consumption opportunities and its wide distribution in the world (Ağaoğlu, 1986; Güleryüz et al., 2001; Aybak, 2005; Özkan, 2012). According to the latest statistical data, in the world strawberry production of 9,175,384 tons, the United States ranks first with a production of 1,211,090 tons, followed by Turkiye with a production of 669,195 tons and Spain with a production of 360,570 tons (TUIK, 2020). Strawberries can be grown naturally at 3255 m above sea level, in cold regions, subtropical regions, Ecuador, other words, and in very different ecological conditions (Ağaoğlu, 1986). Our country has very suitable conditions for strawberry cultivation. Strawberries are grown in almost all regions of our country. However, while most of the strawberry production is obtained from the Mediterranean, Ege and Marmara regions, the lowest amount of production is realized in the Eastern Anatolia Region (Özkaplan, 2010). In order to increase yield in strawberry cultivation, appropriate varieties should be used. Especially planting time affects productivity to a great extent. In commercial vineyard-garden farming, various types of soil cover materials (mulch) are widely used in many plant production. All materials used to cover the soil are called "mulch". In an area covered with plants under natural conditions, the remains such as fallen leaves, branches, etc. also form a natural mulch cover on the soil surface. In the past, materials from nature such as stalk-straw, needle leaves of forest trees were used, while today thin plastic covers (0.01-0.03 mm thick) in different colors are used. It is known that mulching has many benefits in plant cultivation. Mulching is a necessary practice in strawberry cultivation because it prevents weed growth, maintains soil moisture, reduces wind erosion in the soil, provides earliness, increases yield and quality, increases soil temperature and keeps the fruits clean. With mulching, the movement of salts in the soil towards the surface is prevented and the salinity problem of the soil can be reduced. At the same time, mulching reduces tillage practices and thus reduces tillage costs (Kuzucu, 2021). Agricultural management practices, such as mulching and irrigation, can alter the characteristics of vegetation and thus affect the hydrothermal properties of the soil. As a result of high temperatures in the summer months, losses in soil moisture through evaporation create stress conditions in the plant as well as low yields. In order to reduce these conditions, it is necessary to store winter precipitation in the soil in arid areas and to ensure that this accumulated rainwater reaches the plants during the dry period. In order to maintain soil moisture and fertility, mulching is important to reduce evaporation from the soil surface and to keep the plant root zone moist and cool. The only goal in crop production is not only to obtain high yields, but also to reduce production costs is very important in terms of economic and sustainable agriculture (Kang et al., 2002). Mulch application comes to the forefront in reducing irrigation costs, which is one of the important production costs in agricultural activities. One of the plants where soil cover materials are most commonly used in our country is strawberry, which is one of the species with the highest economic value. In addition to its low temperatures, the Eastern Anatolia Region is a high-altitude region where the continental climate prevails and the rainfall during the vegetation period is insufficient for fruit cultivation in economic terms. Some alternative practices come to the forefront in this region in order to tolerate the negative effects of climate. In this study, the effects of mulch use on soil physical properties, plant growth and yield parameters in strawberry cultivation in Erzurum central conditions, one of the high altitude provinces of Eastern Anatolia Region, were investigated.

MATERIAL AND METHOD

Erzurum is located in the Eastern Anatolia region on 39- 55 north latitude and 41-16 east longitude. Erzurum is one of the coldest and highest altitude provinces in Turkiye. Erzurum province, located in the Eastern Anatolia Region, has a harsh continental climate. Winters are cold and snowy, summers are hot and dry. The average annual precipitation in the city center, which is covered with snow for 150 days of the year, is around 400 mm. Erzurum is located at the foot of Palandöken Mountain and on a sloping topography at an altitude of 1850-1980 m above sea level. Table 1 presents the long-term average meteorological data for the central conditions of Erzurum province. Albion and Monterey strawberry varieties with high adaptation potential to Erzurum conditions and neutral day characteristics were used as material in the study. The seedlings used in the experiment were obtained as frigo seedlings. This research was carried out between 2021-2022 and 2022-2023 in an area of approximately 60 m2 in the Research and Application Garden of Atatürk University Faculty of Agriculture, Department of Horticulture. For this purpose; before planting in 2022, the land was leveled and 4 raised beds, each of which was 4 meters long, 30 cm high, 70 cm wide and 40 cm apart, were prepared according to the experimental design, and after the drip irrigation system was placed on them, they were mulched with black plastic (6% UV additive, 18 micron thickness).

Table 1. Some climatic data of Erzurum province for 1991 and 2020 and for long years (Anonymous, 2023)

ERZURUM (1991-2020 Years)	Jan.	Feb.	Mar.	Apr	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Annual
Average Temperature (°C)	-10.2	-8.8	-1.9	5.5	10.5	14.8	19.1	19.5	14.3	8.1	0.2	-7.1	5.3
Average Highest Temperature (°C)	-4.0	-2.4	3.9	12.1	17.7	22.9	27.7	28.5	23.7	16.4	7.3	-1.2	12.7
Average Lowest Temperature (°C)	-15.9	-14.7	-7.5	-0.7	3.4	6.1	9.9	10.0	4.4	0.3	-6.0	-12.4	-1.9
Average Sunshine Time (hours)	3.5	4.3	5.0	6.1	7.6	10.0	10.7	10.2	8.4	6.5	4.8	2.9	6.7
Average Number of Rainy Days	10.6	11.0	12.8	14.9	16.8	10.7	6.9	6.1	5.3	10.3	8.7	11.2	125.7
Monthly Average Total Precipitation (mm)	16.2	19.4	34.9	55.9	72.4	42.1	21.9	16.5	22.7	46.8	25.6	21.3	395.7
Maximum Temperature (°C)	8.0	10.6	21.4	26.5	29.6	32.7	35.6	36.5	33.3	27.0	20.7	14.0	36.5
Lowest Temperature (°C)	-36.0	-37.0	-33.2	-22.4	-7.1	-5.6	-1.8	-1.1	-6.8	-14.1	-34.3	-37.2	-37.2

After mulching, Albion and Monterey strawberry seedlings were planted in four raised beds. A total of 48 seedlings were used in each raised beds, 12 plants in each tubes. Also, a open field parcel was prepared for strawberry seedlings and 24 Albion and Monterey strawberry seedlings were planted in this area at 40x40 intervals. A total of 96 plants were used in open field and raised beds in the study. All of the strawberry seedlings planted in 2022 were established and continued their development. Plant vegetative development parameters and yield values were taken at the end of the vegetation period of 2023. Yield per plant was obtained by multiplying the average weight of 3 fruits taken from each plant during harvest periods by the total number of fruits on the plant. In addition, tillering number, petiole length, number of stolons, leaf width, leaf length, number of fruits per plant, number of clusters, fruit width, fruit size and average fruit weight were determined for the plants grown in mulched system and open field (Özkan, 2012).

According to USDA (1999), the soils used in the research are in the sandy loam class. Sub-samples to be used for basic analyzes were prepared by sieving through 2 mm sieves from soil samples that were duly taken from the mulch and without mulch soil. After air-dried under laboratory conditions. Soil texture was determined by Bouyoucos hydrometer method (Gee and Bauder, 1986), aggregate stability (AS) using Yoder type wet sieving device (Kemper and Rosenau, 1986), particle density were determined by pycnometer method (Blake and Hartge, 1986), organic matter content by Smith Weldon method (Nelson and Sommers, 1982), soil reaction (pH) by glass electrode pH meter (McLean, 1982), lime content by Scheibler calcimeter (Nelson, 1982), electrical conductivity (EC) value with electrical conductivity instrument (Rhoades, 1982), Ca^{++}, Mg^{++}, Na^{+} and K^{+} contents of the soils were determined by (Kacar, 2009) method. Statistical analysis was performed by ANOVA, and differences between means were tested using Duncan’s multiple range test.

RESULTS AND DISCUSSION

In general, more yield is obtained from unit area in summer planting system compared to other planting systems. In the winter planting system, less but high quality products are obtained from the unit area. The spring planting system is practiced in places with very cold winters. In spring planting, low yields can be obtained in the first year and better yields in the second year (Ağaoğlu, 1986, Kaşka et al., 1986, Konarlı, 1986). In this study conducted in the central conditions of Erzurum, where the continental climate prevails, data were taken in the second year after the planting of strawberry seedlings.

Table 2. Plant growth parameters of different strawberry cultivars grown in mulched and non-mulched systems

Cultivation System	Tillering number (item)	Petiole length (cm)	Number of stolon (item)	Leaf width (cm)	Leaf size (cm)
Albion Mulch	2,66a	11,3c	4,33ab	7,8a	7,9a
Monterey Mulch	2,33a	15,88b	5a	7,36a	7,4a
Albion - Without mulch	3,33a	20,5a	4,31ab	7,83a	7,33a
Monterey - Without mulch	2,66a	13,76bc	2,66b	6,26a	6,33b

When yield and pomological development parameters were examined, it was determined that there was a statistical difference between the cultivation method ($p<0.05$).

Plant growth parameters, fruit pomological characteristics and yield values of Albion and Monterey strawberry cultivars were found to be statistically different ($p<0.05$) except tillering number and leaf width values. When the petiole length values were analyzed, it was found that the highest value (20.5 cm) was in Albion variety without mulch and the lowest value (11.3 cm) was in Albion variety with mulching (Table 2). The highest stolon number (4.33 items) and leaf width (7.9 cm) were determined in Albion variety with mulch application, while the lowest value for both parameters was determined in Monterey variety without mulch application.

Table 3. Fruit pomological characteristics and yield values of different strawberry cultivars grown in mulched and non-mulched systems

Cultivation System	Number of fruits per plant (item)	Fruit width (cm)	Fruit size (cm)	Average fruit weight (gr)	Yield (gr/plant)
Albion Mulch	13,66a	2,94a	3,75a	12,56ab	171,5a
Monterey Mulch	14,66a	2,71ab	3,73a	10,56ab	154,8b
Albion - Without mulch	11,0ab	2,9a	3,73a	13,63a	149,9b
Monterey - Without mulch	9,81b	2,36b	2,90b	10,28b	100,8c

When Table 3 was analyzed, it was determined that the highest value with 13.66 fruits per plant was determined in Albion variety with mulch application, while the lowest value with 9.81 fruits per plant was determined in Monterey variety without mulch application. When the fruit width and fruit size values of Albion and Monterey strawberry cultivars were examined, it was determined that the highest value was 2.94 cm and 3.75 cm in Albion cultivar with mulch, respectively, and the lowest value was 2.36 cm and 2.90 cm in Monterey cultivar without mulching, respectively (Table 3). Kapur and Şahiner (2019), reported that when different mulch types were evaluated on fruit size, mulch used in strawberry cultivation significantly increased fruit size as well as its positive effects such as weed control and soil moisture conservation. As a matter of fact, Li et al., (2001), reported that mulching can directly affect the temperature and moisture content of the soil (Acharya et al., 2005) and the grain yield of crops. When the average fruit weights of the varieties were examined, it was observed that the highest value (12.56 g) was in Albion strawberry variety with mulch and the lowest value (10.28 g) was in Monterey strawberry variety without mulch. When Table 3 is examined, it is seen that the highest value (171.5 g/plant) in terms of yield parameters of strawberry varieties is Albion variety applied mulch. This is followed by the Monterey strawberry variety to which mulch is applied, with 154.8 g/plant. Among these 4 groups, the lowest yield values were found to be 149.9 g/plant in Albion variety and 100.8 g/plant in Monterey variety in non-mulched applications. Hassan et al., (2000), investigated the effects of different mulch types on yield and fruit quality with Oso strawberry variety and reported that the best results were obtained from plots mulched with black plastic. Indeed, in a study with Festival, Naya and Senga-Sengana cultivars, it was reported that using black polyethylene mulch provided good weed control, reduced labor requirements by 25-30% and provided 4-8 days of earliness (Nicitochkina and Gusev, 1984).

Table 4. Results of some basic physical and chemical analyzes of the researched soils

Soil Properties	
Sand (%)	54
Silt (%)	34
Clay (%)	12
Texture class	Sandy loam
Particle density (g/cm^3)	2.68
Organic matter (%)	2.65
pH	8.2
EC (dS/m)	0.27
$CaCO_3$ (%)	9.23
Ca, Mg, Na, K (me/100gr)	8.65; 4.56; 0.53; 1.98

Basic soil analysis results are presented in Table 4. In this study, the texture class of the soils was determined as coarse textured in the sandy clay loam texture class (54% sand, 34% silt, 12% clay). Soil organic matter content (2.65%) is in the well class; pH level was found to be 8.2 and neutral, the EC level of the working soils is 0.27 dS/m without salt, the $CaCO_3$ level was determined as 9.23% in the medium calcareous (Ülgen and Yurtsever, 1995) class. According to the available phosphorus contents, the class of the soils was determined as medium (Ülgen and Yurtsever, 1995), Ca^{++}, Mg^{++}, Na^{+}, K^{+} contents were determined as

8.65, 4.56, 0.53, 1.98 me/100gr, respectively. Particle density were determined as 2.68 (g/cm3) (Table 4).

Table 5. Effects of cultivation system on the some properties of soils

Cultivation System	Organic matter (%)	AS (%)	WP (cm/h)	BD (gr/cm3)	Porosity (%)
Mulch	3.14a	34.28a	3.3a	1.23b	55a
Without mulch	2.23b	24.74b	1.9b	1.42a	46b

AS: Aggregate stability, WP: Water permeability, BD: Bulk density

The organic matter of soils was found to be 3.14% in mulched soils and 2.23% in soils without mulch. The reduction in tillage due to mulching allows the soil to maintain its structural properties (Kuzucu, 2021). When Table 5 was examined, it was determined that aggregate stability, water permeability and porosity values of the physical properties of the soils were higher in the mulched soils compared to the soils without mulch. It was determined that the bulk density value decreased depending on the mulch application. It was observed that microbial activity increased in the mulch-applied soils in relation to temperature and humidity and in parallel with this, organic matter content increased significantly (40%). In parallel with this, it was determined that there was an increase of 38.5% in aggregate stability value and 73% in water permeability value. On the other hand, the bulk density value of the soils decreased by 13.3% in the mulch application. The increase in aggregate stability increased soil porosity values by 20%. Different materials used as mulch also benefit the physical, chemical and biological properties of the soil. Mulch covers with organic content decompose over time, increasing the organic matter of the soil with mulching, the effect of sunlight is reduced, moisture in the soil is preserved and microorganism activity in the soil can occur intensively (Koul and Chopra, 2020). Martins and Westphalen (1983), found that mulch applications increased soil temperature, reduced the amount of weeds, increased crop yield and kept the fruits clean.

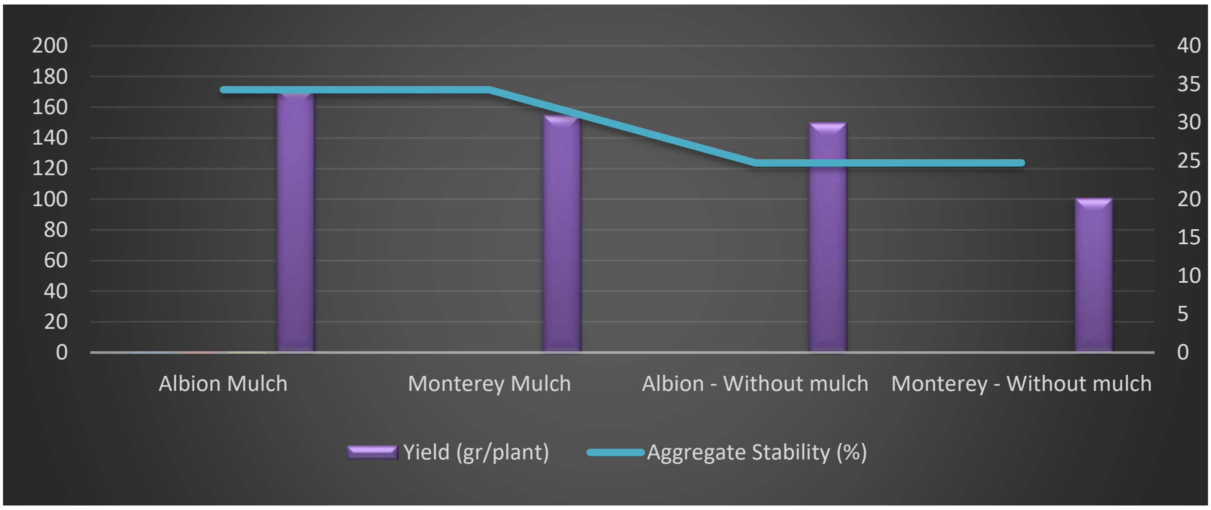

Figure 1. The relationship between yield and soil aggregate stabilitiy depending on cultivation system (mulch and without mulch) in Albion and Monterey varieties

Figure 1 shows that there is a parallel relationship between AS values and yield values. When Figure 1 is examined, it is seen that the aggregate stability values of the soils of Albion and Monterey strawberry varieties in the mulched system are higher than the AS values of the soils in the non-mulched system, and in parallel to this, yield values are higher in the mulched system.

When the soil and plant parameters of the study were evaluated together, it was determined that mulch application improved the soil physical properties by increasing the organic matter content of the soil and as a result of this, plant growth parameters, fruit pomological characteristics and yield values increased in Albion and Monterey strawberry varieties.

CONCLUSION

Strawberry cultivation is an important production branch especially for small and medium-sized enterprises. Since the investments pay off in a short time, small family businesses also prefer to grow strawberries. Although strawberry is one of the species with high adaptability, it has not achieved the targeted economic potential in the Eastern Anatolia Region where the continental climate prevails. Mulch applications, which play an important role in protection against low winter temperatures and late spring frosts, also reduce the cost of cultural practices by providing weed control. In recent years, irregularities in the precipitation regime due to global climate change have made it necessary to limit water use in agriculture. Due to this rainfall irregularity and water scarcity, the desired amount of water cannot be provided to the plant, especially during periods when irrigation water is needed in agricultural production. The development of water resources in our country, planning them with scientific and technical approaches and taking care to protect the environment while doing so will continue to gain more value today and in the future. Nowadays, mulching as one of the basic practices in agricultural production is frequently preferred because it is cheaper, environmentally friendly, safe and easy to apply, as well as reducing water consumption in agriculture and keeping soil temperature at an optimum level for plant growth. Mulch applications have a significant potential in the production of fruit trees, vegetable gardens, field crops, ornamental plants and landscaping, as well as in strawberry cultivation. This study, which investigated the effects of mulch on plant growth and yield values of Albion and Monterey strawberry varieties under Erzurum central conditions, reveals the advantages of mulch applications.

REFERENCES

Acharya, C.L., Hati, K.M., Bandyopadhyay, K.K., 2005. Mulches. In: Hillel D, Rosenzweig C, Pawlson DS, Scow KM, Sorger MJ, Sparks DL, Hatfield J Editors, Encyclopedia of Soils in the Environment, Elsevier Publication, pp. 521-532.

Ağaoğlu, Y.S., 1986. Üzümsü Meyveler. Ankara Üniversitesi Ziraat Fakültesi Yayınları: 984. Ders Kitabı: pp: 290-377. (in Turkish).

Anonimous, 2023. https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?m=ERZURUM (Acces Date:19.November.2023).

Aybak, H.Ç., 2005. Çilek Yetiştiriciliği, Hasad Yayıncılık, pp: 128 (in Turkish)

Blake, G.R., Hartge, K.H., 1986. Bulk density. In A. Klute (ed.), Methods of Soil Analysis. Part 1. 2nd ed. Agronomy monographs. 9. Soil Science Society of America, Madison, 363-382.

Gee, G.W., Bauder, J.W., 1986. Particle-size analysis. In: Klute, A. (Ed.), Methods of soil analysis. Part 1. Physical and mineralogical methods. 2nd edition. American Society of Agronomy. Soil Science Society of America, Madison, pp. 383-411.

Güleryüz, M., Ertürk, Y., Pırlak, L., 2001. Çilek yetiştiriciliğinde organik uygulamalar ve ekolojik çilek yetiştiriciliği, Türkiye 2. Ekolojik Tarım Sempozyumu pp: 319- 332. (in Turkish)

Hassan, G.I., Godara, A.K., Jitender, K., Huchcde, A.D., 2000. Effect of different mulches on the yield and quality of oso grande strawberry. Indian Journal of Agricultural Sciences. 70(3):184–185.

Kacar, B., 2009. Plant and Soil Chemical Analysis III. Soil Analysis. Nobel Publication No:1387. Ankara, 467.

Kang, S.Z., Zhang, L., Liang, Y.L., Hu, X.T., Cai, H.J., Gu, B.J., 2002. Effects of limited irrigation on yield and water use efficiency of winter wheat in the Loess Plateau of China. Agr. Water Manage., 55: 203-216.

Kapur, B., Şahiner, Y., 2019. Effects of ırrigation levels and mulch applications on pomological properties of strawberry. Turkish journal of agriculture-food science and technology, 7(2) : 355-364.

Kaşka, N., Yıldız, A.I., Paydaş, S., Biçici, M., Türemiş, N., Küden, A., 1986. Türkiye için yeni bazı çilek çeşitlerinin adana da yaz ve kış dikim sistemleriyle örtü altında yetiştiriciliğinin verim, kalite ve erkencilik üzerine etkileri. Tübitak Doğa Bilim, 10:1.

Kemper, W.D., Rosenau, R.C., 1986. Aggregate stability and size distribution. Methods of soil analysis. Physical and mineralogical methods. 2nd edition. Agronomy, 9: 425-442.

Konarlı, O., 1986. Çilek. Tarımsal Araştırmaları Destekleme ve Geliştirme Vakfı, yayın Yalova, 12. (in Turkish)

Koul, B., Chopra, M., 2020. Comparative assessment of different types of mulchıng in varıous crops : A Revıew. Plant Archives Vol. 20 (2): 1620-1626.

Kuzucu, M., 2021. Importance of mulching ın dry agricultural areas for soil moisture storage. IJENT., 5 (1): 16-27.

Li, X.Y., Gong, J.D., Gao, Q., Li, F., 2001. Incorporation of ridge and furrow method of rainfall with mulching for crop production under semiari conditions. Agric. Water Manage., 50: 173-183.

Martins, N.L.F., Westphalens, S.L., 1983. Effects of plastic mulchs on yield of two strawberry cultivars and on soil temperature. Agronomia Sulriograndense. 19(2) : 67-88.

McLean, E.O., 1982. Soil pH and Lime Requirement. In: Page, A. (Ed.), Methods of Soil Analysis Part 2. Chemical and microbiological properties. 2nd Edition. Agronomy, 9: 199 - 224.

Nelson, R.E., 1982. Carbonate and gypsum. Methods of soil analysis. Part 2. Chemical and microbiological properties. 2nd edition. Agronomy, 181-197.

Nelson, D.W., Sommers, L.E., 1982. Total carbon, organic carbon, and organic matter.In: Page, A. (Ed.), Methods of soil analysis. part 2. Chemical and Microbiological Properties. Madison, 2nd edition. Agronomy, Wisconsin USA, 1159(9): 539-579.

Nicitochkina, T.D., Gusev A.M., 1984. Growth And cropping of strawberries mulched with black or transparent polyetylene, Hort. Sci. Abs., 54, (7): 422.

Özkan, G., 2012. Erzurum (Merkez) koşullarında organik çilek yetiştiriciliği imkanları üzerinde bir araştırma. Atatürk Univ., Graduate School of Natural and Applied Sciences, doctorate Thesis, Erzurum. (in Turkish)

Özkaplan, C.H., 2010. Perşembe (Ordu) ekolojisinde değişik malç uygulamalarının çilekte verim ve kalite üzerinde etkisi. Graduate School of Natural and Applied Sciences, master Thesis, Ordu. (in Turkish)

Rhoades, J.D., 1982. Soluble salts. In: Page, A. (Ed.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd Edition. Agronomy, 1159 Madison USA, 9: 167-178.

TUİK, (2020). Türkiye İstatistik Kurumu (TUİK), https://tuik.gov.tr, (Date: 23.04.2022).

Ülgen, N., Yurtsever, N., 1995. Türkiye Gübre ve Gübreleme Rehberi (4. Baskı). Başbakanlık Köy Hizmetleri Genel Müdürlüğü Toprak ve Gübre Araştırma Enstitüsü Müdürlüğü Yayınları. Genel Yayın No: 209. Teknik Yayınlar, No: T.66. Ankara pp.230 (in Turkish)

USDA., 1999. United States Department of Agriculture. Soil Taxonomy, A Basic System of Soil Classification for making and ınterpreting soil surveys. Second Edition.

Probiotics in Aquaculture

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ABSTRACT: Probiotics are live microorganisms that, when taken in sufficient amounts, have beneficial effects on the health and physiology of the host. As a result of the use of probiotics, the intestinal flora of humans and animals can be regulated and the development of living things can be accelerated by reducing intestinal problems. Aquaculture plays an important role in the production of food for the world's population, as well as in the livelihoods of those most needed globally. The first application of probiotics in the field of aquaculture was in 1986, when their effects were investigated to increase the development of aquatic organisms. In later periods, probiotics were used to control water quality and bacterial infections. Nowadays, the effects of prebiotics and probiotics in aquaculture are mostly aimed at increasing the digestibility of nutrients, increasing stress resistance and supporting production. In this review, probiotics and probiotic applications used in aquaculture will be mentioned.

Keywords: *Aquaculture,* Health, Probiotics

INTRODUCTION

Although the term probiotic comes from the Greek words pro and bios, it has gained different meanings over time. Dr. While Elie Metchnikoff defined probiotics in 1905 as 'substances that change the body's flora by replacing harmful bacteria in the intestine with beneficial bacteria'; Parker defined it as 'organisms and compounds that support intestinal microbial balance' (Ninawe and Selvin, 2009; Altıntaş et al., 2016). In order for humans and animals to live a healthy life, they need to have a healthy gastrointestinal system. This is achieved by the intestinal microflora. Probiotics, prebiotics, synbiotics are used to strengthen the beneficial microflora of the intestine. Probiotic bacteria provide inhibition of pathogenic microorganisms, increase the digestibility of foods, strengthen the immune system, reduce blood cholesterol levels, and increase the absorption of prebiotics (Sezen, 2013).

Probiotics are used as functional food for human health and as growth promoters and preservatives in animal nutrition. Probiotics settle in the host's gastrointestinal tract, form colonies there, and prevent the colonization of potential pathogens by both producing inhibitory compounds and competing for nutrients and living space (Can et al., 2011). Probiotic microorganisms are used for many purposes (promote digestion, absorption, increase economic growth and and suppress infectious diseases) (Akhter et al., 2015).Probiotics are live microorganisms that regulate the microflora in the intestines (Korkut et al., 2003). Probiotic microorganisms can provide the expected beneficial effect in order to be healthy, they must be taken into the body in quantities of 10^6-10^7 cfu/mL or more and must remain viable during the production and shelf life of the food they contain (Ünal and Erginkaya, 2010; Sezen, 2013). According to the FAO and WHO, probiotics are living microorganisms that, when given in appropriate amounts, provide health benefits to the host. Therefore, it has been expanded of aquaculture by FAO/WHO to cover a wide range of Gram-positive, gram-negative bacteria, bacteriophages, microalgae and yeast through aquaculture application as well as feed supply (Akther et al., 2015). Today, probiotics are widely used in therapeutic, prophylactic and growth supplements in animal production and human health, as well as in health-promoting “functional foods” for humans (Pandiyan et al., 2013). Studies have demonstrated the benefits of probiotics for aquatic animals, such as stimulating growth or improving feed digestion, immune responses and water quality control (Tuan et al., 2013).

PROBIOTICS IN AQUACULTURE

Aquaculture is the world's fastest growing sector. Aquaculture is suffering serious losses due to current diseases. The use of, pesticides, disinfectants and antimicrobial drugs in protecting the health of these products and ensuring their growth has opened the way for the registration of resistant bacterial species. Therefore, with the demand for environmentally friendly, sustainable aquaculture products, research continues on the use of probiotics in the production of aquaculture products (Pandiyan et al., 2013).

Various beneficial probiotics and prebiotics, that have beneficial effects on the host have been used as supplements in aquaculture, including to combat disease, increasing growth, size and weight gain and in some cases acting as alternative antimicrobial compounds (Akther et al., 2015). The use of probiotics in aquaculture already has a long history and has served the goals of more sustainable production from the beginning (Hancz, 2022). The main purpose of using probiotics in the beginning is; regulates or reestablishes the desired relationship between beneficial and pathogenic microorganisms that make up the fish's skin, mucus or intestinal flora is to create (Alak ve Atamanalp, 2012). It is used to prevent pathogens in water and improve water quality by changing the microbial load in water and sediment in order to increase production and fish health. On the other hand, probiotics have been reported to significantly improve the immune system of fish (Can et al., 2011).

Rapid growth in aquaculture has brought about an increase in viral, bacterial, fungal and parasitic diseases along with environmental interactions. This situation has increased the use of antibiotics, pesticides and other chemicals, on the one hand, to support the growth of living things, and on the other hand, to protect and control diseases. The use of antibiotics, especially for preventive purposes, poses a danger in terms of the development of antibiotic-resistant microorganisms and harmful consequences for the aquatic environment and human health. In order to minimize these dangers, new alternative preservatives and methods that do not harm ecology and the environment are needed. Among the alternative solutions produced for this purpose, the use of probiotics and prebiotics has the most important place (Altıntaş et al. 2016). The range of probiotics examined for use in aquaculture has encompassed both Gram-negative, Gram-positive bacteria, bacteriophages, yeasts and unicellular algae (Irianto and Austin, 2002). Probiotics commonly used in aquaculture are *Lactobacillus rhamnosus* and *Carnobacterium*. These microorganisms are gram positive and anaerobic and have pathogenic properties. It has been reported that *Lactobacillus* bacteria are more resistant to stomach pH and can maintain their viability during passage through the digestive tract (Alak and Atamanalp, 2012).

Probiotics in aquaculture, especially to increase production to prevent pathogens in water and improve water quality is used. Other uses of probiotics are as follows (Korkut et al., 2003):

1- It is used to improve the immunity of the cultured species (considered in aquaculture) against pathogenic microorganisms and to increase feeding levels.

2- 2- It is used to increase the population of food organisms in water.

3- Reducing the use of probiotics, antibiotics and chemicals and it is used to prevent the frequent occurrence of diseases.

4- To improve water quality by decomposing toxic substances in water is used.

CONCLUSION

Probiotics, which are widely used in livestock farming, have recently started to be used in aquaculture. In aquaculture, probiotics have been shown to improve growth rates, disease resistance, and water quality. Probiotics are living cells or substrates that provide benefits through stimulation of growth, improved digestion, and improved immune response. Probiotics can also improve water quality.

REFERENCES

Akhter, N., Wu, B., Memon, A. M., Mohsin, M., 2015. Probiotics and prebiotics associated with aquaculture: a review. Fish & shellfish immunology, 45(2), 733-741.

Alak, G., Atamanalp, M., 2012. Su ürünleri yetiştiriciliğinde probiyotik ve prebiyotik kullanımı. Yuzuncu Yıl University Journal of Agricultural Sciences, 22(1), 62-68.

Altıntaş, L., Tutun, H., Sevin, S., Yarsan, E., 2016. Su Ürünleri Yetiştiriciliğinde Prebiyotik ve Probiyotik Kullanımı. Turkiye Klinikleri J. Vet. Sci. Pharmacol. Toxicol-Special Topics, 2(1):29-37

Can, E., Kurtoğlu, İ. Z., Kayım, M., Akhan, S., Kızak, V., Kocabaş, M., Köse, Ö., Demirtaş, N., Delihasan Sonay, F., Othan, A., 2011. Alabalıklarda Probiyotik Uygulamalarının Bugünü ve Geleceği. Türk Bilimsel Derlemeler Dergisi, (1), 45-52.

Hancz, C., 2022. Application of probiotics for environmentally friendly and sustainable aquaculture: a review. Sustainability, 14(22), 15479.

Irianto, A., Austin, B., 2002. Probiotics in aquaculture. Journal of fish diseases, 25(11), 633-642.

Korkut, A. Y., Hoşsu, B., Ferhatoğlu, M., 2003. Probiyotikler ve Su Ürünlerinde Kullanımı. Ege Journal of Fisheries and Aquatic Sciences, 20(3-4): 551-556.

Ninawe, A. S., Selvin, J., 2009. Probiotics in shrimp aquaculture: avenues and challenges. Critical reviews in microbiology, 35(1), 43-66.

Pandiyan, P., Balaraman, D., Thirunavukkarasu, R., George, E. G. J., Subaramaniyan, K., Manikkam, S., Sadayappan, B., 2013. Probiotics in aquaculture. Drug invention today, 5(1), 55-59.

Sezen, A. G., 2013. Prebiyotik, probiyotik ve sinbiyotiklerin insan ve hayvan sağlığı üzerine etkileri. Atatürk Üniversitesi Veteriner Bilimleri Dergisi, 8(3), 248-258.

Tuan, T. N., Duc, P. M., Hatai, K., 2013. Overview of the use of probiotics in aquaculture. International Journal of Research in Fisheries and Aquaculture, 3(3), 89-97.

Ünal, E., Erginkaya, Z., 2010. Probiyotik mikroorganizmaların mikroenkapsülasyonu. Gıda, 35(4), 297-304.

In vitro Methane Production Levels of Carbohydrate Sources in Dogs

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ABSTRACT: This study was aimed to determine the in vitro methane production levels of different carbohydrate sources in dogs using the in vitro fermentation technique. It was determined that sugar beet pulp did not produce methane in the first 12 hours, but methane production in the 24th hour. It was determined that methane production increased in te 24th hour by heat-pressure treatment of sugar beet pulp. The effect of heat-pressure treatment, dog faecal inoculum of different ages and interaction on the methane production of tomato pomace and corn bran in two different incubations of 12 and 24 hours was found to be insignificant (P>0.05). It was determined that the methane level in the total gas produced in wheat bran during 12 (P<0.001) and 24 (P=0.001) hour incubations increased significantly with the application of heat-pressure treatment. It was determined that the heat-pressure treatment applied to the feed had a significant effect on the methane production of barley flour in the 12th and 24th hours of gas production (P<0.05). Methane production in 24-hour gas production of corn flour also increased significantly with the heat-pressure process. Again, age had a significant effect on the methane production of corn flour at the 24th hour, and it was determined that geriatric dog faecal inoculum produced higher methane (P = 0.031). No methane was detected in the gas in the first 12 hours of wheat flour that was not subjected to heat-pressure treatment, but it was determined that it produced 0.5-0.6% methane in fermentations using 2- and 8-year-old dog faecal inoculum when heat-pressure was applied. Again, it was determined that age did not have a significant effect on the 24th hour methane production of rice flour and corn flour (P>0.05). It was determined that heat-pressure treatment had an increasing effect on the 24th hour methane production of oat flour (P = 0.017). It was determined that potato flour also changed with the application of heat-pressure treatment to the feed and dog faecal inoculum of different ages on methane production at the 24th hour (P = 0.027). As a result, it has been revealed that in vitro methane production in dogs varies according to the incubation time and extrusion process of starch sources and structural carbohydrate sources.

Keywords: Dog, in vitro, Food, Methane, Digestion

INTRODUCTION

Dogs are closer to omnivores than cats, which are obligate carnivores. Due to their omnivorous characteristics, dog diets contain more carbohydrates than cat diets. However, on the extent to which different types of carbohydrate source feedstuffs included in the formulation of dog diets are digested at different ages. There is not enough information in the end products of digestion, are not discussed in international scientific committees, especially National Research Council (NRC), the Association of American Feed Control Officials (AAFCO) and the European Pet Food Industry Federation (FEDIAF). Although the in vitro gas production technique is widely used to determine the digestion levels of ruminant feeds; It was first used by Sunvold et al. (1995) to determine the in vitro digestibility level of feed raw materials of dogs. Sunvold et al (1995) performed this in vitro gas production technique with dog feces inoculum. This technique is different from the fermentation technique used in other animals and was first developed by Titgemeyer et al. (1991) by adapting the technique used to detect the digestion of fibrous compounds in human diets to the fermentation of fibrous compounds in dog foods. In recent years, studies using molecular techniques have shown that at least four different bacterial species have been found in the intestinal lumen of dogs: Firmicutes (47.7%), Proteobacteria (23.3%), Fusobacteria (16.6%) and Bacteroidetes (16.6%). has been reported (Suchodolski et al., 2008; Garzia-Mazcorro and Minamoto, 2013). Swanson et al. (2011) determined that the fecal microorganisms of dogs include approximately 35% of Bacteroidetes/Chlorobi and Firmicutes species, approximately 15% of Proteobacteria and 8% of Fusobacteria. The most important short-chain fatty acids formed as a result of carbohydrate digestion in the digestive tract of dogs are butyric, acetic and propionic acids (Swanson et al., 2002). As can be understood, the fermentation of dietary fiber elements in the colon of dogs produces short-chain fatty acids and fermentation gases, carbon dioxide and methane. In this study, it was aimed to determine and compare methane gas production of carbohydrates in dogs of different ages by using inoculums prepared from feces obtained from Labrador Retrievers of different ages (6 months, 2 years and 8 years).

MATERIALS AND METHODS

Ethical approval for this study was received from Erciyes University Animal Experiments Local Ethics Committee with decision no. 14/021. In the study, dog fecal samples were taken from 6 *Labrador Retriever* male dogs: two 6 months old, two 2 years old and two 8 years old. Fecal samples were taken from Labrador Retrievers 10-15 seconds after defecation and placed in a screw cap bottle (Isolab, 500 ml) under CO2 gas and placed in a thermos at 37ºC. In the laboratory, stool samples were diluted 1:10 with pre-heated (37 ºC) anaerobic sterile 0.9% NaCl and mixed under CO2 gas with a laboratory type blender. After the samples were ground to a diameter of 1 mm, they were placed in an autoclave in autoclave petri dishes at 134ºC and 2.1 bar pressure for 14 minutes (4 min holding time + 10 min drying time), close to the conditions applied to commercial extruded feeds (Tran, 2008; Serrano, 1997). It was used in gas production techniques in two different ways: after treatment and cooling during and directly without applying heat and pressure. In the study, Sunvold et al. (1995a;b) and Bosch et al. (2008)'s method developed the gas production technique in which dog feces were used as inoculum. However, in this study, gas production syringes used in the gas production technique of Menke and Steingass (1988) were used. In the study, the methane gas level in the cumulative total gas in different incubations of 12 and 24 hours was injected into a computer-aided methane gas measurement device (Sensors Analysentechnik GmbH&Co. KG, Berlin, Germany) and the methane gas value (%) was determined on the computer.

Two-Way ANOVA method was used to determine the statistical significance of different fecal inoculums and heat-pressure application on the gas production levels of carbohydrate sources used in the study, and Tukey test was applied to control the significance of the difference between groups. All statistical analyzes were performed in SPSS 17.0 (IBM Corp.; Armonk, NY, USA).

RESULTS AND DISCUSSION

In the present study, sugar beet pulp did not produce methane in the first 12 hours, but methane production in the 24th hour. It was determined that methane production increased in the 24th hour by heat-pressure treatment of sugar beet pulp. The effect of heat-pressure treatment, dog faecal inoculum of different ages and interaction on the methane production of tomato pomace and corn bran in two different incubations of 12 and 24 hours was found to be insignificant ($P>0.05$). It was determined that the methane level in the total gas produced in wheat bran during 12 ($P<0.001$) and 24 ($P=0.001$) hour incubations increased significantly with the application of heat-pressure treatment. It was determined that the heat-pressure treatment applied to the feed had a significant effect on the methane production of barley flour in the 12th and 24th hours of gas production ($P<0.05$). Methane production in 24-hour gas production of corn flour also increased significantly with the heat-pressure process. Again, age had a significant effect on the methane production of corn flour at the 24th hour, and it was determined that geriatric dog faecal inoculum produced higher methane ($P = 0.031$). No methane was detected in the gas in the first 12 hours of wheat flour that was not subjected to heat-pressure treatment, but it was determined that it produced 0.5-0.6% methane in fermentations using 2- and 8-year-old dog faecal inoculum when heat-pressure was applied. Again, it was determined that age did not have a significant effect on the 24th hour methane production of rice flour and corn flour ($P>0.05$). It was determined that heat-pressure treatment had an increasing effect on the 24th hour methane production of oat flour (P = 0.017). It was determined that potato flour also changed with the application of heat-pressure treatment to the feed and dog faecal inoculum of different ages on methane production at the 24th hour (P = 0.027) (Tables 1 and 2).

Table 1. The methane production of structural carbohydrate feedstuffs in dogs

		12th and 24th methane production (%)									
		Sugar Beet pulp		Tomato pulp		Wheat bran		Corn bran		Rice bran	
		12	24	12	24	12	24	12	24	12	24
Non-extruded	6 months	0,00	0,15	0,40	0,65	0,00	0,00	0,20	0,30	0,00	0,00
	2 years	0,00	0,15	0,55	0,65	0,00	0,10	0,35	0,40	0,00	0,00
	8 years	0,00	0,20	0,25	0,45	0,00	0,00	0,40	0,45	0,00	0,00
Extruded	6 months	0,00	0,30	0,50	0,50	0,20	0,40	0,30	0,45	0,00	0,00
	2 years	0,00	0,20	0,30	0,55	0,25	0,45	0,25	0,45	0,00	0,00
	8 years	0,00	0,20	0,30	0,55	0,30	0,50	0,20	0,40	0,00	0,30
Age	6 months	-	0,22	0,45	0,57	0,10	0,20	0,25	0,37	-	-
	2 years	-	0,18	0,43	0,60	0,13	0,27	0,30	0,42	-	-
	8 years	-	0,20	0,28	0,50	0,15	0,25	0,30	0,43	-	-
Processing	Non-extruded	-	0,16	0,40	0,58	0,00	0,03	0,32	0,38	-	-
	Extruded	-	0,23	0,37	0,53	0,25	0,45	0,25	0,43	-	-
SEM		-	0,012	0,062	0,020	0,016	0,069	0,030	0,020		-
P values	Processing	-	0,049	0,740	0,360	0,001	0,001	0,154	0,411	-	-
	Age	-	0,645	0,350	0,286	0,125	0,911	0,548	0,753	-	-
	Processing* age	-	0,548	0,350	0,650	0,125	0,604	0,060	0,854	-	-

Sugar beet pulp and rice bran did not produce methane in the first 12 hours. Extruded process: 14 min at 134ºC temperature and 2.1 bar pressure (4 min holding time + 10 min drying time), SH: standard error of the means. A,B: For "applied process", the difference between the averages shown with different letters in the same column is important.

The 50-60% of global methane emissions come from the agriculture and livestock sector (especially ruminant breeding) (Ellis et al., 2007). With the increase in livestock farming, methane emissions have also increased on a global scale, and 17-37% of human-induced methane production consists of enteric methane production of ruminants (Broucek, 2014). The amount of greenhouse gases produced as a result of poultry and pig farming is much lower. No study has been found on the methane production levels of dogs. However, according to the presented project results, it was concluded that dogs are not an important methane producer since the methane level in the total gas they produce in the digestive tract is <1%.

Table 2. The methane production of non-structural carbohydrate feedstuffs in dogs

Uygulanan işlem			12th and 24th methane production (%)											
			Barley flour		Corn flour		Wheat flour		Rice flour		Oat flour		Potato flour	
			12	24	12	24	12	24	12	24	12	24	12	24
	Non-extruded	6 months	0,25	0,35	-	0,00	0,00	0,15	0,00	0,50	0,00	0,40	0,00	0,40
		2 years	0,30	0,45	-	0,40	0,00	0,30	0,20	0,60	0,00	0,50	0,50	0,55
		8 years	0,25	0,45	-	0,55	0,00	0,45	0,20	0,55	0,00	0,50	0,60	0,50
	Extruded	6 months	0,25	0,50	-	0,55	0,00	0,60	0,00	0,55	0,00	0,60	0,00	0,55
		2 years	0,40	0,55	-	0,60	0,50	0,70	0,20	0,50	0,60	0,70	0,60	0,65
		8 years	0,55	0,50	-	0,60	0,60	0,60	0,25	0,50	0,60	0,70	0,60	0,50
	Age	6 months	0,20	0,43	-	0,28^{b}	-	0,38	-	0,53	-	0,50	-	0,48^{b}
		2 years	0,32	0,50	-	0,50^{ab}	-	0,50	-	0,55	-	0,60	-	0,60^{a}
		8 years	0,40	0,48	-	0,58^{a}	-	0,53	-	0,53	-	0,60	-	0,50^{ab}
Processing	Non-extruded		0,23	0,42	-	0,32	-	0,30	-	0,55	-	0,47	-	0,48
	Extruded		0,38	0,52	-	0,58	-	0,63	-	0,52	-	0,67	-	0,57
SH			0,031	0,022	-	0,052	-	0,042	-	0,022	-	0,012		0,022
P values	Processing		0,054	0,024	-	0,009	-	0,001	-	0,207	-	0,017	-	0,027
	Age		0,108	0,252	-	0,031	-	0,119	-	0,630	-	0,230	-	0,027
	Processing* age		0,008	0,512	-	0,067	-	0,119	-	0,098	-	0,128	-	0,178

Extruded process: 14 min at 134ºC temperature and 2.1 bar pressure (4 min holding time + 10 min drying time), SEM: standard error of the means. a,b: The difference between the averages shown with different letters is important.

REFERENCES

Bosch, G., Pellikaan, W. F., Rutten, P. G. P., van der Poel A. F. B., Verstegen, M. W. A., Hendricks, W. H. 2008. Comparative fermentation activity in the canine distal gastrointestinal tract and fermentation kinetics of fiber sources. Journal of Animal Science,86, 2979-2989.

Broucek, J. 2014. Methods of methane measurement in ruminants. Slovak Journal of Animal Science, 47(1): 51-60.

Ellis, J.L., Kebreab, E., Odongo, N.E., McBride, B.W., Okine, E.K., France J. 2007. "Prediction of methane production from dairy and beef cattle". Journal of Dairy Science, 90, 3456–3467.

FEDIAF. 2013. Nutritonal Guidelines for Complete and Complementary Pet Food for Cats and Dogs. The European Pet Food Industry Federation. p. 1-85. Bruxelles.

Menke, H. H., Steingass, H. 1988. "Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid", Animal Research Development, 28, 7-55.

NRC, 2006: Nutrient Requirements of Dogs and Cats. The National Academies Press, Washington D.C., USA.

Serrano, X. 1997. The extrusion-cooking process in animal feeding. Nutritional implications. Ed: Morand-Fehr, P. Feed manufacturing in Southern Europe: New challenges. Zaragoza: CIHEM, p. 107-114.

Suchodolski, J. S., Camacho, J., Steiner, J. M. 2008. "Analysis of bacterial diversity in the canine duopdenum, jejunum, ileum, and colon by comparative 16S rRNA gene analysis", FEMS Microbiology Ecology, 66, 567-578.

Sunvold, G. D., Fahey, G. C., Merchen, N. R., Titgemeyer, E. C., Bourquin, L. D., Bauer, L. L., Reinhart, G. A. 1995a. "Dietary fiber for dogs: IV. fermentation of selected fiber sources by dog fecal inoculum and in vivo digestion and metabolism of fiber-supplemented diets", Journal of Animal Science, 73, 1099-1109.

Sunvold, G.D., Hussein, H.S., Fahey, G.C., Merchen, N.R., Reinhart, G.A. 1995b. "fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using fecal inoculum from cats, dogs, horses, humans, and pigs and ruminal fluid from cattle", Journal of Animal Science, 73, 3639-3648.

Swanson, K. S., Dowd, S, E., Suchodolski, J. S., Middelbos, I. S., Vester, K. A., Barry, K. A., Nelson, K. E., Torralba, M., Henrissat, B., Countinho, P. M., Cann, I. K. O., White, B. A., Fahey G. C. 2011."Phylogenetic and gene-centric metagenomics ot he canine intestinal microbiome reveals similarities with humans and mice", The ISME Journal, 5, 639-649.

Titgemeyer, E. C., Bourquin, L. D., Fahey, G. C., Garleb, K. A. 1991. "Fermentability of various fiber sources by human fecal bacteria", American Journal of Clinical Nutrition, 53: 1418.

Tran, Q. D. 2008." Extrusion processing: effects on dry canine diets" Ph.D. Thesis, Wageningen University and Research Centre, Wageningen, the Netherlands. ISBN: 978-90-8504-903-6.

Comparison of Nutrient Composition, in Vitro Ruminal Fermentation and Ruminal Metagenomic Profiles of Aromatic Plant Pulps*

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ABSTRACT: The aim of this study was to determine the nutrients, in vitro ruminal gas production, digestion variables and fermentation end products of the aromatic plant pulps remaining after the extraction of essential oils of sage, thyme, lavender and yarrow aromatic plants by hydro distillation. In the study, lavender, sage, thyme and yarrow pulps had 4.90%, 10.79%, 7.34% and 7.25% crude protein (CP), 5.20%, 4.94%, 3.86% and 1.90% diethyl extract (EE) and 7.63%, 8.40%, 10.28% and 10.13% ash in dry matter (DM), respectively. Differences were detected between fiber contents, lavender, sage, thyme and yarrow pulps had 56.70%, 55.59%, 57.93% and 60.70% neutral detergent fiber (NDF), 46.38%, 43.73%, 47.73% and 48.03%detergent fiber (ADF) and 10.31%, 10.37%, 10.19% and 12.86% hemicellulose in DM, respectively. The highest non-fiber carbohydrate content was detected in lavender pulp (26.02%) ($P<0.05$). The in vitro total gas level produced by aromatic plant pulp during 24-hour in vitro ruminal fermentation varied between 29.68% and 34.37%, and the highest in vitro total gas production was detected in yarrow pulp ($P<0.05$). In vitro metabolic energy (ME), net energy-lactation (NEL) and organic matter digestion (OMS) values of yarrow pulp were higher than those of other pulps ($P<0.05$). In vitro ruminal ammonia nitrogen (NH_3-N) concentration was highest in thyme pulp (75.14 mg/L) and lowest in sage pulp (60.93 mg/L) ($P<0.05$). Acetic acid molarity of in vitro fermentation fluid was found to be highest in yarrow pulp (67.34 mmol/L) and lowest in lavender pulp (58.28 mmol/L) ($P<0.05$). Butyric acid molarity (12.23 mmol/L) and total short-chain fatty acid (T-SCFA) molarity (102.33 mmol/L) in the in vitro fermentation fluid of lavender pulp were lower than those of other pulps ($P<0.05$). Branched short-chain fatty acid (BSCFA) (iso butyric acid + iso valeric acid) molarity varied between 1.48 and 1.94 mmol/L ($P<0.05$). In metagenomic analysis of the in vitro ruminal fermentation fluid, the relative abundance of total *Archaea* and *Methanobacteria* were lower in thyme pulp than in other pulps ($P<0.05$). The highest relative rate of *Firmicutes* was detected in sage pulp (33.17%) and the lowest in thyme pulp (25.93%) ($P<0.05$). The relative abundances of *Spirochaetota* and *Fibrobacterota* phyla was highest (7.97%) in thyme pulp ($P<0.05$). The relative abundances of *Prevotellaceae* bacteria phyla in thyme and yarrow pulps were higher than those of other pulps ($P<0.05$). The relative abundance of *Ruminococcaceae* bacteria phyla in sage and lavender pulps were higher than those of other pulps ($P<0.05$). As a result, the aromatic plant pulp examined has the potential to be a quality forage in terms of nutrients and in vitro fermentation values. In addition, the difference in their effectiveness on the rumen metagenomic profile (such as methanogenic archaea and fibrolytic and proteolytic bacteria) shows the functional properties of these plant pulps.

Keywords: *Aromatic plant*, Nutrient, Metagenomic profile, Rumen fermentation

INTRODUCTION

Aromatic plant oils are functional additives on which research has been concentrated in recent years due to their positive effects on both animal and human health. In addition to being natural, it also has the potential to be popular in organic animal production because it does not leave residue in animal products. Aromatic oils are produced from various aromatic plants, and the ratio and variety of active ingredients (such as carvacrol, thymol, terpene, thujon, cineole and borneol) in these aromatic oils vary depending on the plant type. Due to their volatile properties, these compounds are also called essential oils (Greathead, 2003; Güler and Dalkılıç, 2005). However, due to their aromatic content and to avoid name confusion with volatile organic/fatty acids (such as acetic, propionic and butyric acid), the term aromatic oil will be more understandable and valid. There are plants such as thyme, sage, yarrow, lavender, and St. John's wort that are cultivated as aromatic oil plants in Türkiye. Since aromatic oils from these plants will be extracted from the flower and leaf parts, the hydrodistillation method with water is widely used instead of methods such as solvent extraction. At the end of this distillation method, aromatic oil is obtained, but waste aromatic pulp is released into the external environment as a by-product (Baydar, 2009; Benli and Yiğit, 2005). The use of aromatic plant pulps, which contain active ingredients and have possibly increased digestibility, in rations and reveal their effects on fermentation end products; It is important for waste control and search for alternative feed materials. The hypothesis of the study is that these waste plant pulps have the potential to be used in rations in terms of nutrients and in vitro ruminal fermentation values. The aim of the study was to determine the nutritional content and in vitro digestion value of aromatic plant pulps.

MATERIAL AND METHODS

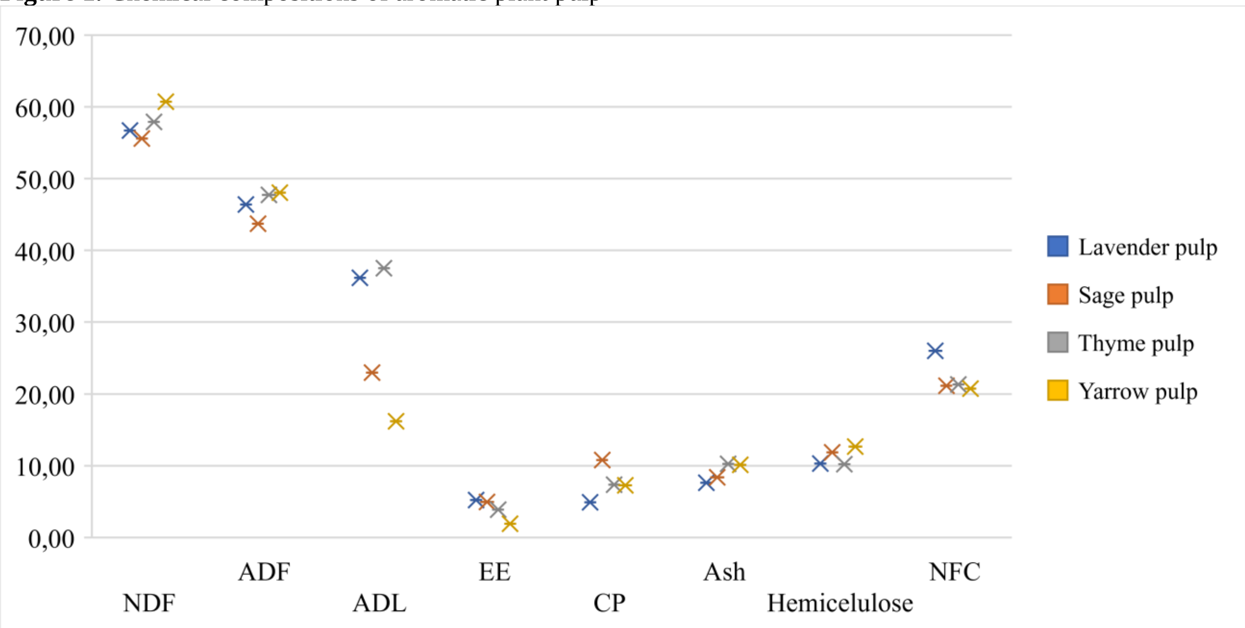
Dried aromatic plant pulps were analysed for crude protein (CP), diethyl ether extract (EE), ash, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents (AOAC, 1995; Van Soest et al., 1991). The *in vitro* ruminal fermentation values of the samples of four aromatic plant pulp were analysed using an *in vitro* gas production technique (Menke and Steingass, 1988). Rumen fluid, used for the preparation of in vitro fermentation inoculum, was collected and pooled from two beef cattle *(Brown Swiss-Simmental* mix breed). After 24-hour of incubation, the total gas volume was recorded from the calibrated scale of anaerobic glass fermenter (Model Fortuna, Germany) in the *in vitro* glass fermenter. The TMR samples were analysed in quadruplicate. The grinded TMR samples (200 ± 10 mg DM) were incubated in the rumen fluid inoculum plus buffer mixture (1:2, v/v) (30 ml) in anaerobic fermenter (Model Fortuna, Germany) for 24 h at 39 ºC (Menke et al. 1979). The total gas volume (at 24h) in the end of incubation was recorded. The molarities of total short-chain fatty acid (tSCFA), branched short-chain fatty acids (BCFA) and straight short-chain fatty acids (SCFA) in the *in vitro* fermentation fluid were determined by using a gas chromatograph device with flame ionisation detector (GC-FID) (Thermo Trace 1300, Thermo Scientific, USA)

(Ersahince and Kara, 2017). The estimated digestion values (metabolic energy (ME), net energy lactation (NE_L) and organic matter digestion (OMD)) o were calculated using the equations of Menke and Steingass (1988). The study's raw data were analysed with the SPSS 17.0 package program. Tukey test from multiple compromising test was used to detect the significant differences at $P < 0.05$.

RESULTS AND DISCUSSION

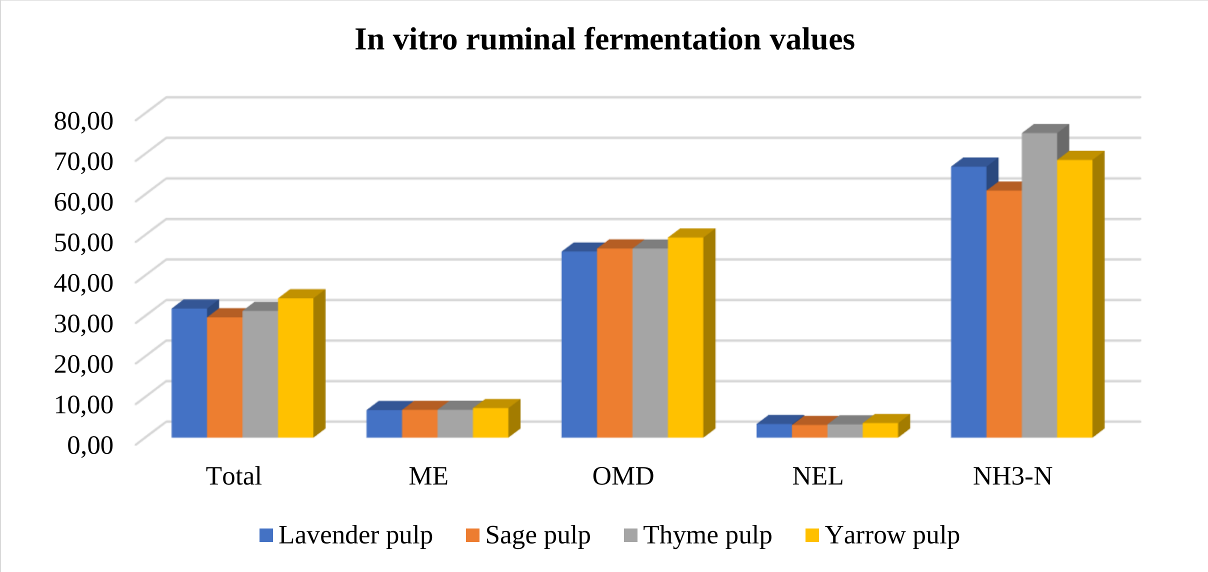
In the study, lavender, sage, thyme and yarrow pulps had 4.90%, 10.79%, 7.34% and 7.25% crude protein (CP), 5.20%, 4.94%, 3.86% and 1.90% diethyl extract (EE) and 7.63%, 8.40%, 10.28% and 10.13% ash in dry matter (DM), respectively. Differences were detected between fiber contents, lavender, sage, thyme and yarrow pulps had 56.70%, 55.59%, 57.93% and 60.70% neutral detergent fiber (NDF), 46.38%, 43.73%, 47.73% and 48.03%detergent fiber (ADF) and 10.31%, 10.37%, 10.19% and 12.86% hemicellulose in DM, respectively (Figure 1).

Figure 1. Chemical compositions of aromatic plant pulp



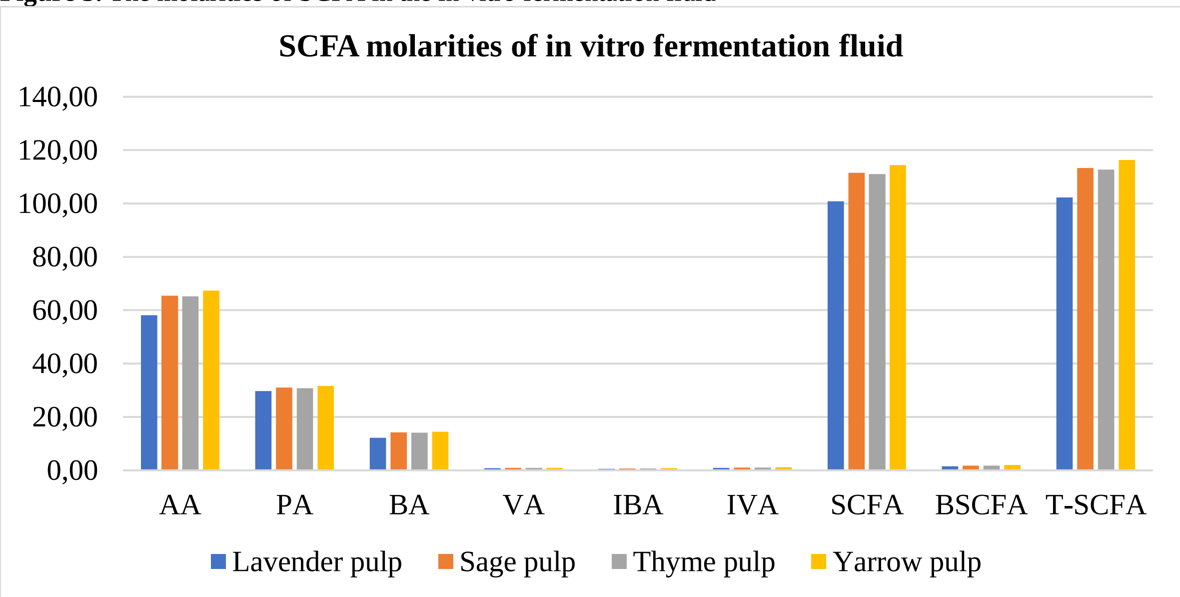
The in vitro total gas level produced by aromatic plant pulp during 24-hour in vitro ruminal fermentation varied between 29.68% and 34.37%, and the highest in vitro total gas production was detected in yarrow pulp ($P<0.05$). In vitro metabolic energy (ME), net energy-lactation (NEL) and organic matter digestion (OMS) values of yarrow pulp were higher than those of other pulps ($P<0.05$). In vitro ruminal ammonia nitrogen (NH_3-N) concentration was highest in thyme pulp (75.14 mg/L) and lowest in sage pulp (60.93 mg/L) ($P<0.05$) (Figure 2).

Figure 2. In vitro fermentation parameters of aromatic plant pulps



Acetic acid molarity of in vitro fermentation fluid was found to be highest in yarrow pulp (67.34 mmol/L) and lowest in lavender pulp (58.28 mmol/L) ($P<0.05$). Butyric acid molarity (12.23 mmol/L) and total short-chain fatty acid (T-SCFA) molarity (102.33 mmol/L) in the in vitro fermentation fluid of lavender pulp were lower than those of other pulps ($P<0.05$). Branched short-chain fatty acid (BSCFA) (iso butyric acid + iso valeric acid) molarity varied between 1.48 and 1.94 mmol/L ($P<0.05$).

Figure 3. The molarities of SCFA in the in vitro fermentation fluid



In metagenomic analysis of the in vitro ruminal fermentation fluid, the relative abundance of total *Archaea* and *Methanobacteria* were lower in thyme pulp than in other pulps ($P<0.05$). The highest relative rate of *Firmicutes* was detected in sage pulp (33.17%) and the lowest in thyme pulp (25.93%) ($P<0.05$). The relative abundances of *Spirochaetota* and *Fibrobacterota* phyla was highest (7.97%) in thyme pulp (P<0.05). The relative abundances of *Prevotellaceae* bacteria phyla in thyme and yarrow pulps were higher than those of other pulps (P<0.05). The relative abundance of *Ruminococcaceae* bacteria phyla in sage and lavender pulps were higher than those of other pulps (P<0.05).

As a result, there are differences between aromatic plant pulps in terms of nutritional composition. Sage pulp was more advantageous in terms of CP value, and ADL content was lower in sage and yarrow. In vitro fertilization values were higher in yarrow and thyme pulp. Lavender pulp was more advantageous than others in terms of organic acid profile in the in vitro fermentation broth. As a result, yarrow, thyme and sage can be used as a source of good quality forage.

REFERENCES

AOAC. 1995. Association of Official Analytical Chemists. Washington (DC): Association of Official Analytical Chemists.

Baydar, H. 2009. Tıbbi ve aromatik bitkiler bilimi ve teknolojisi (Genişletilmiş 3.Baskı). Süleyman Demirel Üniversitesi Yayın No: 51 (ISBN: 975-7929--79-4), pp. 1-347.

Benli M, Yiğit N. 2005. Ülkemizde yaygın kullanımı olan kekik (Thymus vulgaris) bitkisinin antimikrobiyal aktivitesi. Orlab On-line Mikrobiyoloji Dergisi 03:1-8.

Ersahince A, Kara K. 2017. Nutrient composition and *in vitro* digestion parameters of Jerusalem artichoke (Helianthus tuberosus L.) herbage at different maturity stages in horse and ruminant. J of Animal Feed Science. 26(3), 213-225,

Greathead, H., 2003. Plants and plant extracts for improving animal productivity. Proceedings of the nutrition Society, 62(2), 279-290

Güler T, Dalkılıç B. 2005. Aromatik Bitkilerin Organik (Ekolojik) Hayvancılıkta Kullanım imkânı. Doğu Anadolu Bölgesi Araştırmaları. 13-7

Menke K.H., Raab L., Salewski A., Steingass H., Fritz D., Schneider W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. J Agric Sci. 93, 217-222.

Menke, H.H., Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim Res Develop. 28(1), 7-55.

Van Soest P.V., Robertson J.B., Lewis B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74(10), 3583-3597.

Genomic Insights in to the Fugu (*Takifugu rubripes*) *crhbp* Gene: An in Silico Analysis for Functional Characterization

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ABSTRACT: An in-silico analysis has been conducted to gain deeper insights into the functional characteristics of the fugu *crhbp* gene. This analysis involves exploring the genomic organization of the gene, conducting in silico experiments to predict its structure and potential functions, and comparing it with orthologous genes in other species. Understanding the functional implications of the fugu *crhbp* gene can provide valuable insights into its biological significance and potential implications in fish and vertebrates including human health and disease. This article aims to present a comprehensive analysis of the fugu *crhbp* gene, shedding light on its structure, function, and evolutionary importance through in silico methods. The researchers applied various bioinformatics tools and databases, including BLAST searches, multiple sequence alignments, and phylogenetic tree construction, to identify conserved domains and predict potential protein structures and functions. By employing these methods, they were able to elucidate crucial information pertaining to the physiological role of the *crhbp* gene in fugu. The findings of this study provide valuable genetic knowledge that can be leveraged for further investigations into this specific gene and its associated biological processes.

Keywords: Fugu, Crhbp, Bioinformatics studies, in Silico analysis

INTRODUCTION

Bioinformatics research encompasses subjects like protein structures, functions, enzyme behaviors, and pathways, and it plays a crucial role in the examination of genomic information (Kumar et al., 2008).

The fugu *crhbp* gene is a member of the chromobox (CBX) gene family and has been of great interest in the field of genomics due to its potential role in various biological processes (Vincenz and Kerppola 2008). Fugu (*Takifugu rubripes*) is a species of pufferfish that has a compact 400-Mb genome that is ~7.5 times smaller than the human genome but contains a similar number of genes (Brunner et al., 1999). The pufferfish (. *rubripes*) is an ideal model organism for genetic and developmental biology studies due to its small genome, which is the smallest among vertebrates, making genomic research more accessible. Pufferfish is particularly used in transplantation and microinjection studies (Brenner et al., 1993; Crnogorac-Jurcevic et al., 1997; Yamanoue et al., 2009; Uji et al., 2011). It offers several advantages for genomic research compared to other vertebrates. This is because the pufferfish genome is only 400 Mb in size, which is considerably smaller than the human genome (3.000 Mb) (Watabe and Ikeda, 2006). It is known that the pufferfish genome has more genes compared to lungfish and coelacanths due to teleost-specific whole-genome duplication (tsWGD) (Van de Peer, 2004). The small genome size, which facilitates gene identification and analysis, contains regular sequences similar to other vertebrates, requiring less effort to obtain comparable data.

The study of the fugu *crhbp* gene is important for gaining genomic insights and understanding its functional characteristics (Clark, 2003). This research provides an opportunity to delve into the intricacies of this gene and its potential implications. Through in silico analysis, we can utilize computational methods to explore and predict the functions of the fugu *crhbp* gene.

With the availability of the *Fugu rubripes* genome sequence, we can compare the fugu *crhbp* gene with its orthologs in other vertebrate genomes, such as humans. Utilizing this comparative genomics approach, we can identify conserved sequences and gene synteny between Fugu and humans, providing further evidence of the functional importance of the crhbp gene across different species. Additionally, by characterizing the complete coding sequence and gene structure of the fugu *crhbp* gene, we can analyze its amino acid sequences and determine potential functional domains or motifs.

MATERIAL AND METHODS

In Silico Analysis

Sequence Alignment and Homology Modeling

Sequence alignment is a crucial method for assessing and recognizing similarities within genetic sequences. In our research, we will utilize sequence alignment tools, specifically the BLOSUM62 matrix algorithm (Gromiha in 2010), to investigate genetic variations within the crhbp/CRHBP gene and pinpoint regions that are conserved.

```
                              10        20        30        40        50        60
                      ....|....|....|....|....|....|....|....|....|....|....|....|
Fugu Crhbp        1   ---MERTFREQLFVLLLSLSVLKGDCR--YMENNEISKDELYSFFNSELKREAPEELMFR
Medaka Crhbp      1   ---.G.......CF....V...R.C..--DL......-..F..L..P.PR.DP.DAFVY.
Stickleback Crhbp 1   MRV.A........F.V..AC.....S.--HI.........FF...D......T...F.Y.
Zebrafish Crhbp   1   ---.SA.S.A..CF....VTA.R.HA.FLD.QD....PEG.L.LLS......L...FVY.
Goldfish Crhbp    1   ---.SG.S.A..CF....VTA.R.HA.FLDIQD....PEG.L.LLS......L...FVY.
Commoncarp Crhbp  1   ---.SG.S.A..CF....VTA.R.HA.FLDIQD....PEG.L.LLS......L...FVY.
HUMAN Crhbp       1   ---.SPN.KL.CHFI.IF.TA.R.ES.--.L.LR.AADYDPFLL.SAN....LAG.QPY.
Mouse Crhbp       1   ---.SPN.KL.CHFI.IL.TA.R.ES.--.L.VQ.AAVYDPLLL.SAN...DLA..QPY.

                              70        80        90       100       110       120
                      ....|....|....|....|....|....|....|....|....|....|....|....|
Fugu Crhbp        56  RPLRCLDMIAVEGRFTFTAERPQLSCAAFFIAEPNEVITVDYDGVDIDCSGGDFITVFDG
Medaka Crhbp      55  .....M..V....Q......T.H....T.LM...S.L.S.EFGR................
Stickleback Crhbp 59  ........V......S.A.DS...R....LV...DQL...ELER.H....A...V.....
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Zebrafish Crhbp     58 .A......V....Q..........N..V...G..TD..SIE..S.N...R.....K....
Goldfish Crhbp      58 .A......V....Q..........N..V...G..SDI.S.E..S.N...R.....K....
Commoncarp Crhbp    58 .A......V.I..Q..........N..V...G..SDI.SIE..S.N...R.....K....
HUMAN Crhbp         56 .A......LSLQ.Q.....D....H......S..E.F..IH..Q.S...Q....LK....
Mouse Crhbp         56 .A......LSLP.Q.....D....H......G..E.F..IH..L.S...Q....LK....

                              130       140       150       160       170       180
                       ....|....|....|....|....|....|....|....|....|....|....|....|
Fugu Crhbp         116 WVMKGEKFPSSQDHPLPLYERYVDYCDSGSLRRSVRSSQNVAMVFFRVHNPGSTFTLTVR
Medaka Crhbp       115 ............................APA..................SA..S......
Stickleback Crhbp  119 ..K.............AA..........--PK...............L.AA..G.A....
Zebrafish Crhbp    118 ......................T...ET.VS.PI.........L...L.QS..S..V.F.
Goldfish Crhbp     118 ..........T...........S...ET.VT.PI.........L...L.QS..S..V.F.
Commoncarp Crhbp   118 ..........T........K..S...ET.VT.PI.........L...L.QS..S..V.F.
HUMAN Crhbp        116 .IL..............SA...I.F.E..LS...I........I.....E..NG....IK
Mouse Crhbp        116 .IL..............TMK..T.F.E..LT...I..............E..NG..I.IK

                              190       200       210       220       230       240
                       ....|....|....|....|....|....|....|....|....|....|....|....|
Fugu Crhbp         176 KHINPFPCNVISQSPEGAYTMVIPQQHRNCSFSIIYPVEIDISEFSLGQINHFT-KRSRP
Medaka Crhbp       175 ..V.L....A.......S........R.......L...A.....YR..RHSS.P-..IM.
Stickleback Crhbp  177 ..A..........AT..S.......ER..............V......HD.GHP-RS.T.
Zebrafish Crhbp    178 .L........V..T...SF..I..................Q.G.L....H.DL--...IL
Goldfish Crhbp     178 .L........V..T...SF..I..................Q.G.L....H.DL--...IF
Commoncarp Crhbp   178 .L........V..T...SF..I..................Q.G.L....H.DL--...IL
HUMAN Crhbp        176 TDP.L........T.N.KF.L.V.H.............V.K..DLT..HV.GLQL.K.SA
Mouse Crhbp        176 TDP.L........T.S.RF.L.V.Y..Q..........A.K..DLT..HLHGLQL.KPAA

                              250       260       270       280       290       300
                       ....|....|....|....|....|....|....|....|....|....|....|....|
Fugu Crhbp         235 ACAETGDFVQLLGGNGIDTSKLLPITDLCVSFTGPTHLKVGCDNTVVRVVSSGKFVSRVS
Medaka Crhbp       234 G.T.SE.....................I.T.......M.I........M....S......
Stickleback Crhbp  236 G...S.........S.........V....A..S...RV......A...M....R......
Zebrafish Crhbp    236 G..GS....E......M....MF.MA...Y..N..AQM..........M.......N...
Goldfish Crhbp     236 G..GS....E......M....MF.MA...Y..N..AQM..........M.......N...
Commoncarp Crhbp   236 G..GS....E......M....MY.MA...Y..N..AQM........I.M.......N...
HUMAN Crhbp        236 G.EGI....E....T.L.P..MT.LA...YP.H..AQM..........M.....H.N..T
Mouse Crhbp        236 G.GG.....E....T.L.P..MM.LA...YP.L..AQM.IS...A...M.....HIN..T

                              310       320
                       ....|....|....|....|....|..     Identity      Similarity
Fugu Crhbp         295 FSYRLLDSQELQTIKLNNVEDFCFNN-     100           100
Medaka Crhbp       294 ....A.....................-     78            87
Stickleback Crhbp  296 ......QRP...S.............-     76            85
Zebrafish Crhbp    296 .Q....GH....QM.G.S...V.LRA-     69            81
Goldfish Crhbp     296 .Q....GH....QM.G.S...V.LRA-     68            81
Commoncarp Crhbp   296 .Q....GH....QM.G.S...V.LRA-     67            81
HUMAN Crhbp        296 .E..Q.EPY..ENPNG.SIGE..LSGL     57            74
Mouse Crhbp        296 .E..Q.EPF..E.STG.SIPEY.LSSL     55            73
```

Figure1. Comparing the Identity and Similarity Rates of Crhbp Protein Sequences from Fugu with Those of Crhbp/CRHBP Protein Sequences in Other Vertebrates

We carried out a BLAST analysis using cDNA sequences of the *crhbp* gene obtained from the NCBI database to confirm the functionality of the *crhbp* gene. Additionally, we examined the conservation of this gene in medaka, zebrafish (*Danio rerio*), and humans (*Homo sapiens*) by identifying the chromosomal regions where the fugu *crhbp* genes are located and comparing them with the gene regions in zebrafish and humans. This analysis enabled us to identify corresponding gene regions in zebrafish and humans, ultimately helping us assess the conservation of gene synteny based on these identified gene regions.

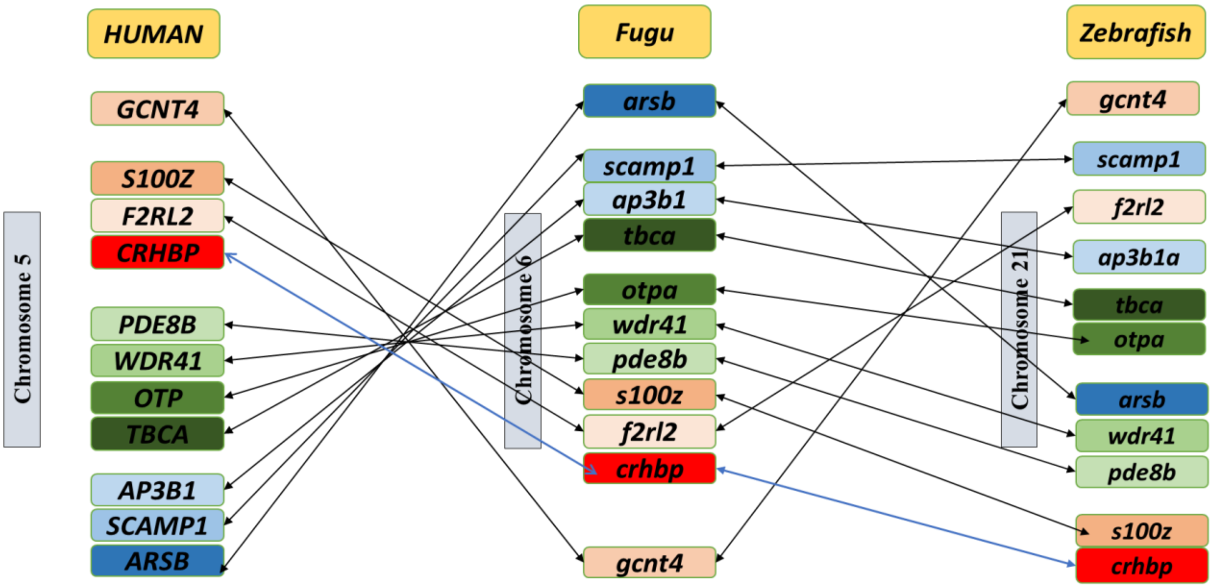

Figure 2. Conserved gene synteny in fugu

To establish the phylogenetic relationship of the fugu *crhbp* gene with other aquatic model organisms and vertebrates, we conducted a phylogenetic tree analysis using the maximum likelihood method. To accomplish this, we utilized software tools such as CLUSTALW, BioEdit (accessible at http://www.mbio.ncsu.edu/bioedit/page2.html), and MEGA6 (Tamura et al., 2013). The resulting phylogenetic tree was constructed using protein sequences from fugu (*Takifugu rubripes*) and other organisms, including, zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), common carp (*Cyprinus carpio*), goldfish (*Carassius auratus*), platyfish (*Xiphophorus maculatus*), tetraodon (*Tetraodon nigroviridis*), human (*Homo sapiens*), and mouse (*Mus musculus*) *crhbp/CRHBPgenes* according to maximum likelihood method using MEGA10 program.

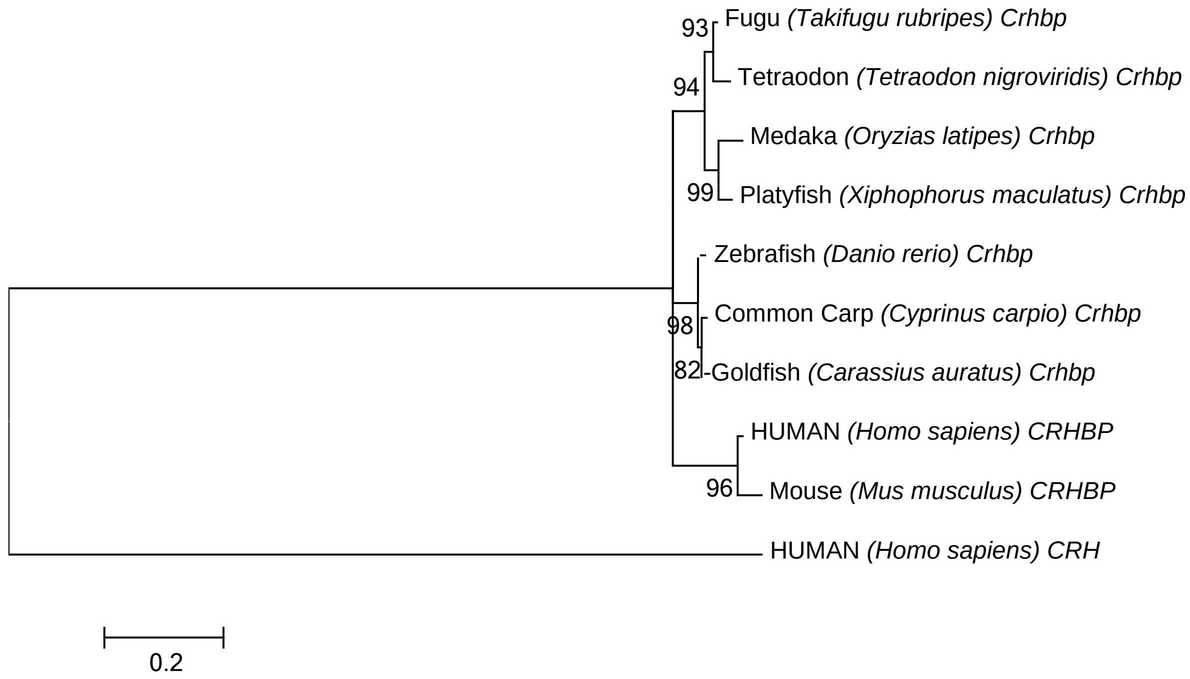

Figure 3. Phylogenetic relationship between fugu and some vertebrates

We conducted in silico analyses using the longest cDNA transcripts of the medaka *crhbp* gene. To determine the gene structure of crhbp genes, examine the arrangement of exons and introns, and identify the nucleotides responsible for encoding proteins along with the 5'-3' ends, we relied on data from the Ensembl database. Furthermore, we documented and included in Table 1 information about the transcription start point (+1), the presence of a TATA box, and the location of the poly A tail.

```
Fugu crhbp ENSTRUG00000018344
5’gaaaacaagcttattttggtgtcatttgcccaaaATATgggtggatgctttgattcac
CTTTTCTGCTTTTGCCTTCATCTTTGTTATCAAAAAAAGTCAATTTTGTATAAGTGTTGC
TTCATTTTCCCACTCTTCTAAAGAAAAGGGCCATGTAGCTCTGCAGAACCGATCAGAAAT
TGCATGTAAACTTACTATATATAGTCTTTTTTTTTTTTTTTTCTTTTTTTTTTAAGAAAGA
CGAACTAATTACTAGAATTAGTGCCCATTATTCCCTGTGAACTCCACTGAGGTAACCATA
CTGGACATTAATTGATTTTTAAATCAAATATACAAACCTTTTCGTCCCGGTAAGTCAATA
GTTTATTCAAAATGTGTCCCTATCAAAGACAGGTCAGAAAGACAACTGTAGAGGCATTTA
AATAACTTTTTAGCCCCGGGACATCGTTTAATGAGCGCGCTGTTTGAAGCACGATGACGT
CATGAAGTTTGATCGTCTTTCAGGAATCAACTGATAATCTCTCTCTCTCTCTCTCTCTCT
CTCTCTTTCTCTCTCTCCCCCTTCAGCGTGTGTCCATCCTCACCCTCGTCACAGCCTCCC
AATTGGTTGGATGTGAGAGGGGAGGAGGGGGGGCCGTGGCGGTGGCGCTGAGGGGGGTCC
ACATTAGCCCAGTTGATCCTTGCGCAGAAGTTGGACCCATAAAAAGGGTCGCGGCTTGGG
AGCGCGCTTCAGACCGTCCTCGCTCAGGTGCGGACAGAGACGCGCGTCATGGAGCGCACT
                                                -M--E--R--T-
TTCCGGGAGCAGCTCTTCGTCCTGCTGCTGAGCCTGTCCGTCCTCAAGGGAGACTGCAGG
-F--R--E--Q--L--F--V--L--L--L--S--L--S--V--L--K--G--D--C--R-
TATATGGAGgtgag’N120’ttaagAACAACGAAATCTCTAAAGACGAACTGTACTCATT
-Y--M--E-                -N--N--E--I--S--K--D--E--L--Y--S--F
TTTCAACTCGGAACTTAAAAGAGAAGCACCTGAGGAATTAATGTTCCGCAGACCTTTACg
--F--N--S--E--L--K--R--E--A--P--E--E--L--M--F--R--R--P--L--
taag’N121’cacagGATGCCTGGACATGATTGCAGTGGAGGGTCGGTTCACCTTCACAG
               R--C--L--D--M--I--A--V--E--G--R--F--T--F--T--
CAGAGCGTCCTCAGCTCAGCTGTGCAGCCTTCTTCATAGCAGAGCCGAATGAAGTGATCA
A--E--R--P--Q--L--S--C--A--A--F--F--I--A--E--P--N--E--V--I--
```

```
CTGTGGATTATGACGGTGTTGACATCGACTGCAGTGGAGGAGACTTCATCACGgtaga'N
T--V--D--Y--D--G--V--D--I--D--C--S--G--G--D--F--I--T-
363'tgaagGTGTTTGATGGCTGGGTAATGAAAGGAGAGAAGTTCCCAAGCTCCCAGGAT
         -V--F--D--G--W--V--M--K--G--E--K--F--P--S--S--Q--D-
CATCCTCTGCCCCTGTACGAGCGCTACGTGGATTACTGTGACTCTGGGTCGCTGAGGAGA
-H--P--L--P--L--Y--E--R--Y--V--D--Y--C--D--S--G--S--L--R--R-
AGCGTGCGCTCCTCGCAGAACGTGGCCATGGTCTTTTTCCGCGTCCACAACCCTGGCAGC
-S--V--R--S--S--Q--N--V--A--M--V--F--F--R--V--H--N--P--G--S-
ACGTTCACCCTGACCGTCAGGAAGCACATCAATCCTTTCCgtgag'N291'tgcagCCTG
-T--F--T--L--T--V--R--K--H--I--N--P--F--                P--C
TAATGTCATCTCCCAGTCACCAGAGGGCGCTTACACGATGGTGATCCCGCAGCAGCACAG
--N--V--I--S--Q--S--P--E--G--A--Y--T--M--V--I--P--Q--Q--H--R
GAACTGCAGCTTCTCCATCATTTACCCAGTGGAGATCGACATCTCCGAGTTCAGCCTGGG
--N--C--S--F--S--I--I--Y--P--V--E--I--D--I--S--E--F--S--L--G
ACAGATCAACCACTTTACAAAGgtaaa'N471'cacagAGGTCCAGACCAGCGTGCGCAG
--Q--I--N--H--F--T--K-                -R--S--R--P--A--C--A--
AAACAGGAGACTTTGTGCAGCTGCTCGGAGGAAATGGGATCGATACATCGAAGTTGCTGC
E--T--G--D--F--V--Q--L--L--G--G--N--G--I--D--T--S--K--L--L--
CCATCACGGACCTTTGCGTCTCCTTCACGGGGCCCAgtaag'N63'tgcagCTCACCTGA
P--I--T--D--L--C--V--S--F--T--G--P--               T--H--L--
AGGTCGGCTGCGACAACACGGTGGTCAGGGTGGTGTCCAGCGGGAAGTTCGTCAGCCGGG
K--V--G--C--D--N--T--V--V--R--V--V--S--S--G--K--F--V--S--R--
TGTCGTTCAGCTACAGGCTACTGGACAGTCAGGAGCTGCAGACCATCAAACTCAACAACG
V--S--F--S--Y--R--L--L--D--S--Q--E--L--Q--T--I--K--L--N--N--
TGGAGGATTTCTGCTTCAACAACTGAcacaacgtgtttcatttgactgtttttaaaaaga
V--E--D--F--C--F--N--N--*-
attAAATTAtttgacttaaactgacaaaatcactctgattattattcactgtaatgtcca
tatgaactaattttacaaaaaaaa3'
```

Table 1. Gene structure of fugu *crhbp* gene

RESULTS AND DISCUSSION

To explore the orthology between the fugu and *crhbp/CRHBP* genes of other vertebrates, we computed the sequence identity-similarity ratio. We accomplished this by aligning the putative amino acid sequences encoded by the fugu *crhbp* gene with *crhbp/CRHBP* sequences from various vertebrate species, including zebrafish, common carp, goldfish, stickleback, medaka, fugu, human, and rat. To determine the sequence identity and similarity, we employed the CLUSTAL W multiple alignment tool with the BLOSUM62 matrix algorithm. The percentage of sequence identity varied among the analyzed crhbp genes, but the medaka crhbp gene showed the highest degree of sequence identity and similarity with the medaka, with values of 78% identity and 87% similarity, as depicted in Figure 1.

To identify conserved gene synteny between the fugu crhbp gene and the crhbp/CRHBP genes in zebrafish and humans, we manually examined the Ensembl genome database. We identified the conserved genes and their respective positions in the fugu genome and then checked if they were also present on the chromosomes of humans and zebrafish. Our research in the Ensembl database revealed the presence of highly conserved genes that are depicted in the synteny diagram in Figure 2.

The phylogenetic relationships were represented in Figure 3, where a tree was constructed using protein sequences from various species, including fugu (*Fugu rubripes*) medaka (*Oryzias* *latipes*), zebrafish (*Danio rerio*), common carp (*Cyprinus carpio*), goldfish (*Carassius auratus*), platyfish (*Xiphophorus maculatus*), tetraodon (*Tetraodon nigroviridis*), human (*Homo sapiens*), and mouse (*Mus musculus*), focusing on the *crhbp/CRHBP* genes. The tree was generated using the maximum likelihood method with the MEGA10 program. Notably, fugu clustered together with other teleost fishes, while organisms such as humans and Mouse formed a distinct group. In this arrangement, the human *CRH* gene served as the outgroup.

CONCLUSION

With the availability of the *Fugu rubripes* genome sequence, we can compare the fugu *crhbp* gene with its orthologs in other vertebrate genomes, such as humans. Utilizing this comparative genomics approach, we can identify conserved sequences and gene synteny between fugu and humans, providing further evidence of the functional importance of the *crhbp* gene across different species. Additionally, by characterizing the complete coding sequence and gene structure of the fugu *crhbp* gene, we can analyze its amino acid sequences and determine potential functional domains or motifs. Furthermore, by analyzing the noncoding regions of the fugu *crhbp* gene, we can identify potential transcriptional regulatory elements and gain insights into the regulatory mechanisms that govern the expression of this gene. These genomic insights and functional characterizations of the fugu *crhbp* gene will contribute to our understanding of its role in various biological processes, such as development, immune response, or disease susceptibility. By conducting in silico analysis on the fugu *crhbp* gene, we can explore its genomic features and identify potential functional elements.

REFERENCES

Brenner, S., Elgar, G., Sanford, R., Macrae, A., Venkatesh, B., Aparicio, S. (1993). Characterization of the pufferfish (Fugu) genome as a compact model vertebrate genome. Nature, 366(6452), 265-268. doi:10.1038/366265a0

Brunner, B., Todt, T., Lenzner, S., Stout, K., Schulz, U., Ropers, H. H., Kalscheuer, V. M. (1999). Genomic structure and comparative analysis of nine Fugu genes: conservation of synteny with human chromosome Xp22. 2–p22. 1. Genome Research, 9(5), 437-448. doi:10.1101/gr.9.5.437

Clark, M. S. (2003). Genomics and mapping of teleostei bony fish. Comparative and functional genomics, 4(2), 182-193. doi:10.1002/cfg.259

Crnogorac-Jurcevic, T., Brown, J. R., Lehrach, H., Schalkwyk, L. C. (1997). Tetraodon fluviatilis, a new puffer fish model for genome studies. Genomics, 41(2), 177-184. Doi:10.1006/geno.1997.4646

Crnogorac-Jurcevic, T., Brown, J. R., Lehrach, H., Schalkwyk, L. C. (1997). Tetraodon fluviatilis, a new puffer fish model for genome studies. Genomics, 41(2), 177-184. doi:10.1006/geno.1997.4646

Gromiha, M. M. Protein bioinformatics: from sequence to function, academic press, Printed in India, 2010.

Kumar, S., Nei, M., Dudley, J., Tamura, K. (2008). MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Briefings in bioinformatics, 9(4), 299-306. doi: 10.1093/bib/bbn017.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Sudhir, Tamura K., Stecher G., Peterson D., Filipski A., Kumar S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0 Molecular Biology and Evolution, 30. doi:10.1093/molbev/mst197.

Uji, S., Kurokawa, T., Hashimoto, H., Kasuya, T., Suzuki, T. (2011). Embryogenic staging of fugu, Takifugu rubripes, and expression profiles of aldh1a2, aldh1a3 and cyp26a1. Development, growth and differentiation, 53(5), 715-725. Doi:10.1111/j.1440-169X.2011.01281.x

Van de Peer, Y. (2004). Tetraodon genome confirms Takifugu findings: most fish are ancient polyploids. Genome biology, 5(12), 1-4. Doi:10.1186/gb-2004-5-12-250

Vincenz, C., and Kerppola, T. K. (2008). Different polycomb group CBX family proteins associate with distinct regions of chromatin using nonhomologous protein sequences. Proceedings of the National Academy of Sciences, 105(43), 16572-16577. Doi:10.1073/pnas.0805317105

Watabe, S., and Ikeda, D. (2006). Diversity of the pufferfish Takifugu rubripes fast skeletal myosin heavy chain genes. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 1(1), 28-34. Doi:10.1016/j.cbd.2005.12.001

Yamanoue, Y., Miya, M., Matsuura, K., Miyazawa, S., Tsukamoto, N., Doi, H., Takahashi, H., Mabuchi, K., Nishida, M., Sakai, H. (2009). Explosive speciation of Takifugu: another use of fugu as a model system for evolutionary biology. Molecular biology and evolution, 26(3), 623-629. Doi:10.1093/molbev/msn283.

Management of Male Infertility with some Antioxidant Supplements

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ABSTRACT: The worldwide prevalence of infertility is estimated to afflict about 15% of couples, with a range of 2.5% to 12% attributed primarily to male causes. A disparity between reactive oxygen species (ROS) and antioxidant concentrations constitutes oxidative stress (OS). OS causes peroxidation of lipids and damage to spermatozoa, thus reducing the quality of seminal parameters. OS is one of the most frequently reported pathologies, affecting approximately fifty percent of infertile males. Furthermore, variations in antioxidant and ROS levels resulting from intrinsic and extrinsic factors associated with OS, lifestyle, diet, genetics, and genetics also play a role in male infertility. Therefore, increased levels of ROS may cause infertility via DNA damage, lipid peroxidation, enzyme inactivation, and protein oxidation in spermatozoa. Lifestyle changes and antioxidant treatment may help treat OS-related infertility. The damage caused by OS to sperm is reduced by antioxidants such as vitamins E and C, coenzyme-Q10, carnitines, N-acetylcysteine, selenium, zinc, and folic acid. This review aims to describe the current understanding of managing male infertility, which is known to be caused by oxidative stress, and manage it with some natural antioxidants.

Keywords: Male infertility, Sperm, Oxidative stress, Antioxidant, Free radicals

INTRODUCTION

Infertility is increasingly recognized as a significant public health issue on a worldwide scale. It is a disease that is characterized by an inability to establish a clinical pregnancy after one year of regular and unprotected sexual activity (Ali et al.,2021). About 15% of couples worldwide experience infertility; between 2.5% and 12% of these cases are attributed to male causes. The prevalence of male factor infertility exhibits significant regional variability, with rates ranging from 20% to 70%. Male infertility is a multifaceted condition that may be attributed to several factors, including genetic predispositions, the presence of varicocele, diseases affecting the reproductive system, obstructive or non-obstructive azoospermia, male hypogonadism, and the presence of anti-sperm antibodies (Agarwal et al., 2021).

Oxidative stress occurs from the dysregulation between ROS and antioxidants and is recognized as a significant contributor to male infertility. This condition is associated with elevated levels of sperm DNA fragmentation and aberrant semen parameters (Kumar & Singh, 2018). Hence, the use of antioxidants as a means to mitigate the excessive generation of ROS is a viable avenue for enhancing the overall quality of semen. The use of antioxidant supplements has the potential to result in higher rates of live births and clinical pregnancies while also reducing the incidence of miscarriages (de Ligny et al., 2022).

ROS is thought to be a contributing agent in between 30 and 80 percent of cases of male factor infertility. The overproduction of ROS and OS has repeatedly been linked to low reproductive potential in men who aspire to become fathers. These mechanisms have been shown to correlate with sperm DNA fragmentation, poor fertilization and embryo development, low implantation rates, high miscarriage rates, and pregnancy loss (Ali et al.,2021). ROS can gain electrons from the structures around them to achieve ground state, which results in these highly reactive molecules becoming free radicals (Goldfarb, 1999). Compared to spermatozoa, the seminal plasma has a much higher concentration of antioxidants, which semen possesses in enormous quantities (Shiva et al., 2011). They are shielded from the potentially harmful effects of OS due to this. Antioxidants, both enzymatic and nonenzymatic, are added to the seminal plasma as a dietary supplement (Bansal & Bilaspuri, 2010).

As a result, the use of antioxidant supplements seems to be a viable choice for lowering ROS levels and enhancing the quality of sperm. At this time, there needs to be more consensus about the advantages, adverse effect profile, and optimal dosage of antioxidant supplements (Ali et al., 2021). According to a recent consensus guideline published by the European Society for Human Reproduction and Embryology, there is inadequate evidence to justify using antioxidant supplementation (Barratt et al., 2017). According to recent research, the most frequent components found in commercially accessible dietary supplements are vitamin E, zinc, vitamin C, folic acid, and selenium (Ali et al., 2021). Research on dietary supplements has consistently shown that using antioxidants is associated with enhanced semen characteristics. The administration of vitamin E, selenium, N-acetylcysteine, or carnitine has shown positive effects on sperm motility after therapy. The administration of vitamins C and E have shown a significant decrease in DNA fragmentation compared to a placebo (Steiner et al., 2020).

Vitamine C

Vitamin C in sperm cells inhibits agglutination and protects against DNA damage induced by ROS molecules (Angulo et al.,2011). Although vitamin C supplementation plays an important role, its impact on male fertility remains unclear. Several studies have shown no impact on fundamental semen characteristics or DNA fragmentation when used alone; however, enhancements are seen only when combined with additional antioxidants such as vitamin E or selenium (Torres-Arce et al., 2021). Vitamin C, when administered to mice at a dosage comparable to the therapeutic dose for humans (10mg/Kg), has been shown to decrease MDA levels, raise sperm count, and improve the fraction of normal sperm population (Gadallah and Abeel, 2018). Vitamin C, like E, might be a membrane defender against ROS. The addition of Vitamin C did not enhance the quantity of sperm. However, it did positively impact the movement and structure of the sperm (Cyrus et al., 2015).

Vitamine E

Based on the results obtained from in vitro experiments, there is a potential for vitamin E to protect against oxidative damage and loss of motility in spermatozoa. A randomized control trial demonstrated the efficiency of vitamin E in the treatment of an infertile male suffering from an increased level of ROS. Additionally, research has shown that vitamin E treatment effectively reduced the concentration of MDA in spermatozoa to a level consistent with normospermic values. Moreover, this therapy has been shown to improve sperm motility and raise the probability of successful pregnancies (Beygi et al., 2021). This antioxidant has several roles in male fertility, including testosterone production and telomerase activity regulation (Torres-Arce et al., 2021). A recent research on rats investigated the impact of noise-induced stress and nicotine exposure on sperm viability. The study showed that vitamin E had a beneficial effect on sperm viability in rats exposed to either of these stressors (Bisong et al., 2018; Miyazawa et al., 2019). Vitamin E is the main antioxidant in spermatozoa, protecting the cell membrane from ROS. Insufficient levels of Vitamin E lead to improper sperm production. Vitamin E has a beneficial impact on the functioning of the testis and sperm. However, the use of vitamin E supplements has not significantly improved the overall quality of sperm (Rengaraj et al., 2015; Ener et al., 2016).

Folic acid (Vitamin B9)

Since the available data is insufficient to indicate a conclusive enhancement in spermatic parameters, it does warrant more investigation in this area, particularly considering that up to 23% of men between the ages of 50 and 70 have suboptimal Folic Acid levels (Fenech, 2012).

Folic acid only acts as a DNA safeguard when its levels exceed 36 nmol/L. The impact of insufficient vitamin B9 on the integrity of sperm DNA remains unknown based on current understanding (Torres-Arce et al., 2021).

Selenium

Selenium (Se) is a cofactor in antioxidative enzymes that are important for neutralizing and preventing the creation of ROS during normal spermatogenesis, mitochondrial function, and capacitation. Glutathione peroxidase is a crucial selenoprotein vital in several redox processes related to male reproduction. Spermatozoa integrate it into the mitochondrial membrane to balance ROS formation throughout the motility phase (Mintziori et al., 2020; Cilio et al., 2022).

Selenium positively associated with specific semen characteristics, including progressive motility, total motility, sperm concentration, total sperm count, and normal morphology. Elevated levels of seminal selenium correlate with increased live birth rates and a greater likelihood of pregnancy (Wu et al., 2020; Torres-Arce et al., 2021).

Zinc

Both the motility of human sperm and the response of acrosomes are significantly influenced by zinc. It is commonly believed that zinc, which is integrated into sperm, helps support sperm motility, membrane stability, and antioxidant capability. Additionally, zinc is thought to defend against sperm decondensation (Riffo et al., 1992; Kerns et al., 2018). Zinc plays a role in several aspects of human male fertility, including the reduction of MDA levels, the enhancement of sperm total motility, progressive motility, sperm concentration, chromatin integrity, and the maintenance of normal sperm morphology (Torres-Arce et al., 2021).

N-Acetylcysteine

Some studies have demonstrated that NAC may increase a man's fertility. Human testicular cells cultured in vitro with NAC incubation showed a 68% reduction in apoptotic rate compared to controls without NAC (Erkkilä et al., 1999; Torres-Arce et al., 2021). Following the administration of NAC, it has been shown that the overall antioxidant capacity of seminal fluid increases while the number of ROS molecules experiences a reduction (Oeda et al., 1997; Ciftci et al., 2009; Jannatifar et al., 2019; Jannatifar et al., 2020; Ghafarizadeh et al., 2021). N-acetylcysteine (NAC) has been scientifically shown to enhance many sperm characteristics, including volume, motility, count, concentration, and normal morphology. Negative processes that NAC may help reduce include sperm viscosity, liquefaction time, and DNA fragmentation (Salas-Huetos et al., 2018).

Carnitines

Carnitines are found in large concentrations in the epididymis, where they are continuously released. The correlation between carnitines and sperm quality is well demonstrated. Multiple studies have shown a clear correlation between carnitines and sperm motility (Torres-Arce et al., 2021). An investigation has shown evidence of a favorable link between seminal l-carnitine and sperm count levels, motility, and morphology (Ahmed et al., 2017). In case-control research comparing men who were fertile and infertile, it was shown that the fertile group had greater levels of seminal carnitine (108.43 mg/L), higher sperm counts (66.66 X 106), and higher motility (50.45%) compared to the infertile group. Specifically, the fertile group had seminal carnitine levels of 80.6 mg/L, sperm counts of 52.56 X 106, and motility of 32.31% (Sheikh et al., 2023).

Coenzyme-Q10 (CoQ10)

The fact that there is a direct association between sperm count and ubiquinol and an inverse correlation between hydroperoxide and ubiquinol, respectively, is evidence of the important function that CoQ10 plays in male fertility and the redox state. In a clinical investigation that was conducted in 2015, it was discovered that CoQ10 might be used as a protective entity against oxidative stress as well as DNA damage (Torres-Arce et al., 2021). The most significant substantial increase in sperm concentration and motility was shown in a trial that used 300 mg of CoQ10 daily for 26 weeks (Safarinejad, 2012).

CONCLUSION

Antioxidant supplements that are sold to treat male infertility are thought to be capable of improving semen parameters (concentration, motility, and morphology), sperm quality, and live birth rates. According to the findings of this research, using antioxidant supplements may help reduce the harm that oxidative stress causes to sperm.

REFERENCES

Agarwal, A., Leisegang, K., Majzoub, A., Henkel, R., Finelli, R., Selvam, M. K. P., ... & Shah, R. (2021). Utility of antioxidants in the treatment of male infertility: clinical guidelines based on a systematic review and analysis of evidence. The world journal of men's health, 39(2), 233.

Ahmed, S. H., Ahsan, S., Iqbal, T., & Burney, S. A. (2017). Relationship of seminal free L-Carnitine with functional spermatozoal characteristics: results from an observational study conducted in a tertiary care hospital of Karachi, Pakistan. Journal of Pakistan Medical Association, 67(2), 280-284.

Ali, M., Martinez, M., & Parekh, N. (2021). Are antioxidants a viable treatment option for male infertility?. Andrologia, 53(1), e13644.

Angulo, C., Maldonado, R., Pulgar, E., Mancilla, H., Córdova, A., Villarroel, F., ... & Concha, I. I. (2011). Vitamin C and oxidative stress in the seminiferous epithelium. Biological research, 44(2), 169-180.

Bansal, A. K., & Bilaspuri, G. S. (2010). Impacts of oxidative stress and antioxidants on semen functions. Veterinary Medicine International, 200, 1–7. https://doi.org/10.4061/2011/686137

Beygi, Z., Forouhari, S., Mahmoudi, E., Hayat, S. M., & Nourimand, F. (2021). Role of oxidative stress and antioxidant supplementation in male fertility. Current Molecular Medicine, 21(4), 265-282.

Bisong, S. A., Ukoh, I. E., Nna, V. U., & Ebong, P. E. (2018). Vitamin E attenuates nicotine- and noise-induced reproductive impairment in male albino Wistar rats. Andrologia, 50(7), e13050.

Ciftci, H., Verit, A., Savas, M., Yeni, E., & Erel, O. (2009). Effects of N-acetylcysteine on semen parameters and oxidative/antioxidant status. Urology, 74(1), 73-76.

Cilio, S., Rienzo, M., Villano, G., Mirto, B. F., Giampaglia, G., Capone, F., ... & Crocetto, F. (2022). Beneficial effects of antioxidants in male infertility management: A narrative review. Oxygen, 2(1), 1-11.

Cyrus, A., Kabir, A., Goodarzi, D., & Moghimi, M. (2015). The effect of adjuvant vitamin C after varicocele surgery on sperm quality and quantity in infertile men: a double blind placebo controlled clinical trial. International braz j urol, 41, 230-238.

de Ligny, W., Smits, R. M., Mackenzie-Proctor, R., Jordan, V., Fleischer, K., de Bruin, J. P., & Showell, M. G. (2022). Antioxidants for male subfertility. Cochrane Database of Systematic Reviews, (5).

Ener, K., Aldemir, M., Işık, E., Okulu, E. M. R. A. H., Özcan, M. F., Uğurlu, M., ... & Özayar, A. (2016). The impact of vitamin E supplementation on semen parameters and pregnancy rates after varicocelectomy: a randomised controlled study. Andrologia, 48(7), 829-834.

Erkkilä, K., Hirvonen, V., Wuokko, E., Parvinen, M., & Dunkel, L. (1998). N-acetyl-L-cysteine inhibits apoptosis in human male germ cells in vitro. The Journal of Clinical Endocrinology & Metabolism, 83(7), 2523-2531.

Fenech, M. (2012). Folate (vitamin B9) and vitamin B12 and their function in the maintenance of nuclear and mitochondrial genome integrity. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 733(1-2), 21-33.

Gadallah, K., & Abeel, A. M. (2018). Role of antioxidants in the treatment of male infertility. Surg Med Open Acc J, 1(2), 1-10.

Ghafarizadeh, A., Malmir, M., Naderi Noreini, S., & Faraji, T. (2021). Antioxidant effects of N-acetylcysteine on the male reproductive system: A systematic review. Andrologia, 53(1), e13898.

Goldfarb, A. H. (1999). Nutritional antioxidants as therapeutic and preventive modalities in exercise-induced muscle damage. Canadian Journal of Applied Physiology, 24(3), 249–266. https://doi.org/10.1139/h99-021

Jannatifar, R., Parivar, K., Roodbari, N. H., & Nasr-Esfahani, M. H. (2019). Effects of N-acetyl-cysteine supplementation on sperm quality, chromatin integrity and level of oxidative stress in infertile men. Reproductive Biology and Endocrinology, 17(1), 1-9.

Jannatifar, R., Parivar, K., Roodbari, N. H., & Nasr-Esfahani, M. H. (2020). The effect of N-acetyl-cysteine on NRF2 antioxidant gene expression in asthenoteratozoospermia men: a clinical trial study. International Journal of Fertility & Sterility, 14(3), 171.

Kerns, K., Zigo, M., & Sutovsky, P. (2018). Zinc: A necessary ion for mammalian sperm fertilization competency. International journal of molecular sciences, 19(12), 4097.

Kumar, N., & Singh, A. K. (2018). Reactive oxygen species in seminal plasma as a cause of male infertility. Journal of gynecology obstetrics and human reproduction, 47(10), 565-572.

Mintziori, G., Mousiolis, A., Duntas, L. H., & Goulis, D. G. (2020). Evidence for a manifold role of selenium in infertility. Hormones, 19, 55-59.

Miyazawa, T., Burdeos, G. C., Itaya, M., Nakagawa, K., & Miyazawa, T. (2019). Vitamin E: regulatory redox interactions. IUBMB life, 71(4), 430-441.

Oeda, T., Henkel, R., Ohmori, H., & Schill, W. B. (1997). Scavenging effect of N-acetyl-L-cysteine against reactive oxygen species in human semen: a possible therapeutic modality for male factor infertility?. Andrologia, 29(3), 125-131.

Rengaraj, D.; Hong, Y.H. Effects of Dietary Vitamin E on Fertility Functions in Poultry Species. Int. J. Mol. Sci. 2015, 16, 9910–9921.

Riffo, M., Leiva, S., & Astudillo, J. (1992). Effect of zinc on human sperm motility and the acrosome reaction. International journal of andrology, 15(3), 229-237.

Safarinejad, M. R. (2012). The effect of coenzyme Q 10 supplementation on partner pregnancy rate in infertile men with idiopathic oligoasthenoteratozoospermia: an open-label prospective study. International urology and nephrology, 44, 689-700.

Salas-Huetos, A., Rosique-Esteban, N., Becerra-Tomás, N., Vizmanos, B., Bulló, M., & Salas-Salvadó, J. (2018). The effect of nutrients and dietary supplements on sperm quality parameters: a systematic review and meta-analysis of randomized clinical trials. Advances in Nutrition, 9(6), 833-848.

Sheikh, N., Goodarzi, M. T., Al-Havaejee, H. B., Safari, M. R., Amiri, I., Najafi, R., & Hadeie, J. (2023). L-carnitine level in seminal plasma of fertile and infertile men. Journal of Research in Health Sciences, 7(1), 43-48.

Shiva, M., Gautam, A. K., Verma, Y., Shivgotra, V., Doshi, H., & Kumar, S. (2011). Association between sperm quality, oxidative stress, and seminal antioxidant activity.

Clinical Biochemistry, 44(4), 319–324. https://doi.org/10.1016/j.clinbiochem.2010.11.009

Steiner, A. Z., Hansen, K. R., Barnhart, K. T., Cedars, M. I., Legro, R. S., Diamond, M. P., ... & Network, R. M. (2020). The effect of antioxidants on male factor infertility: the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial. Fertility and sterility, 113(3), 552-560.

Torres-Arce, E., Vizmanos, B., Babio, N., Marquez-Sandoval, F., & Salas-Huetos, A. (2021). Dietary antioxidants in the treatment of male infertility: counteracting oxidative stress. Biology, 10(3), 241.

Wu, S., Wang, M., Deng, Y., Qiu, J., Zhang, X., & Tan, J. (2020). Associations of toxic and essential trace elements in serum, follicular fluid, and seminal plasma with In vitro fertilization outcomes. Ecotoxicology and environmental safety, 204, 110965.

Effect of Variety on Organic Matter Digestibility, Chemical Composition, Metobolizable Energy and *In vitro* Gas Production of Barley (*Hordeum vulgare* L.)

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ABSTRACT: In this study the effect of variety on organic matter digestibility (OMD), chemical composition, in vitro gas production (IVGP) and metobolizable energy (ME) of barley (*Hordeum vulgare* L.) were investigated. 5 different variety (Olgun, Sladoran, Bozlak, Sarıbey, Asutay) were used in the study. Crude protein (CP), fiber insoluble in acid solvents (ADF), fiber insoluble in neutral solvents (NDF), IVGP, ME, OMD of barley variety are 12.52-14.89%, respectively. It varied between 4.68-6.53, 24.19-38.15%, 54.82-62.28 mL 200 mg-1, 8.23-9.32 mJ kg-1 KM, 56.77-63.66%. According to the results obtained, the effect of the variety was found to be statistically significant in terms of the examined traits. Sarıbey variety stands out in terms of HP, ADF and NDF content. Olgun variety had higher values than Asutay in terms of IVGP, ME and OMD. According to the results obtained from the study, it was seen that barley variety can be used in ruminant animal feeding. Beside to chemical analyses, use to in vitro gas production technique is quite useful and easy in determining variety differences in the way of in vitro gas production, OMD and ME.

Keywords: Digestibility, Gas production, Metabolizable energy, variety

INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important cereal grain for ruminant in many regions of the World (Guney, 2019). Barley, one of the highest feed values among the grains used as animal feed. Today, barley is generally uses as animal feed (grain and straw), food and malt industry. Barley consumption in Turkey is 90% as animal feed, the rest of uses in malt and food industry (Kandemir ve Altuntaş, 2012). Barley, used extensively in animal nutrition, is not only source of starch, but also valuable feed source in terms of vitamins and minerals. (Alkan and Kandemir, 2015; Biel ve Jayncno, 2013). Barley is a delicious feed for ruminants. It is use in ration prepare both dairy and beef cattle. In order to prepare a balanced ration for ruminants and implement a successful feeding program, it is important to determine the energy values and nutrient digestibilities of these varieties. As well as the differences in the content of barley varieties in term of nutrient composition (Guney, 2019).

In vitro gas production technique is widely used in determining the nutritional value of feeds. In vitro gas production technique is a remarkable method; In recent years, many studies have been found on this subject, (Güney, 2019; Atalay and Kamalak, 2019; Kurt et al., 2022), more studies are being conducted to determine the nutritional value of feeds. Indeed, the nutrients and energy content of barley used as feed raw material. Therefore, feed value varies greatly due to genetic and environmental conditions. (Reynolds ve ark., 1992; Sanford and ark., 2003).

This study was conducted to determine the chemical composition of some barley varieties grown in Turkey, as well as their *in vitro* gas production (IVGP), organic matter digestibility (OMD), and metobolizable energy (ME) contents.

MATERIAL AND METHOD

Barley grain samples were obtained from agronomic trials carried out in experimental area of Muş Alparslan University between 2020 and 2021. Samples of 5 barley varieties were randomly collected from each block in the experimental plots with 3 replications. Dry matter (DM), crude ash (CA), ether extract (EE), and crude protein (CP) contents of the grains were determined according to the method reported by AOAC (1990). ADF and NDF contents were determined by Van Soest et al., (1991) according to the method reported. An *in vitro* gas production technique was used to determine the TG production of barley grain samples (Menke et., 1979).

Samples (0.2 grams) mixed with buffer solution were fermented with rumen fluid from sheep for 24 hours at 39°C. (Menke ve Steingass, 1988). Gas production is arranged according to standard feed and blind measurement. Metabolizable energy and organic matter digestibility of barley grains were determined using the equations given below Menke and Steingass (1998).

ME (Mj kg^{-1} DM) = 1.06+0.1570GP+ 0.084CP+0.220EE-0.081CA

OMD (%) = 28.49+ 0.7967GP+ 0.325CP

GP: Gas production of 200 mg sample at 24 h incubation (ml)

CP: Crude protein (%)

EE: Ether extract (%)

CA: Crude ash (%)

The data obtained in the study were evaluated statistically (Tukey 5%) using one-way analysis of variance (One-way ANOVA).

RESULTS AND DISCUSSION

The chemical compositions of barley grains are given in Table 1. There was no statistical difference between DM and EE contents of barley varieties (P>0.05). It was determined that the differences in the other chemical compositions (CA, CP, ADF, NDF) of the cultivars were significant (P<0.05). The CA contents of barley cultivars ranged from 3.01-3.75 %, the highest values in Sladoran and the lowest values in Asutay. The CA content of barley grains was determined by Muruz and Çelik (2022) 1.59-2.06%, Sirat andBaha (2020), 2.00-2.31%, Kurt et al., (2022) 3.04%. The CP contents of barley cultivars ranged from 12.52-14.89%, the highest values in Sarıbey and lowest values in Sladoran. The CP content of barley grains was determined by Alijosius et al., (2016), 10.37-11.93%, Sevim et al., (2017) 12.60-13.10%, Fant et al., (2020) 9.1-15.2%. Elgün et al., (2001) reported that the protein rate in feed barley should be over 12%. Accordingly, it can be said that the barley varieties used in the study have forage properties. The ADF and NDF contents of barley cultivars ranged from 4.68-6.53%, 24.19-38.15% respectively. The highest and lowest ADF in Sladoran and Bozlak, the highest NDF values in Bozlak, the lowest values in Sladoran and Sarıbey. The ADF and NDF contents barley grains was determined by Alkan and Kandemir, (2015), 3.65-6.49%, 23.4-42.41%, Alijosius et al., (2016), 6.31-6.86%, 18.76-20.87%, Güney (2019), 6.53-9.07%, 19.77-26.61%, Kurt et al., (2022) 6.70-43.64%. It was concluded that the differences observed between the current study and similar studies are related to differences such as variety, environment, soil conditions, as well as the amount of hull in the grains. As a matter of fact, it has been reported that the total husk ratio in barley varies between 15-25% of the grain weight and the NDF ratio of hull can be more than 70% (Rode ve Beauchemin, 1995; Zhao ve ark., 2015).

Table 1. Chemical compositions of barley varieties (% DM)

Variety	DM	EE	CA	CP	ADF	NDF
Olgun	91.64	1.45	3.12^{b}	14.46^{ab}	5.82^{bc}	34.17^{ab}
Sladoran	92.03	1.92	3.75^{a}	12.52^{c}	6.53^{a}	24.19^{c}
Bozlak	91.55	1.67	3.20^{b}	14.69^{a}	4.68^{d}	38.15^{a}
Sarıbey	92.27	1.83	3.29^{b}	14.89^{a}	5.65^{c}	26.38^{c}
Asutay	92.03	1.58	3.01^{b}	13.76^{b}	6.49^{ab}	31.27^{b}
SEM	0.424	0.269	0.405	1.362	1.069	1.272
Sig.	NS	NS	**	**	**	**

**abc Column means with common superscripts do DM: Dry matter (%), CA: Crude ash (% of DM), **not differ (P>0.05.) EE: Eter extract (% of DM), CP: Crude protein (% of DM), ADF: Acid detergent fiber (% of DM), NDF: Neutral detergent fiber (% of DM), SEM: standart eror mean, Sig: Significant level, **: P<0.01, *: P<0.05, NS: Non- significant.

In vitro TG, ME and OMD of barley varieties were given in Table 2. These values were affected at significant levels in terms of varieties (P<0.05). The *in vitro* TG of the grains of barley cultivars ranged from 54.82-62.28 mL with the highest values in Olgun and Sladoran and the lowest values in Asutay. The *in vitro* TG content of barley grains was determined by Sevim et al., (2017) 64.00-72.50 mL, Muruz and Çelik, (2020) 42.23- 46.51 mL. The ME values of the cultivars ranged from 8.23-9.32 Mj kg^{-1} DM, the highest values in Sladoran and the lowest values in Asutay. ME values of barley grains was determined by Sevim et al., (2017) 11.75-12.86 Mj kg^{-1} DM, Muruz and Çelik (2020), 7.74-8.44 Mj kg^{-1} DM. The OMD values of cultivars ranged from 56.77-63.66%, the highest values in Sladoran and the lowest values in Asutay. OMD values of barley grains was determined by Muruz and Çelik (2020), 51.79-56.14%. Within the data obtained from the *in vitro* gas production values, it was determined that the ME and OMD with higher total gas production were in the same direction. As a matter of fact, it has been reported that OMD will be high in feed materials with a high amount of gas formed as a result of the 24-hour incubation period (Kılıç, 2005).

Table 2. *n vitro* total gas production, metabolizable energy and organic matter digestibilty of triticale cultivars.

Variety	TG	ME	OMD
Olgun	62.28^{a}	9.03^{ab}	61.87^{ab}
Sladoran	62.11^{a}	9.32^{a}	63.66^{a}
Bozlak	58.81^{ab}	8.94^{bc}	61.27^{bc}
Sarıbey	57.76^{bc}	8.67^{c}	59.54^{c}
Asutay	54.82^{c}	8.23^{d}	56.77^{d}
SEM	1.091	0.579	0.673
Sig	**	**	**

**abc Column means with common superscripts do not differ (P>0.05.) TG: Total gas, ME: Metabolizable energy, OMD: Organic matter digestibilty, SEM: standart eror mean, Sig: Significant level, **: P<0.01, *: P<0.05.

CONCLUSION

Varieties have a significant effect on chemical composition, *in vitro* total gas production, ME, and OMD. It has been determined that the Olgun variety, which has high values in terms of CP, ME, and OMD according to chemical composition and fermentation parameters, can be used successfully in animal feeding. But before large implication, yield of barley varieties should be tested. It is suggested that this study should be supported by in vivo studies.

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Statement of Conflict of Interest

In this study is not conflict of interest.

REFERENCES

Alijosius, S., Svirmickas, G.J., Kliseviciute, V., Gruzauskas, R., Sasyee, V., Raceviciute-Stupeliene, A., Dauksiene, A., Dailidaviviene, J., 2016. The chemical composition of different barley varieties grown in Lıthuania. Veterınarıja Ir Zootechnıka, 73 (95): 9-13.

Alkan, R.A., Kandemir, N., 2015. Tokat yerel arpa çeşidi içinden seçilen saf hatların bazı gıda, yem ve tarımsal özellikler bakımından varyasyonları. Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 24 (2):124-139.

AOAC, 1990. Official method of analysis. Association of Official Analytical Chemists, 15th ed., DC, USA, Washington; pp.66-88.

Atalay, A. İ., Kamalak, A., 2019. Olgunlaşma dönemlerinin sirken (Chenopodium album) otunun kimyasal kompozisyonuna, besleme değerine ve metan üretimine etkisi. Türk Tarım ve Doğa Bilimleri Dergisi, 6(3): 489-493.

Biel, W., Jacyno, E., 2013. Chemical composition and nutritive value of spring hulled barley varieties. Bulgarian Journal of Agricultural Science, 19 (4): 721-727.

Elgün, A., Türker, S., Bilgiçli, N., 2001. Tahıl ve ürünlerinde analitik kalite kontrolü. Selçuk Üniversitesi Ziraat Fakültesi Gıda Mühendisliği Bölümü, Yayın No: 2, Konya.

Fant, P., Ramin, M., Jaakkola, S., Grimberg A., Carlsson, A. S., Huhtane, P., 2020. Effects of different barley and oat varieties on methane production, digestibility, and fermentation pattern in vitro. Journal of Dairy Science, 103(2): 1404-1415.

Güney, M., 2019. Effect of grain processing at different barley varieties on nutrient composition, starch contents and in vitro digestion parameters. Progree in Nutrition, 21(3): 715-721.

Kandemir, N., Altuntaş, F.R., 2012. Tokak Yerel Arpa Çeşidi İçinden Seçilen Saf Hatların Bazı Gıda, Yem ve Tarımsal Özellikler Bakımından Varyasyonları. Gaziosmanpaşa Üniversitesi Bilimsel Araştırma Projesi Sonuç Raporu.

Kılıç, Ü., 2005. Ruminant beslemede kullanılan bazı yem ham maddelerinin in vitro gaz üretim tekniği kullanılarak bazı fermentasyon ürünlerinin ve enerji içeriklerinin belirlenmesi. Ondokuzmayıs Üniversitesi, Fen Bilimleri Enstitüsü, Doktora tezi, Samsun.

Kurt, Ö., Kamalak, A., Atalay, A. İ., Kaya, E., Kurt, A. N., 2022. Ruminant Beslemede Kullanılan Bazı Kaba ve Kesif Yemlerin in Vitro Gaz Üretimlerinin Belirlenmesi. Türk Tarım ve Doğa Bilimleri Dergisi, 9(2): 406-412.

Marwat, S.K., Hashimi, M., Khan, K.U., Khan, M.A., Shoaib, M., Fazal-ur-Rehman., 2012. Barley (*Hordeum vulgare* L.) A Prophetic Food Mentioned in Ahadith and its Ethnobotanical Importance. American-Eurasian Journal of Agricultural and Environmental Sciences. 12(7):835-841.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. J Agric Sci (Camb), 93:217-222.

Menke, K.H., Steingass, H., 1998. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. Anim. Res. Dev, 28:7–55.

Muruz, H., Çelik, C., 2020. Bazı arpa (*Hordeum vulgare* L.) çeşitlerinin in vitro gaz üretimi, organik madde sindirilebilirliği, besin maddeleri içerikleri ve enerji değerlerinin karşılaştırılması. Anadolu Tarım Bilimleri Dergisi, 35(1): 133-139.

Reynolds, W.K., Hunt, C.W.J., Eckert, W., Hall, M.H., 1992. Evaluation of the feeding value of barley as affected by variety and location using near infrared reflectance spectroscopy. Proceedings, Western Section, American Society of Animal Science, 43: 498.

Rode, L.M., Beauchemin, K.A., 1995. Utilization of barley based diets for feedlot cattle. Proceedings, Western Section, American Society of Animal Science, 46:606−607.

Sanford, B. J., Grove, A.V., Hunt, C.W., 2003. Evaluation of barley characteristics that are associated with digestible energy for beef cattle. The Professional Animal Scientist, 19: 281–285.

Sevim, B., Ayaşan, T., Ülger, İ., Ergül, Ş., Aykanat, S., Coşkun, A. M., 2017. Farklı maltlık arpa çeşitlerinin besin değerlerinin in vitro gaz üretim tekniği kullanılarak tespiti. Turkish Journal Of Agriculture-Food Science And Technology, 5(10):1216-1220.

Sirat, A., Bahar, B., 2020. Gümüşhane ekolojik koşullarında bazı altı sıralı arpa çeşitlerinin tane kalite özellikleri ve besin değerlerinin belirlenmesi. Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi, 6(2):325-335.

Van Soest, P.V., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci, 74(10): 3583- 3597.

Zhao, Y.L., Yan, S.M., He, Z., Anele U.C, Swift M.L., A Mcallister, T., Yang, W., 2015. Effects of volume weight, processing method and processing index of barley grain on in situ digestibility of dry matter and starch in beef heifers. Animal Feed Science and Technology, 199: 93-103. Agrios, G.N., 2005. Plant Pathology. 5th Edition, Elsevier Academic Press, New York, 952 p.

Utilization of Herbs and Herbal Extracts in Beef Meatballs

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ABSTRACT: Beef meatballs, among the most widely consumed meat products globally, stand out as a delectable snack celebrated for their rich nutritional profile and distinctive taste and aroma properties. However, the production processes and nutritional content of beef patties create an environment conducive to microbial growth, significantly compromising their shelf life. This susceptibility to spoilage prompts the exploration of additives with antioxidant and antimicrobial properties, aimed at extending shelf life, enhancing quality, and ensuring food safety. To mitigate potential health risks associated with synthetic antioxidants, this study advocates for the preference of natural alternatives. Synthetic antioxidants have been linked to health concerns such as cancer, neurodegenerative diseases, and cardiovascular issues. Given that plants are abundant sources of antioxidants, they have gained prominence in meat and meat product preservation. Laden with phenolic acids, terpenes, and flavonoids, plants offer natural compounds that effectively contribute to the longevity and quality of beef meatballs. This study compiles an extensive review of the plants and plant extracts utilized in beef meatball production, examining their effects on the overall quality and characteristics of the meatballs within the existing literature.

Keywords: Beef patties, Plant, Plant extracts, Antioxidant

INTRODUCTION

Meat products are integral components of human nutrition. They are consumed all over the world because they are the primary source of high biological value protein, fatty acids, vitamins, and mineral substances (Bagheri et al., 2021; Rahman et al., 2021; García-Valencia et al., 2022; Asghar et al., 2023).

However, their susceptibility to rapid spoilage arises from factors such as high moisture concentration, heme pigments, and certain oxidizing agents (Ouerfelli et al., 2019). Microbial proliferation and oxidation reactions cause some quality losses (Abdallah et al., 2023) and undesirable changes, such as loss of color and nutrients and the formation of toxic compounds. Diverse additives, possessing antioxidant and antimicrobial properties, can prevent these adverse occurrences through various ways (Ouerfelli et al., 2019). Opting for natural additives is preferable over synthetic chemical substances due to the potential health risks associated with the latter (Al-Juhaimi et al., 2020; Ahmed et al., 2022). In this context, essential oils obtained from plants, spices (thyme, cinnamon, sage, clove, lemon-grass, rosemary, etc.), some plant extracts, and enzymes obtained from animal sources (lysozyme, lactoferrin, etc.) can be used in order to extend the shelf life of meat and meat products and prevent quality losses. In recent years, research on food additives containing natural antioxidants and antimicrobials, including organic acids (such as sorbic, citric, and ascorbic acid, etc.) has gained significant popularity (Aziz and Karboune, 2018; Cabi Çarşı et al., 2019; Abdallah et al., 2023).

Natural herbal additives have been used for many purposes in various areas, such as pharmaceuticals, medicine, and hygiene, since ancient times. They are now widely used in the food industry (Abdallah et al., 2023). Herbal additives are regarded as natural preservatives owing to their robust antioxidant, antifungal, and antimicrobial properties (Hygreeva et al., 2014). This implies that they also positively contribute to human health (Aziz and Karboune, 2018; Demir, 2021).

REVIEW OF STUDIES EMPLOYING HERBS AND HERBAL EXTRACTS IN MEATBALL

Meatball is the meat product that is generally consumed by turning beef or lamb into minced meat, adding fat (tallow, tail fat, etc.) and various spices to the desired shape and size, and then cooking it (Aydemir and Arslan, 2023). Meatballs and similar foods are among the world's most popular processed meat products (Mizi et al., 2019). It is a popular choice in fast food restaurants and households alike due to its ease of preparation and cooking (Folake, 2019; Bagheri et al., 2021; Aydemir and Arslan, 2023). These types of foods are economical and inexpensive compared to other meat products, leading to an increase in consumption (Mizi et al., 2019). In addition, changes in consumers' ready-made food preferences increase meatball production and consumption (Hajrawati et al., 2021).

Some factors affect meatball production, such as meat type, nutritional value, cost, and religious reasons (Turgut et al., 2017). Beef is the predominant choice for meatballs, constituting nearly 60% of the meat used in production (Abustam et al., 2019). In this context, the current review study focuses on beef patties.

Although meatballs are an easy-to-prepare meat product, their shelf life is relatively short (Folake, 2019; Asghar et al., 2023). The muscle membrane's primary structure and integrity degenerate, and the total surface area increases after mincing. Correspondingly, deterioration accelerates and shelflife decreases (Mostafa and Azab, 2022). In addition, the beef's high myoglobin and iron content bring into peroxide formation. As a result of lipid oxidation accelerated by the increase in surface area, the product's nutritional value decreases and the pigments deteriorate (Hajrawati et al., 2021). In addition to lipid oxidation and protein oxidation, other oxidation events can also cause a decrease in digestibility and produce genotoxic and cytotoxic derivatives of amino acids (Wahab et al., 2019). Moreover, through protein oxidation, the Fe^{2+} in the myoglobin heme group is oxidized to Fe^{3+} and for that reason, the oxymyoglobin in the muscle turns into metmyoglobin. This causes the turning of color from red to brown (Wang et al., 2022). This situation can negatively affect the products' water retention capacity, texture, and cell structure (Turgut et al., 2017).

Another main reason for deterioration is microbial spoilage. Meat and meat products present a suitable habitat for the proliferation of common foodborne pathogens, which pose a risk to human health by making foods hazardous and easily perishable (Demir, 2021). At the same time, it causes the formation of toxic compounds in psychrotrophic and mesophilic microorganisms. These microorganisms are initially present in high numbers during meat processing, leading to issues such as tissue deterioration, loss of color and nutrients, and a shortening of the shelf life (Mizi et al., 2019; Ouerfelli et al., 2019).

Meat and meat products are generally consumed in cooked form. During cooking, hydroperoxides contain some volatile organic compounds such as aldehydes, alkanes, alcohols, esters, and acids (Domínguez et al., 2019). These compounds (heterocyclic aromatic amines, acrylamide, hydroxymethylfurfural, etc.) cause losses in color, texture, flavor, and nutritional value of the product (Karwowska et al., 2021; Sarv et al., 2023). For the reasons mentioned here, meatballs can be formulated with different spices and ingredients to improve aroma, color, taste, and appearance and get more attention from consumers (Parvin et al., 2023). In addition, consumers increasingly prefer healthier, enriched, and functional meat products (Kotecka-Majchrzak et al., 2021).

Substances with antioxidant/antimicrobial properties can be used in order to protect meat and meat products from spoilage, preserve meat quality, and reduce exposure to harmful compounds for humans (Ouerfelli et al., 2019; Yu et al., 2024). Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), ascorbic acid, tocopherol, and gallic acid are among the most commonly used antioxidants in foods (Hajrawati et al., 2021; García-Valencia et al., 2022). These substances inhibit the formation or spreading of free radicals by various mechanisms including scavenging species that initiate peroxidation, chelation of metal ions, quenching of O_2 that prevents peroxide formation, breaking the autooxidative chain reaction, stimulating antioxidative enzyme activities, and reducing localized oxygen concentrations. They play a significant role in delaying and preventing oxidative processes (Dorman et al., 2003). The compounds with the most effective antioxidant properties can block the chain reaction of free radicals. They generally contain more aromatic rings with one or more -OH groups and can donate H to free radicals produced during oxidation (Aziz and Karboune, 2018). However, consumers have not entirely accepted these synthetic antioxidants due to their potential toxicity and carcinogenic effects (Parvin et al., 2020; Al-Juhaimi et al., 2020; Mostafa and Azab, 2022). Therefore, the food industry is looking for natural alternatives with strong antimicrobial/antioxidant properties in order to meet the demands of consumers and restore confidence in food safety (Dua et al., 2012; Islam et al., 2012; Aziz and Karboune, 2018; Mokhtar and Eldeep, 2020). Among natural alternatives, plants and extracts obtained from plants stand out (Wang et al., 2020; Rasak et al., 2021). Natural alternative herbs are "dietary components humans use to supplement the diet by increasing total dietary intake" (Manessis et al., 2020). Herbal additives are composed of phenolic compounds, phenolic acids (rosmarinic acids, caffeic acids and gallic acid), phenolic diterpenes (carnosol and carnosic acid), flavonoids (quercetin, catechin, apigenin, naringenin, kaempferol, hesperetin), essential oils (carvacrol, thymol, eugenol, menthol) and many other bioactive chemicals (Hygreeva et al., 2014; Wang et al., 2020; Hajrawati et al., 2021; Beya et al., 2021). Thanks to these compounds, they prevent oxidation reactions, microbial development and various toxic compounds in meat products caused by cooking (Cabi Çarşi et al., 2019; Al-Juhaimi et al., 2020; Demir, 2021; Ahmed et al., 2022; Ahmed et al., 2022; Aydemir and Arslan, 2023; Abdallah et al., 2023; Elhassaneen et al., 2023; Aoudeh et al., 2023; Ekiz et al., 2023; Elbir et al., 2023; Aydemir et al., 2024). It is also reported that phenolic compounds obtained from plants positively affect human health by inhibiting various enzymes and that the extracts have antiallergic, antiglycemic, anticholesterol, anti-inflammatory, antithrombotic, and sedative properties (Karabulut and Yemiş, 2019). Herbal additives possess several advantageous propertie

1. They scavenge free radicals, preventing chain inhibition.
2. They break chain reactions and decompose peroxide compounds.
3. They achieve this by minimizing localized oxygen concentrations and binding to some chain-initiating catalysts (Unar et al., 2022).

Within the scope of the current review study, the plants and plant extracts used in beef meatballs, which are widely produced and consumed worldwide, have been compiled. The physicochemical, microbiological, and sensory properties they provide to beef meatballs are studied. Table 1 includes the plants and plant extracts used, their extraction methods, amounts of use in the product, and application results.

Table 1. Plants and plant extracts used in meatballs, their extraction methods, amounts of use in the product, and the application results

Plant material	Extraction solvent	Amount added	Storage processes	Results	References
Black cumin (*Nigella sativa* L.) seeds	Powdery	2.0%	16 days at 4°C	Black cumin seed exhibited antimicrobial properties, notably slowing the microbial growth rate starting from the eighth day of storage. Its impact was particularly pronounced on lactic acid bacteria, yeast, and mold.	Aydemir and Arslan, 2023
Clove (*Syzygium aromaticum*) bud	Methanol	0, 2.0, 4.0, 6.0%	21 days at 4°C	The extract added at 4.0% and 6.0% increased beef patties' phenolic content and antioxidant activity, resulting in reduced lipid oxidation and microbial spoilage during the storage period. It has significantly improved the cooking properties of beef burgers. In addition, the sensory acceptability of burgers containing 2.0% and 4.0% extract was higher than the other groups.	Ahmed et al., 2022
Bay leaves (*Laurus nobilis L.*) or sage (*Salvia officinalis*) +whey protein isolate	Ethanol:Water (80:20, v/v)	2.0, 4.0%	7 days at 2°C	The TBA values of the meatballs supplemented with sage were found to be lower than the TBA values of the meatballs supplemented with bay leaf. Additionally, total phenolic content increased significantly in edible film groups with extract added compared to those without extract.	Akcan and Serdaroğlu, 2022
Acacia nilotica	Water	0, 0.5, 1.0, 2.0%	14 days at 4°C	It was observed that burgers formulated with 1.0% and 2.0% extract had a lower rate of TBA increase and microbial spoilage during the storage period.	Al-Juhaimi et al., 2020
Poppy seed (*Papaver somniferum*)	70% Ethanol	2.0%	14 days at 4°C	It has been reported that combining poppy seed extract and gamma irradiation increased the shelf stability of beef patties under different storage and packaging conditions.	Asghar et al., 2023
Black garlic	Different combinations of aging temperature and duration (30, 45, 60 days at 60°C; 30, 45, 60 days at 70°C; 30, 45, 60 days at 80°C) in a climatic chamber	0.5, 1.0%	-	Black garlic reduced the PAH contents of the samples by 38.17-94.12% compared to raw garlic. Additionally, beef patties enriched with black garlic reduced human exposure to PAHs from beef patties.	Aoudeh et al., 2023

Black cumin (*Nigella sativa* L.) seeds	Methanol	2.0%	16 days at 4ºC	While it was determined that CML formation increased as the storage period progressed, the highest increase was observed in the control group. It has been proven that adding black cumin seeds to meatballs effectively prevents CML formation.	Aydemir et al., 2024
Hyssop (*Echinophora sibthorpiana*) powder	Dried plant	0.1, 0.25, 0.50, 1.0, 2.0%	5 days at 4ºC	Adding 2.0% hyssop powder significantly reduced lipid oxidation in beef patties compared to the control. Raw meatballs' hardness, gumminess, and chewiness values increased with Hyssop powder concentration. TMAB of samples containing hyssop powder was lower than the control on the first and third days.	Cabi Çarşi et al., 2019
Green tea	Water	0.5, 1.0%	14 days at 4°C	A statistically significant decrease was detected in the free fatty acidity, peroxide value, and TBARS levels of meatballs on different days of storage.	Demir, 2021
Cocoa pod shells	Pyrolysis and distillation	0.5, 1.0, 2.0, 3.0%	60 h at room temperature	Within the scope of the research, it was concluded that liquid smoke produced from cocoa pod shells can be used as a natural preservative for beef meatballs. However, liquid smoke concentrations affect the maximum storage time of meatballs.	Desvita et al., 2023
Cumin (Cuminum cyminum)	Powdery	0.5, 1.0%	-	It was determined that using cumin in meatballs increased the pH value and decreased the TBARS value. On the other hand, the cooking temperature (150 and 250ºC) and adding cumin to the meatballs caused an increase in the total HAA content. In this case, it was revealed that the addition of cumin to meatballs caused an increase in the total HAA content, probably due to its pro-oxidant effect. However, it was determined that using cumin in specific amounts could reduce the formation of the MeIQx compound, which has an antioxidant effect.	Ekiz et al., 2023
Chia seeds	Powdery	0, 0.5, 1.0, 1.5%	-	The study stated that the use of chia seeds increased the total HAA content, and the highest level was found in meatballs prepared with 1.0% chia seeds.	Elbir et al., 2023
Onion skin (*Allium cepa* L.), Potato peel (*Solanum tuberosum*, L.), Marjoram (*Origanum*	Water	0.25%	12 days at 4ºC	Plant extracts reduced the TBA and TVBN amounts in the samples. The sensory analysis results, especially the acceptability scores, showed significant advantages of using all extracts in meat products sensitive to rancidity.	Elhassaneen et al., 2023

marjorana L.), Fennel (*Foeniculum vulgare*), Cinnamon (*Cinnamomun verum*), Black seed (*Nigella sativa*), Olive leaf (*Olea europaea*), Basil (*Ocimum basilicum*)				In addition, using mixtures of these extracts gave more effective results than using the extracts alone.	
Artichoke (*Cynara scolymus* L.)	Ethanol (80% v/v)	27.3 mg phenolic compound/100 g meat (optimized using response surface methodology)	7 days at 2ºC	Artichokes showed high antioxidant activity against lipid and protein oxidation during storage. Artichoke inhibited lipid and protein oxidation in raw beef patties much more than BHT.	Ergezer and Serdaroğlu, 2018
Pinto bean	In flour	6.0, 12.0, 18.0%	-	As concentration increased, cooking loss decreased. The natural color of the pinto bean flour caused only minor changes in the color of the cooked patty, resulting in a color close to that of a regular cooked beef patty. Pinto bean flour worked just as well as raw flour in general softening, protein retention, and moisture retention.	Felker et al., 2023
Cemba (*Albizia lebbeckoides* [DC.] Benth)	In flour	0.5, 1.0, 1.5%	-	Adding 1.0% and 1.5% cemba increased meatballs' b* value and antioxidant activity. The addition of 1.5% cemba also increased crude fiber significantly.	Hajrawati et al., 2021
Moringa oleifera	Moringa leaf extracts	0.1, 0.2, 0.3%	60 days at -20ºC	Considering the softness, juiciness, general acceptability, cooking loss, FFA, POV, and TBARS value, it was concluded that Moringa oleifera leaf extract up to 0.3% could replace BHA in meatball preservation without deteriorating its quality. In the sensory evaluation, 0.2% Moringa leaf extract gave better results.	Islam et al., 2018
Garlic (*Allium sativum*), Onion (*Allium cepa* L.), Red chilli (*Capsicum frutescens*), Paprika (*Capsicum annuum*), ginger (*Zingiberofficinale*	Powdery	0.5%	-	Control meatballs had the highest HAA content compared to all other meatballs. PAHs could only be inhibited by ginger.	Lu et al., 2018

Rosc.), and Black pepper (*Piper nigrum* L.)					
Salvia (sage) and high-pressure processing (HPP) [300 MPa (10 min, 9.9°C) and 600 MPa (10 min, 10.2°C)]	Powdery	0.3, 0.6%	60 days at 2°C	No detrimental effects of sage on the sensory properties of burgers were observed. Adding sage powder to meatballs had no significant effect on antimicrobial activity. It was concluded that sage powder was effective as an antioxidant that delayed lipid oxidation in HPP-treated beef burgers at 60 days of cold storage.	Mizi et al., 2019
Neem (*Azadirachta indica* L.)	50% Ethanol (v/v)	0.7%	11 days at 4°C	It has been determined that *A. indica* limits color loss, reduces metmyoglobin formation, and significantly affects bacterial growth and hexanal content.	Ouerfelli et al., 2019
Galangal	Powdery	0.1, 0.25, 0.5%	7 days at 4°C	The lowest TBA value was observed in samples containing 0.5% galangal powder. When the conjugated diene results were examined, it was determined that the highest value was obtained in the group to which 0.5% galangal powder was added. There was no significant difference between the samples to which other galangal powder was added. As a result of the research, it was reported that galangal can be used as a natural antioxidant.	Palamutoğlu and Kasnak, 2019
Moringa oleifera	Powdery	0.5, 1.0, 1.5%	-	Adding M*oringa oleifera* reduced the cooking loss and increased the antioxidant activity of meatballs. While there was no significant change in the nutritional content, moisture content, ash, crude protein, and crude fat of the meatballs, it was determined that the crude fiber content increased significantly.	Rasak et al., 2021
Sumac	Powdery	0.5%	-	The use of sumac caused a decrease in pH, cooking loss, lipid oxidation level, and total HAA amount in the samples.	Savaş et al., 2023
Matcha+ whey protein isolate (5.0%)	Ethanol	3.0, 6.0, 9.0%	14 days at 4°C or 60 days at 18°C	The edible coating process did not negatively affect color, texture, physicochemical composition, or consumer preferences. It has also been shown that Matcha extract in whey protein isolate formulation inhibits lipid oxidation and microbial growth and can potentially extend the shelf life of patties by preserving their physicochemical properties.	Bilecen Şen and Kılıç, 2021

Lemon balm (*Melissa officinalis* L.) and Wild thyme (*Thymus serpyllum* L.)	Water	6.0%	-	Lemon balm-enriched patties processed by hot air convection were characterized by higher polyphenol content, while those obtained by steam convection showed high flavonoid concentration. However, it has been shown that herbal aqueous extracts open possibilities for the development of functional foods with specific purposes, both in terms of lactogenic activity determined by FT-IR analysis and other beneficial properties that contribute to the human body's well-being.	Tănase et al., 2022
Rosemary, Aloe vera,	Aloe vera-pulp of the leave Rosemary-water	10 ml/100 g	21 days at 4^{o}C	All groups remained suitable for consumption until 21 days of storage, except for the control group of beef meatballs. Therefore, the results showed that plant extracts could be used to extend products' shelf life and beef patties' quality characteristics, possibly by delaying microbial spoilage.	Unar et al., 2022
Clove, Sage	Water	0.1, 0.25, 0.5%	30 days at 4^{o}C	It has been determined that the 0.5% plant extract mixture is highly effective against lipid and protein oxidation, has a strong antimicrobial property in beef burger patties, and is a promising natural antioxidant and antimicrobial that replaces synthetic preservatives in meat processing.	Wahab et al., 2019
Camellia sinensis L.	-	1.0%	90 days at -18^{o}C	It has been determined that adding 1.0% *Camellia sinensis* L. extract to meatballs is an effective strategy for delaying oxidative changes. After 90 days of frozen storage, antioxidant activity in the group supplemented with 1.0% *Camellia sinensis* L. was determined to be two times higher than in the control group.	Wojtasik-Kalinowska et al., 2021
Rosemary (*Rosmarinus officinalis*), Turmeric (*Curcuma longa*), and Bay leaf (*Laurus nobilis*)	Powdery	0.5%	-	Using plant extracts in meatball production significantly reduced cooking and water loss and positively affected textural quality and amino acid retention.	Yu et al., 2024
Paprika, Cloves, Piper cubeba	Powdery	Paprika powder and extract- 2.0, 4.0, 8.0%;	15 days at 3^{o}C	Sensory evaluation of cooked meatballs containing red pepper, clove, and piper cubeba powders and their extracts did not reveal any adverse effects on the consumer. The current study showed that the shelf life	Zaher et al., 2023

		Clove powder and extracts 0.5, 1, 1.5%; Piper cubeba powder and extract- 1.0, 2.0, 3.0%		increased and Enterobacteriaceae and S. aureus contamination levels decreased in meatballs treated with red pepper, cloves, and piper cubeba.	
Clove extract	Water	0.1%	10 days at 4ºC	Adding clove extract to meatballs significantly limited lipid oxidation, but the antioxidant effects of clove extract on lipid oxidation were more pronounced than its effects on protein oxidation.	Zahid et al., 2019

CONCLUSION

With the increasing demand for quickly produced and nutritious foods, meatballs have become popular in the meat industry. With this demand, efforts have accelerated to improve beef meatballs' quality, safety, and shelf life. In this context, compounds with antioxidant/antimicrobial properties obtained naturally from plants have gained importance. Herbal additives instead of synthetic preservers to increase safety and quality can provide favorable properties to the products regarding physicochemical, microbiological, and shelf life. However, to obtain the desired effects from plants with this feature, it is sometimes necessary to use them in high amounts. This may cause undesirable organoleptic changes in the products. In addition, there are products where the molecular mechanism that causes antioxidant/antimicrobial activity is not fully explained, which may cause nutritional and toxicological problems. As a result, it is possible to say that natural antioxidants should be characterized precisely, their antioxidant capacities should be measured, and their safe limits should be determined. It is also envisaged that plant-derived antioxidant/antimicrobial compounds should be added to meat and meat products by using new technologies, which should be made sustainable.

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Statement of Conflict of Interest

The authors declare that they do not have any conflict of interest.

Authors' Contributions

PA and KÇT conceptualized, planned, and implemented the research. PA, KÇT, and ÖÇ conceptualized, reviewed, and edited the research.

REFERENCES

Abdallah, R., Mostafa, N.Y., Kirrella, G.A.K., Gaballah, I., Imre, K., Morar, A., Herman, V., Sallam, K.I., Elshebrawy, H.A., 2023. Antimicrobial effect of moringa oleifera leaves extract on foodborne pathogens in ground beef. Foods, 12: 766.

Abustam, E., Said, M.I., Yusuf, M., 2019. Organoleptic characteristics of bali beef meatballs based on collagen concentration in UKKMB and time of maturation. IOP Conf. Series: Earth and Environmental Sci, 255: 012005.

Ahmed, I.A.M., Babiker, E.E., Al-Juhaimi, F.Y., Bekhit, A.E.A., 2022. Clove polyphenolic compounds improve the microbiological status, lipid stability, and sensory attributes of beef burgers during cold storage. Antioxid, 11: 1354.

Akcan, T., Serdaroğlu, F.M., 2022. Edible protein films containing bay leaves (Laurus Nobilis L.) and sage (Salvia Officinalis) extracts: Preventing oxidation and application on refrigerated cooked meatballs. DEUFMD, 24 (72): 927-938.

Al-Juhaimi, F.Y., Almusallam, I.A., Ahmed, I.A.M., Ghafoor, K., Babiker, E.E., 2020. Potential of *Acacia nilotica* fruit flesh extract as an anti-oxidative and anti-microbial agent in beef burger. J. Food Process. Preserv, 44: e14504.

Aoudeh, E., Oz, E., Oz, F., 2023. Effect of beef patties fortification with black garlic on the polycyclic aromatic hydrocarbons (PAHs) content and toxic potency. Food Chem, 428: 136763.

Asghar, Z., Arshad, M.S., Khalid, W., Saeed, F., Imran, A., Suleria, H.A.R., 2023. Impact of gamma irradiation and poppy seed extract on quality and storage stability of beef patties. Int. J. Food Prop, 26 (1): 1645-1662.

Aydemir, M.E., Arslan, A., 2023. Investigation of the effects of jerusalem thorn (*Paliurus spina-christi* mill.), oriental hackberry (*Celtis tournefortii* L.) fruits and black cumin (*Nigella sativa* L.) seed on microbial quality and physicochemical properties of meatballs. Harran Üniv. Vet. Fak. Der, 12 (1): 027-034.

Aydemir, M.E., Arslan, A., Takım, K., Altun, S.K., Yılmaz, M.A., Çakır, O., 2024. Inhibitory effect of *Paliurus spina-christi* Mill., *Celtis tournefortii* L. and *Nigella sativa* L. on N^{ε}–(Carboxymethyl) lysine in meatballs. Meat Sci, 207: 109362.

Aziz, M., Karboune, S., 2018. Natural antimicrobial/antioxidant agents in meat and poultry products as well as fruits and vegetables: A review. Crit. Rev. Food Sci. Nutr, 58 (3): 486-511.

Bagheri, R., Ariaii, P., Motamedzadegan, A., 2021. Effects of chitosan incorporated with basil seed gum and nettle (*Urtica dioica* L.) essential oil on the quality of beef burger during refrigerated storage. J. Food Meas. Charact, 15: 256-264.

Beya, M.M., Netzel, M.E., Sultanbawa, Y., Smyth, H., Hoffman, L.C., 2021. Plant-based phenolic molecules as natural preservatives in comminuted meats: A review. Antioxidants, 10: 263.

Bilecen Şen, D., Kılıç, B., 2021. Effects of edible coatings containing acai powder and matcha extracts on shelf life and quality parameters of cooked meatballs. Meat Sci, 179: 108547.

Cabi Çarşi, Ş., Sariçoban, C., Cabi, A., 2019. Effects of addition Hyssop (*Echinophora sibthorpiana*) powder on some quality properties of beef patties. J. Agroaliment. Processes Technol, 25 (2): 89-100.

Demir, T., 2021. Effects of green tea powder, pomegranate peel powder, epicatechin and punicalagin additives on antimicrobial, antioxidant potential and quality properties of raw meatballs. Molecules, 26: 4052.

Desvita, H., Faisal, M., Mahidin, M., Suhendrayatna, 2023. Natural antimicrobial properties of liquid smoke derived from cocoa pod shells in meatball preservation. S. Afr. J. Chem. Eng, 46: 106-111.

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F.J., Zhang, W., Lorenzo, J.M., 2019. A comprehensive review on lipid oxidation in meat and meat products. Antioxid, 8: 429.

Dorman, H.J.D., Peltoketo, A., Hiltunen, R., Tikkanen, M.J., 2003. Characterisation of the antioxidant properties of deodorised aqueous extracts from selected Lamiaceae herbs. Food Chem, 83: 255-262.

Dua, A., Gupta, S.K., Mittal, A., Mahajan, R., 2012. A study of antioxidant properties and antioxidant compounds of cumin (*Cuminum cyminum*). Int. J. Pharm. Biol. Sci. Arch, 3 (5): 1110-1116.

Elbir, Z., Ekiz, E., Aoudeh, E., Oz, E., Savaş, A., Brennan, C., Proestos, C., Khan, M.R., Elobeid, T., Brennan, M., Oz, F., 2023. Enhancing effect of chia seeds on heterocyclic amine generation in meatball. Int. J. Food Sci. Technol, 58: 2560-2572.

Elhassaneen, Y., ElBassouny, G.M., Hassan, R.H., Meharam, E.B., 2023. Application of natural extracts in beef meatballs to prevent chemical and bacteriological spoilage agents, and extend its storage life. Am. J. Food Sci, 11 (4): 118-130.

Ergezer, H., Serdaroğlu, M., 2018. Antioxidant potential of artichoke (*Cynara scolymus* L.) byproducts extracts in raw beef patties during refrigerated storage. J. Food Meas. Charact, 12: 982-991.

Felker, F.C., Kenar, J.A., Singh, M., Winkler-Moser, J.K., Byars, J.A., 2023. Comparison of raw and excess steam jet-cooked/drum-dried pinto bean flours and their effects on ground beef patties. J. Food Process. Preserv, 5915625.

Folake, A.O., 2019. Meat quality and consumers acceptability of meat balls treated with different plant extracts. Int. J. Adv. Sci. Res, 4 (6): 23-26.

García-Valencia, S., Soto-Simental, S., Ocampo-López, J., Ayala-Martínez, M., 2022. Diet supplementation with dalbergia palo-escrito hexane extract in fattening rabbits: its effect on productive performance, carcass traits, meat characteristics and meatballs shelf-life. Ital. J. Anim. Sci, 21 (1): 1533-1542.

Hajrawati, H., Malaka, R., Prahesti, K.I., Arifin, T.S.M., Rani, Y.A.A., 2021. Evaluation of physico-chemical properties and antioxidant activity of bali beef meatballs added Cemba (Albizia lebbeckoides [DC.] Benth). IOP Conf. Series: Earth and Environmental Science, 788: 012110.

Hygreeva, D., Pandey, M.C., Radhakrishna, K., 2014. Potential applications of plant based derivatives as fat replacers, antioxidants and antimicrobials in fresh and processed meat products. Meat Sci, 98: 47-57.

Islam, F., Hossain, M.A., Rahman, M.F., Hashem, M.A., Rahman, M., Azad, M.A.K., 2018. Effect of synthetic or herbal preservatives on the quality of beef meatballs at different shelf life periods. SAARC J. Agric, 16 (1): 23-34, Karabulut, G., Yemiş, O., 2019. Fenolik bileşiklerin bağlı formları ve biyoyararlılığı. Akademik Gıda, 17 (4): 526-537.

Karwowska, M., Stadnik, J., Stasiak, D.M., Wojciak, K., Lorenzo, J.M., 2021. Strategies to improve the nutritional value of meat products: Incorporation of bioactive compounds, reduction or elimination of harmful components and alternative technologies. Int. J. Food Sci. Technol, 56: 6142-6156.

Kotecka-Majchrzak, K., Kasałka-Czarna, N., Spychaj, A., Mikołajczak, B., Montowska, M., 2021. The effect of hemp cake (*Cannabis sativa* L.) on the characteristics of meatballs stored in refrigerated conditions. Molecules, 26: 5284.

Lu, F., Kuhnle, G.K., Cheng, Q., 2018. The effect of common spices and meat type on the formation of heterocyclic amines and polycyclic aromatic hydrocarbons in deep-fried meatballs. Food Control, 92: 399-411.

Manessis, G., Kalogianni, A.I., Lazou, T., Moschovas, M., Bossis, I., Gelasakis, A.I., 2020. Plant-derived natural antioxidants in meat and meat products. Antioxid, 9: 1215.

Mizi, L., Cofrades, S., Bouc, R., Pintado, T., López-Caballero, M.E., Zaidi, F., Jiménez-Colmenero, F., 2019. Antimicrobial and antioxidant effects of combined high pressure processing and sage in beef burgers during prolonged chilled storage. Innov. Food Sci. Emerg. Technol, 51: 32-40.

Mokhtar, S.M., Eldeep, G.S.S., 2020. Impact of mango peel extract on the physicochemical properties, microbiological stability and sensory characteristics of beef burgers during cold storage. EJFS, 48 (2): 245-258.

Mostafa, H.B., Azab, E.F.E., 2022. Efficacy of green coffee as an antioxidant in beef meatballs compared with ascorbic acid. Food Chem: X, 14: 100336.

Ouerfelli, M. Villasante, J., Kaâb, L.B.B., Almajano, M.P., 2019. Effect of neem (Azadirachta indica L.) on lipid oxidation in raw chilled beef patties. Antioxidants, 8: 305,

Palamutoğlu, R., Kasnak, C., 2019. Antioxidant activity of galangal powder and the effect of addition on some quality characteristics of meatball. MJEN, 7 (2): 136-140.

Parvin, R., Seo, J-K., Eom, J-U., Ahamed, Z., Yang, H-S., 2023. Inhibitory and antioxidative capacity of nutmeg extracts on reduction of lipid oxidation and heterocyclic amines in pan-roasted beef patties. Meat Sci, 197: 109064.

Parvin, R., Zahid, A., Seo, J-K., Park, J., Ko, J., Yang, H-S., 2020. Influence of reheating methods and frozen storage on physicochemical characteristics and warmed-over flavor of nutmeg extract-enriched precooked beef meatballs. Antioxid, 9: 670.

Rahman, M.H., Alam, M.S., Monir, M.M., Ahmed, K., 2021. Comprehensive effects of black cumin (*Nigella sativa*) and synthetic antioxidant on sensory and physicochemical quality of beef patties during refrigerant storage. J. Agricultural Sci. Food Res., 4: 100145.

Rasak, A.N.M., Hajrawati, H., Ningrum, E.M., Suharyanto, 2021. Physical characteristic and antioxidant activity of beef meatballs with addition of *Moringa oleifera* (Lam.) leaves powder. IOP Conf. Series: Earth and Environmental Science, 788: 012111.

Sarv, V., Kerner, K., Venskutonis, P.R., Rocchetti, G., Becchi, P.P. Lucini, L., Tanavots, A., Bhat, R., 2023. Untargeted metabolomics and conventional quality characterization of rowanberry pomace ingredients in meatballs. Food Chem: X, 19: 100761.

Savaş, A., Ekiz, E., Elbir, Z., Demir Savaş, B., Proestos, C., Elobeid, T., Khan, M.R., Oz, F., 2023. Advantageous effects of sumac usage in meatball preparation on various quality criteria and formation of heterocyclic aromatic amines. Separations, 10: 29.

Tănase, L-A., Nistor, O-V., Andronoiu, D-G., Mocanu, G-D., Dediu, A. V. B., Botez, E., 2022. Different types of meatballs enriched with wild thyme/lemon balm aqueous extract-complex characterization. Molecules, 27: 3920.

Turgut, S.S., Işıkçı, F., Soyer, A., 2017. Antioxidant activity of pomegranate peel extract on lipid and protein oxidation in beef meatballs during frozen storage. Meat Sci, 129: 111-119.

Unar, N.D., Soomro, A.H., Marri, A., Khaskheli, G.S., Miano, T.F., Shaikh, N., Shah, A.H., Kumar, D., 2022. Efficacy of some plant extracts in extending keeping quality of beef patties during storage. Pure Appl. Biol, 11 (1): 340-348.

Wahab, M.G.A.E., Sohaimy, S.A.E., Ibrahim, H.A., El-Makarem, H.S.A., 2019. Effect of natural antioxidant extracts on oxidative and microbiological stability of beef burger. Alex. J. Vet. Sci, 60 (1): 141-154.

Wang, C., An, X., Gao, Z., Li, Z., Tıan, S., Lu, Y., 2022. Effects of ethanolic extract from onion skin on the quality characteristics of beef patties during refrigerated storage. J. Food Sci. Technol, 42: e118121.

Wojtasik-Kalinowska, I., Onopiuk, A., Szpicer, A., Wierzbicka, A., Półtorak, A., 2021. Frozen storage quality and flavor evaluation of ready to eat steamed meat products treated with antioxidants. CYTA–J. Food, 19 (1): 152-162.

Yu, Z., Lu, Y., Wei, F., Zhang, Y., Dong, L., Wang, S., 2024. The impact of natural spices additions on hazards development and quality control in roast beef patties. Food Chem, 435: 137644.

Zaher, H., El-Sherbiny, H., El-Khateeb, A.Y., Elkenany, R., Zakaria, A., 2023. Preservative effect of selected natural flavoring spices and their extracts on microbial quality of meatballs during cold storage. EJVS, 54 (5): 997-1014.

Zahid, A., Seo, J-K., Parvin, R., Ko, J., Yang, H-S., 2019. Comparison of butylated hydroxytoluene, ascorbic acid, and clove extract as antioxidants in fresh beef patties at refrigerated storage. Food Sci. Anim. Resour, 39 (5): 768-779.

Fish Oil

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ABSTRACT: Nutrition is necessary for living things to survive. Nutrition is a psychological, sociological and economic event as well as a physiological one. Healthy nutrition is the consumption of nutrients in sufficient and balanced amounts for growth and development, maintenance of life, protection, improvement and development of health, and increasing the quality of life. It is known that when any of the nutrients are not taken or are taken too little or too much, development and growth are prevented and health deteriorates. The importance of nutrition for the healthy development and functioning of the human body and protection from diseases has become an increasingly emphasized issue in recent years. Fats are one of the most important elements required for human nutrition. Since the human body cannot synthesize essential fatty acids, they must be taken with food. Fish oils differ from animal and plant oils in high amounts of polyunsaturated fatty acids (PUFA). This review will focus on the composition, use and health importance of fish oil.

Keywords: Consumption, Fish oil, Health, Nutrition

INTRODUCTION

Nutrition is one of the basic needs of living things and is of great importance for the body (Ünsal, 2019). Nutrition is one of the most important issues emphasized today. Today, while millions of people in the world are constantly fighting against death and diseases caused by hunger and malnutrition, others lose their lives at an early age due to disorders caused by excessive and malnutrition. Thus, inadequate and unbalanced nutrition problems are among the main factors that deteriorate people's health (Baysal, 2004). Aquatic products are important in human nutrition.

Unfortunately, while the annual per capita consumption of aquatic products in Turkey in 2019 was 6.3 kg, the world average is 22 kg. Although aquatic product consumption in Turkey varies by region, the annual per capita consumption of aquatic products in 2021 is determined as 6.5 kg. Per capita seafood consumption in the world reached 20.5 kg in 2019. While fish consumption per capita in low-income, food-deficient countries was determined as 5.4 kg in 2019, it was determined as 15.2 kg in middle-income countries and 26.5 kg in high-income countries. It is seen that the consumption habits of aquatic products in Turkey are behind even underdeveloped countries (Çöteli, 2021).

Nowadays, people pay much attention to their nutrition and are careful to choose healthy foods in their diet. Among these foods, fish and other aquatic products rich in polyunsaturated fatty acids take the first place (Kaya et al., 2004).

Fish oil is an almost unique natural source of long-chain omega-3 fatty acids, as it contains eicosapentaenoic acid (EPA) and Decosahexaenoic acid (DHA). Farm animals can accumulate trace amounts of these fatty acids in milk and eggs (Uçar et al., 2019). Fish, especially oily fish, are rich in EPA and DHA. Although white-fleshed fish contain some omega-3 fatty acids, they contain less EPA and DHA than fatty fish. The fatty acid contents of fish lipids depend on various factors such as nutrition, geographical region, environmental temperature, season, body length, and lipid content. While freshwater fish contain more n-6 polyunsaturated fatty acids than marine fish, they contain less n-3 fatty acids. The n-6 content of fish obtained through aquaculture is higher than that obtained by natural hunting (Kocatepe and Turan, 2018).

Omega-3 fatty acids have effects against many diseases, from heart diseases to cancer, from AIDS to brain-related disorders, and play a vital role in the central nervous systems (Caygil et al., 1996; Mol, 2008; Weitz et al., 2010).

Fish oils are the richest source of Omega-3 fatty acids. Therefore, it will provide the nutritional requirement 3 times a week (200-300 g); It is recommended to consume fish rich in fatty acids such as herring, mackerel, sardine and salmon (Arıman Karabulut and Yandı, 2006).

COMPOSITION OF FISH OILS

A quality fish oil has a homogeneous, sediment-free appearance. Its consistency is fluid. It is clear yellow in color, has a fishy smell and does not taste bitter. It is not desired that the moisture content in fish oil be more than 1%. If it is too much, oxidation (rancidity) accelerates. The peroxide value in fish oil is very important and it is not desired to exceed 20 meq /1000g (Korkut et al., 2007). Fish oils are liquid at room temperature and solidify below 10-15°C. The composition of fish oil depends on the type of fish. While pelagic fish generally store fat in the body, not in the liver, demersal fish store fat in the liver (Uçar et al., 2019).

Since saturated fatty acids are solid at room temperature, they can accumulate in the body. Polyunsaturated fatty acids are liquid at room temperature and are also very important for the continuity of human life. Therefore, they are called essential fatty acids and are divided into two groups: omega (ω)-6 and omega (ω)-3 fatty acids. ω-3 is abundant in flaxseed, walnuts and especially plankton and oily fish. EPA and DHA are the most important fatty acids in fish oils. EPA and DHA must be taken externally. Because they cannot be synthesized by the body, they are called essential fatty acids (Kaya et al., 2004). Chemical, physical and nutritional characteristics of fatty acids are detected by the number of carbon atoms in the molecule, the number of double bonds between carbon atoms and the position of the carbon atoms. When fatty acids are considered in terms of physical properties, it is stated that all unsaturated fatty acids with carbon numbers up to 10 are liquid at room temperature and become volatile. Also, they do not dissolve in water. Fatty acids with longer carbon chains are solid. Fats and oils consist of fatty acid chains. Omega-3; It is the name given to a group of polyunsaturated fats with a long chain structure. Unsaturated fatty acids contain various numbers of double bonds between carbon atoms in their molecular arrangements (Arıman Karabulut and Yandı, 2006).

USE OF FISH OIL

Producing fish oil from fresh fish is very important. The main use of fish oil in the aquaculture industry is aquaculture. It is used in more feed rations, especially for carnivorous fish such as trout and marine species. The selection of raw materials according to the nutritional needs of the fish species grown and their return on feed costs has also gained importance. The most important nutritional source, which is one of the raw materials used in fish feed and contains the most omega 3 fatty acids such as EPA and DHA, is fish oil (Korkut et al., 2007; Uçar et al., 2019). In addition, use in human foods and capsules has also found a use in the increasingly important field called nutraceuticals. It is also used as a carrier for pesticides, in dyes and in leather production (Uçar et al., 2019).

THE IMPORTANCE OF FISH OIL ON HUMAN HEALTH

Fats provide energy, form the cell membrane surrounding the cell, and control biochemical events (chemical messages, cell development and division, blood pressure and coagulation, immune reactions) (Arıman Karabulut and Yandı, 2006). Fish is a rich source of omega-3, have important physiological, structural roles in various neurological, immune, cardiovascular systems. Since humans cannot synthesize PUFA, must be consumed in the diet. Conversion from the parent PUFA to the more biologically active, DHA and EPA, is inefficient and some of the preformed long chains are consumption of PUFA is important for optimal health (Oken and Belfort, 2010). The known effects of fish oil are attributed to DHA and EPA. The purpose of consuming fish oil is to get DHA and EPA found in fish oil-derived products. These two omega-3 fatty acids are of primary importance for the human body, as DHA is the most common fatty acid in the human brain and is responsible for the eventual development of the nervous system. Omega-3 fatty acids increase vascular permeability and cause vasodilatation, resulting in lower blood pressure (Özkan ve Koca 2006; Gholami et al., 2020).

It has been reported that the use of fish oil has a triglyceride-lowering effect. It is also stated that fish oil can have a healing effect on the symptoms of rheumatism. Nutrition affects the risk of cancer types, especially esophageal, stomach, colon, breast, lung and prostate cancer. It has also been reported that longer chain n-3 polyunsaturated fatty acids such as EPA and DHA have protective effects on breast, colon and prostate cancers (Günebak Şahin, 2015). It is known that taking n-3 fatty acids with the diet reduces the risks of cancer and cardiovascular disease, LDL cholesterol, reduces joint and muscle inflammation, and may be beneficial in preventing and managing AIDS. These also have positive effects on maintaining glycemic control in diabetic patients (Mol, 2008; Arsava, 2023). Scientific data has revealed that consumption of ω-3 content of fish or fish oils reduces the risk of coronary heart disease, reduces hypertension, reduces certain heart rhythm disorders and sudden deaths, reduces the rate of diabetes and reduces joint pain due to rheumatism. It is clear that PUFAs play a vital role in regulating reproductive system, vision and nervous system functions (Kaya et al., 2004).

REFERENCES

Arıman Karabulut, H. A., Yandı, İ., 2006. Su Ürünlerindeki Omega-3 Yağ Asitlerinin Önemi ve Sağlık Üzerine Etkisi. Ege J. Fish. Aqua. Sci, 23(3): 339-342. (in Turkish with abstract in English)

Arsava, E. M., 2023. Vasküler sağlık için balık yağı kullanımı. Turk J. Cerebrovasc. Dis, 29(2): 54-58.

Caygill, C. P. J., Charlett, A., Hill, M. J., 1996. Fat, fish, fish oil and cancer. Br. J. Cancer, 74(1): 159-16.

Çöteli, F., 2021. Ürün Raporu Su Ürünleri 2022. TEPGE Yayın No: 355, Ankara. (in Turkish)

Gholami, N., Abotorabi, S., Lalooha, F., Oveisi, S., 2020. Effects of fish oil supplementation on pregnancy outcomes in pregnant women referred to Kosar Hospital. Iran. J. Pharm. Res, 19(3): 241-247.

Günebak Şahin Ç. T., 2015. Balık Yağları Hakkında Güncel Yaklaşımlar. https://gncturkiye.wordpress.com/2015/04/14/balik-yaglari-hakkinda-guncel-yaklasimlar/ (Accessed Date:19 September 2023).

Kaya, Y., Duyar, H. A., Erdem, M. E., 2004. Balık yağ asitlerinin insan sağlığı için önemi. Ege J. Fish. Aqua. Sci, 21 (3-4): 365-370. (in Turkish with abstract in English)

Kocatepe, D., Turan, H., 2018. Balık yağları, DHA, EPA ve sağlık. Türkiye Klinikleri J Public Health-Special Topics, 4(1): 62-67.

Korkut, A. Y., Kop, A., Demir, P., 2007. Balık Yemlerinde Kullanılan Balık Yağı ve Özellikleri. Ege J. Fish. Aqua. Sci, 24(1): 195-199. (in Turkish with abstract in English)

Oken, E., Belfort, M. B., 2010. Fish, fish oil, and pregnancy. Jama, 304(15): 1717-1718.

Özkan, Y., Koca, S. S., 2006. Hiperlipidemi tedavisinde omega-3 yağ asitinin (balık yağı) etkinliği. Fırat Tıp Dergisi, 11(1): 40-44.

Uçar, Y., Durmuş, M., Ceylan, Z., 2019. Balık yağı üzerine. Detay Yayıncılık, Ankara.246 p.

Ünsal, A., 2019. Beslenmenin önemi ve temel besin öğeleri. Kırşehir Ahi Evran University J. Health Sci, 2(3): 1-10.

Weitz, D., Weintraub, H., Fisher, E., Schwartzbard, A. Z., 2010. Fish oil for the treatment of cardiovascular disease. Cardiol. Rev, 18(5): 258-263.

Effect of Light Intensity on the Amino Acid Composition of Cyanobacteria

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ABSTRACT: Utilizing algae as an alternative feedstuff has significant promise in poultry production and can reduce polluting gas emissions from animal feeding operations. This study aimed to investigate the influence of light intensities on the amino acid profile of *Synechococcaceae* to assess its potential as a feed ingredient for poultry nutrition. *Synechococcaceae* was cultivated under three different light intensities: 60, 100, and 200 μmol m^{-2} s^{-2}. *Synechococcaceae* showed the highest lipid content of 32.3% at the light intensity of 100 μmol m^{-2} s^{-1}. Carbohydrate content increased with higher light intensities, and lipid content peaked at 100 μmol m^{-2} s^{-1} and decreased at 200 μmol m^{-2} s^{-1}. Furthermore, the study demonstrated that amino acid content of *Synechococcaceae* increased with elevated light intensity. Glutamic acid was the most abundant amino acid at each of light intensity. Light intensity significantly affected the content of individual amino acids. In conclusion, this study provides valuable insights into the relationship between light intensity and the nutritional composition of *Synechococcaceae*, offering promising prospects for its utilization as poultry feed ingredient. These findings contribute to developing more nutritious and sustainable poultry feed formulations while considering color attributes relevant to the food industry.

Keywords: *Synechococcaceae,* Sustainability, Protein, Amino acid, Light intensity

INTRODUCTION

The usage of microalgae has gained significant attention in various industries due to their unique properties and potential applications. Microalgae are microscopic photosynthetic organisms found in diverse aquatic environments, including freshwater and marine habitats. They have attracted interest as a sustainable and renewable resource for various applications, including food and feed production, biofuel production, wastewater treatment, and pharmaceuticals. One of the prominent areas where microalgae have shown great potential is in animal feed production. Traditional feed sources, such as soybean meal and fishmeal, are associated with environmental concerns and limited availability. Microalgae offer a promising alternative as a feed ingredient due to their high nutritional value and sustainable production potential. They are rich in proteins, essential amino acids, vitamins, minerals, and bioactive compounds, making them a valuable source of nutrients for livestock and poultry (Chaves et al. 2021).

The nutritional composition of the microalgae varies at different environmental factors such as light intensity, carbon and nitrogen sources, and the culture conditions (pH and temperature) (Atta et al. 2013). Light intensity plays a crucial role in the growth and biochemical composition of microalgae, including the content and composition of amino acids, lipids, pigments, and fatty acids (Nzayisenga et al. 2020; Ogbonna et al. 2022; Lamminen 2021; Zainan et al. 2022). Different microalgae species may exhibit varying responses to light intensity, leading to differences in their biochemical composition. Also, cultivation modes, such as using different nutrient media or variations in carbon dioxide concentration, can also affect the amino acid composition of microalgae. Understanding the effects of light intensity and cultivation mode on the amino acid composition of microalgae is essential for optimizing their cultivation conditions and harnessing their potential for various applications, such as biofuel production and food supplementation (Sui and Harvey 2021).

The nutritional composition of microalgae can be tailored to meet the specific requirements of different animal species. By manipulating the cultivation conditions, such as light intensity, nutrient availability, and carbon dioxide concentration, the amino acid profile and fatty acid composition of microalgae can be optimized to enhance their nutritional value for animal feed. This customization allows for the formulation of feed with balanced amino acid profiles, essential fatty acids, and other bioactive compounds that promote animal health and performance (Chaves et al. 2021).

The amino acid composition of microalgae is a crucial aspect of their biochemical profile, as it directly influences their nutritional value and potential applications in various industries. The amino acid composition of microalgae can vary significantly depending on factors such as species, cultivation conditions, and nutrient availability. Nitrogen and phosphorus are essential macronutrients that play a vital role in the growth and metabolism of microalgae, including the synthesis of amino acids. Studies have shown that manipulating nitrogen and phosphorus concentrations in the growth medium can influence the amino acid composition of microalgae, with specific amino acids being more abundant under certain nutrient conditions (Yaakob et al. 2014). Understanding the factors that affect the amino acid composition of microalgae is crucial for optimizing their cultivation conditions and harnessing their potential as a sustainable source of protein and other valuable bioactive compounds.

To evaluate the effect of the light intensities on the amino acids profile for algae usage as a feed ingredient in poultry rations, *Synechococcaceae* (AQUAMEB-32) were produced at three different light intensities (60, 100, and 200 μmol m^{-2} s^{-2}).

MATERIAL AND METHODS

Algae Cultivation and medium

Synechococcaceae (AQUAMEB-32) strain obtained from the AQUAMEB Culture Collection of Algae was isolated from Lake Uluabat, Turkey. This strain was used due to its high growth rate and adaption to a wide range of light intensities. *Synechococcaceae* strain was inoculated in 250 mL Erlenmeyer flasks containing 100 mL of Bold's Basal medium (BBM). The composition of the BBM can be found in Uguz et al. (Uguz et al. 2022). The prepared medium was adjusted for pH (6.8-7.0) with 0.5 M HCl or 0.5 M NaOH and sterilized by placing it into an autoclave for 20 minutes at 121°C before use. The algal strain started from a 100-mL culture and doubled every seven days with a freshly prepared BBM. During cultivation, the culture was continuously aerated with air at an airflow rate of 0.5 LPM per liter of culture. The daylight LED lamps illuminated the algal culture at three different light intensities (60, 100, and 200 µmol m^{-2} s^{-2}).

Experimental Procedure

In order to test the effect of different light intensities on the amino acid and nutrient content of *Synechococcaceae* as a supplementary nutrient for animal feed, the algal cultures started from a 100 mL volume, and BBM was periodically added to achieve a final culture volume of 10 L. Six rectangular PBRs (inner dimensions: 500 mm × 100 mm × 350 mm) were used to operate control and treatment experiments. *Synechococcaceae* was grown under three light intensities 60, 100, and 200 µmol m^{-2} s^{-1}. Each experiment was run for seven days in triplicates with control tanks at a light intensity of 60-70 µmol m^{-2} s^{-1} (24 h light photoperiod).

During the cultivation, the treatment PBRs was continuously aerated with air (containing 1200 ppm CO_2) at a rate of 0.5 L min^{-1} per liter of culture volume. The flow meter (Bass Instruments, Turkey) adjusted the airflow rate for each PBR. The light intensity was measured with a LI-COR quantum sensor (LI-COR, Lincoln, Nebraska). Environmental conditions, including pH, temperature, water level (maintained with deionized water), aeration rate, and light intensity, were monitored and controlled throughout the experiments. PH was maintained daily in the range of 7.0±0.2 by 0.5 M HCl or 0.5 M NaOH. A room temperature of 24±2°C was also maintained during the experimental period. The temperature and pH of the algal culture were measured using a digital pH meter (Hanna Instrument, HI98128). Sampling was done every 24h periodically, and cell counts were measured using hemocytometers under an optical microscope to investigate algal growth. In the batch culture tests, no nutrients were added at any point throughout the growing time. A dilution rate of 0.1 (Kang and Wen 2015) was employed for the PBRs for the continuous cultivation mode experiments. Ten percent of the total working volume (10 L) was removed daily from each PBR, and the same amount of BBM was added back to each PBR.

Determination of Amino Acids

The microalgal biomass was harvested for amino acid profile analysis at the end of each experiment.

The pellet was freeze-dried and stored at −40 °C in a lyophilizer for further experiments. The amino acid LC–MS/MS analysis was performed by an Agilent LC–MS mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) accoding to the method described by Uğuz and Sozcu (2023). The targeted amino acid concentrations were determined using electrospray ionization (ESI) and multiple reactions monitoring (MRM).

Analytical methods

The cell concentration (cells L^{-1}), dry algal biomass concentration (mg L^{-1}), and specific growth rate (d^{-1}) were determined according to the procedures of Uguz et al. (Uguz et al. 2022). Briefly, the cell concentration was measured using hemocytometers under an Olympus optical microscope. Gravimetric analysis was used to calculate the dry algal biomass content. A known volume of an algal sample was vacuum-filtered, and the filter was then dried for three h at 80ºC in a laboratory oven (Osabutey et al. 2023). Calculating the specific growth rate involved normalizing the increase in cell concentration by the initial cell concentration.

The procedures of the Association of Official Analytical Chemists (AOAC, 2006) were followed to determine the chemical composition of harvested algal biomass. Lipid content was analyzed with acid hydrolysis (AOAC, Official Method 954.02, 2006), The crude protein was determined using the Kjeldahl method, using 6.25 as a conversion factor to calculate protein content (AOAC, Official Method 984.13 (A–D), 2006). Carbohydrate composition was determined from their alditol acetates via gas–liquid chromatography (GLC) and via gas–liquid chromatography–mass spectrometry (GLC–MS) based on the procedure by Oxley et al. (Oxley D, Currie G C 2004).

Statistical Analyses

The collected data were exposed to one-way Analysis of Variance (ANOVA) using JMP (version 13) followed by the least significant difference (LSD) Student comparison tests to compare the differences between treatments, where significant differences were observed ($p < 0.05$). Results are given as the mean and standard error of the mean (SEM). Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

The nutritional quality of *Synechococcaceae* at different light intensities was investigated as a protein source based on its nutrition and amino acid composition. Table 1 shows the nutrient compositions of *Synechococcaceae* produced at different light intensities. The protein content of the *Synechococcaceae* at 60, 100, and 200 µmol m^{-2} s^{-2} were 15.7±0.06%, 14.6±0.06%, and 16.9±0.06%, respectively. Among the three light intensities, *Synechococcaceae* had the highest protein content at 200 µmol m^{-2} s^{-2} ($P<0.001$). In contrast to the findings of the present study, previous research indicated that the algal biomass obtained from several species, including *Synechococcus sp.*, *Anabena cylindrical*, *Chlorella vulgaris*, and *Spirulina* exhibited a crude protein content ranging from 43% to 73% (Lum, Kim, and Lei 2013). *Scenedesmus, Chlorella, Tetraselmis, Arthrospira*, *Skeletonema*, and *Phaeodactylum* are the most commonly used algae species for protein-rich feed additives in animal nutrition (Mobin and Alam 2017; Spolaore et al. 2006). Eilam et al. (Eilam et al. 2023) reported that microalgae can produce 2.5 to 7.5 tons of protein per hectare yearly.

The carbohydrate content of *Synechococcaceae* showed significant differences between light intensities. The carbohydrate content of the *Synechococcaceae* at 60, 100, and 200 µmol m^{-2} s^{-2} were 2.6±0.06%, 4.0±0.06%, and 18.4±0.06%, respectively. The carbohydrate content of cyanobacteria and algae depends on various factors such as nutrient availability, light intensity, and growth stage and conditions. Christaki et al. (Christaki, Florou-Paneri, and Bonos 2011) investigated the carbohydrate content of three microalgae and reported 8–14% for *Spirulina maxima*, 2–17% for *Chlorella vulgaris*, 32% for *Diacronema vikianum*, and 10–17% for *Isochrysis galbana*. Carbohydrates have a significant role in the composition of algal biomass, owing to their considerable nutritional and pharmaceutical value. For example, a beta-1-3-glucan, classified as a soluble fiber, is mostly present in *Chlorella sp.* This compound has been shown to possess antioxidant properties that reduce blood cholesterol levels, as Spolaore et al. (Spolaore et al. 2006) and De Morais et al. (De Morais et al. 2015) reported.

Table 1. Nutrient compositions of *Synechococcaceae* produced at different light intensities

Light Intensity	Lipids (%)	Protein (%)	Carbohydrate (%)
60 µmol m^{-2} s^{-1}	28.6^{b}	15.7^{b}	2.6^{c}
100 µmol m^{-2} s^{-1}	32.3^{a}	14.6^{c}	4.0^{b}
200 µmol m^{-2} s^{-1}	$22{,}3^{c}$	16.9^{a}	18.4^{a}
SEM	±0.06	±0.06	±0.06
P VALUES	*<0.0001*	*<0.0001*	*<0.0001*

[a-c] Differences in letters within columns indicate significant differences among the experimental groups

Algae have attracted much attention as sources of biodiesel feedstock due to their high lipid content. Microalgae can accumulate lipids up to 50% of their dry weight, which is much higher than that in terrestrial oil crops. The lipid content and fatty acid composition of microalgae vary significantly depending on species, growth conditions, and culture methods. Under stress conditions such as nutrient deprivation, some microalgal species can accumulate lipids up to 70% of their dry weight (Mata, Martins, and Caetano 2010). This high lipid content makes microalgae promising feedstocks for biodiesel production. The current results showed that the protein and carbohydrate contents increased with the elevated light intensities. However, the lipid content achieved the highest value at the light intensity of 100 μmol m^{-2} s^{-1}, and then decreased at the light intensity of 200 μmol m^{-2} s^{-1}. The nutritional content, including protein, carbohydrates, and lipids, varies in algal growth conditions (Madeira et al. 2017). The nutritional value of microalgae in animal feed has been extensively studied and demonstrated to be significant for animals, including chickens, pigs, cows, and aquaculture (Furbeyre et al. 2017; Bonos et al. 2016).

Proteins derived from algae are abundant in essential amino acids, which account for 7% of total proteins. Other essential amino acids, which include isoleucine, phenylalanine, threonine, and valine, usually constitute 4% of proteins (Patel et al. 2019). The essential and non-essential amino acid composition (mg/100 mg of dried weight) of *Synechococcaceae* produced at different light intensities (60-200 μmol m^{-2} s^{-1}) in this study is illustrated in Tables 2 and 3. Current results show that the amino acid content of *Synechococcaceae* increased with elevated light intensity. The most abundant amino acid in mg/100 mg (1.04 for 60 μmol m^{-2} s^{-1}, 1.23 for 100 μmol m^{-2} s^{-1}, and 2.58 for 200 μmol m^{-2} s^{-1}) was Glutamic acid for each of light intensity. Each amino acid content was significantly affected by light intensity ($P<0.0001$). The amino acid composition of algae is essential for their potential use as animal feed. The essential and non-essential amino acid profiles determine the nutritional value and suitability of algae as a feedstock. The presence of essential amino acids in algae makes them a potential source of high-quality protein for animal feed. The amino acid composition of algae can be tailored to meet the specific nutritional requirements of different animal species. Parjikolaei et al. (Parjikolaei et al. 2016) investigated the amino acid composition of nine North Atlantic red macro algae species. They found that the dominating essential amino acids were lysine and leucine, while the dominating non-essential amino acids were aspartic acid, glutamic acid, and arginine. The amino acid composition varies between algae species depending on growth conditions and environment. However, essential amino acids like lysine and leucine tend to be present in high amounts, making algae a potential source of these limiting amino acids for animal feed.

Table 2. Essential amino acid compositions of *Synechococcaceae* produced at different light intensities (60-200 µmol m^{-2} s^{-1}) (mg/100 mg of dry weight)

Light Intensity	Arginine	Threonine	Valine	Isoleucine	Leucine	Methionine	Lysine	Phenylalanine	Histidine	Glutamic Acid	Glycine
60	0.51^c	0.16^c	0.21^c	0.22^b	0.68^c	0.08^c	0.36^c	0.45^c	0.1^b	1.04^c	0.7^c
100	0.65^b	0.54^b	0.34^b	0.22^b	0.76^b	0.17^b	0.44^b	0.57^b	0.1^a	1.23^b	0.77^b
200	0.97^a	0.75^a	0.78^a	0.56^a	1.38^a	0.22^a	0.85^a	0.82^a	0.29^a	2.58^a	1.36^a
SEM	0.0004	0.0003	0.0001	0.001	0.005	0.0005	0.0002	0.004	0.0001	0.002	0.001
P Values	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*

[a-c] Differences in letters within columns indicate significant differences among the experimental groups

The amino acid composition of algae plays a crucial role in determining their nutritional value and potential applications. Algae are known to contain both essential and non-essential amino acids, which are important for various biological processes in animals. The essential amino acids are those that cannot be synthesized by animals and must be obtained from their diet. On the other hand, non-essential amino acids can be synthesized by animals, but their presence in the diet can still be beneficial. The non-essential amino acids in algae can contribute to overall animal protein synthesis and metabolic processes. In this study, the highest contents for each non-essential amino acid were achieved at 200 µmol m^{-2} s^{-1} except ornitine. The most abundant amino acid was Aspartic acid (1.92 mg/100 g) at each of light intensity.

Table 3. Non-essential amino acid compositions of *Synechococcaceae* produced at different light intensities (mg/100 mg of dry weight)

Light Intensity	Serine	Proline	Alanine	Tyrosine	Aspartic Acid	Ornitine
60 µmol m^{-2} s^{-1}	0.43^c	0.22^c	0.50^c	0.1^c	0.55^c	0.29^a
100 µmol m^{-2} s^{-1}	0.46^b	0.34^b	0.79^b	0.39^b	1.31^b	0.30^a
200 µmol m^{-2} s^{-1}	0.52^a	0.94^a	1.69^a	0.74^a	1.92^a	0.12^b
SEM	0.0005	0.0002	0.005	0.005	0.003	0.0001
P Values	*0.0003*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*

[a-c] Differences in letters within columns indicate significant differences among the experimental groups.

The biosynthesis and functional properties of carotenoids originating from algae have been the subject of extensive research. Tetra terpene pigments with the colors yellow, orange, red, and purple are called carotenoids. The pigments known as carotenoids are found in photosynthetic bacteria, certain types of fungi and archaea, algae, plants, and mammals. They are the most abundant pigments found in nature (Maoka 2011). Studies have highlighted the distribution, biosynthesis, and functions of carotenoids in different algal species, shedding light on the diverse pathways and enzymes involved in carotenoid production (Takaichi 2011; Christaki et al. 2013; Igreja et al. 2021). Furthermore, the production of carotenoids from algae has gained prominence in biotechnological applications due to the increasing demand for natural products and the potential health benefits associated with these compounds (Christaki, Florou-Paneri, and Bonos 2011). Carotenoids play a crucial role in animal nutrition, offering a range of benefits that contribute to overall health and well-being.

In this study, the assessment of color in algal biomass was conducted by employing colorimetric analysis to determine the values of lightness (*L**), redness (*a**), and yellowness (*b**) indexes. The color characteristics of *Synechococcaceae* produced at different light intensities are presented in Table 4. The lightness (*L**) value was found to be the highest, with a value of 39.32 in algae biomass produced from *Synechococcaceae* that was illuminated at the light intensity of 100 µmol m^{-2} s^{-1} cultivated ($P < 0.001$). An *L** value represents the darkness to lightness, ranging from 0 to 100. The a* value is used to evaluate the characteristics of red and green, while the b* value serves as an indicator for the characteristics of yellow and blue. According to the analysis of a* and b* values, the color characteristics of the algae samples in this study are within the spectrum of green and blue colors. These color characteristics play a significant role in egg yolk and meat pigmentation.

These natural pigments, commonly found in algae, are known for their antioxidant properties, immune system support, and enhancement of animal reproductive and growth performance (Takaichi 2011; Mikami and Hosokawa 2013). The diverse carotenoid composition in algae, including fucoxanthin and astaxanthin, has been linked to improved pigmentation, immune response, and reproductive success in various animal species (Wade et al., 2015; Moreno et al., 2016). Furthermore, the utilization of carotenoids in animal feed has gained attention due to their potential to enhance the nutritional value of the feed and improve animal health (Igreja et al. 2021; Machmudah et al. 2018). The biotechnological production of carotenoids from algae presents an opportunity to incorporate these valuable compounds into animal feed formulations, thereby positively impacting animal health and performance (Sedkova et al. 2005; Pierce et al. 2015). The ability to optimize carotenoid-enriched feed through fermentation and extraction processes offers a sustainable approach to enhancing the nutritional content of animal diets (Ananda and Vadlani 2010).

Table 4. Color characteristics of *Synechococcaceae* produced at different light intensities (mg/100 mg of dry weight)

Light Intensities	**L*-value**	**a*-value**	**b*-value**	**C^*_{ab}-value**	**α°-value**
60 µmol m^{-2} s^{-1}	21.04[b]	-4.2[b]	8.82[b]	9.95[b]	115.33[a]
100 µmol m^{-2} s^{-1}	39.32[a]	-5.8[c]	15.52[a]	16.6[a]	110.4[b]
200 µmol m^{-2} s^{-1}	21.00[b]	-0.74[a]	2.88[c]	2.98[c]	103.9[c]
SEM	1.28	0.23	0.66	0.65	0.82
P Values	*<0.0001*	*<0.0001*	*<0.0005*	*<0.0011*	*0.6*

[a-c] Differences in letters within columns indicate significant differences among the experimental groups.

CONCLUSION

In conclusion, this study highlights the intricate relationship between light intensity and the nutritional composition of *Synechococcaceae*, shedding light on its potential as a valuable feed ingredient for poultry diets. These findings provide valuable insights for optimizing microalgae cultivation conditions to enhance their nutritional content, ultimately contributing to developing more nutritious and sustainable poultry feed formulations.

Despite the numerous advantages of microalgae as a feed ingredient, challenges still need to be addressed. Microalgae cultivation and processing costs can be a barrier to widespread adoption, and further research is needed to optimize production methods and reduce production costs. Additionally, the regulatory framework for using microalgae in animal feed must be established to ensure that safety and quality standards are met. Understanding and optimizing the amino acid profiles of algae can contribute to developing sustainable and nutritionally balanced feed formulations for various animal species.

REFERENCES

Ananda, Nanjundaswamy, and Praveen V. Vadlani. 2010. "Production and Optimization of Carotenoid-Enriched Dried Distiller's Grains with Solubles by Phaffia Rhodozyma and Sporobolomyces Roseus Fermentation of Whole Stillage." *Journal of Industrial Microbiology and Biotechnology* 37 (11). https://doi.org/10.1007/s10295-010-0765-y.

Atta, Madiha, Ani Idris, Ataullah Bukhari, and Suzana Wahidin. 2013. "Intensity of Blue LED Light: A Potential Stimulus for Biomass and Lipid Content in Fresh Water Microalgae Chlorella Vulgaris." *Bioresource Technology* 148. https://doi.org/10.1016/j.biortech.2013.08.162.

Bonos, E, E Kasapidou, A Kargopoulos, A Karampampas, E Christaki, P Florou-Paneri, and I Nikolakakis. 2016. "Spirulina as a Functional Ingredient in Broiler Chicken Diets." *South African Journal of Animal Science* 46 (1): 94. https://doi.org/10.4314/SAJAS.V46I1.12.

Chaves, Andreia A.M., Cátia F. Martins, Daniela F.P. Carvalho, David M. Ribeiro, Madalena Lordelo, João P.B. Freire, and André M. de Almeida. 2021. "A Viewpoint on the Use of Microalgae as an Alternative Feedstuff in the Context of Pig and Poultry Feeding—a Special Emphasis on Tropical Regions." *Tropical Animal Health and Production*. https://doi.org/10.1007/s11250-021-02800-5.

Christaki, Efterpi, Eleftherios Bonos, Ilias Giannenasa, and Panagiota Florou-Paneria. 2013. "Functional Properties of Carotenoids Originating from Algae." *Journal of the Science of Food and Agriculture*. https://doi.org/10.1002/jsfa.5902.

Christaki, Efterpi, Panagiota Florou-Paneri, and Eleftherios Bonos. 2011. "Microalgae: A Novel Ingredient in Nutrition." *Http://Dx.Doi.Org/10.3109/09637486.2011.582460* 62 (8): 794–99. https://doi.org/10.3109/09637486.2011.582460.

Eilam, Yahav, Hamdan Khattib, Noam Pintel, and Dorit Avni. 2023. "Microalgae—Sustainable Source for Alternative Proteins and Functional Ingredients Promoting Gut and Liver Health." *Global Challenges* 7 (5): 2200177. https://doi.org/10.1002/GCH2.202200177.

Furbeyre, H., J. Van Milgen, T. Mener, M. Gloaguen, and E. Labussière. 2017. "Effects of Dietary Supplementation with Freshwater Microalgae on Growth Performance, Nutrient Digestibility and Gut Health in Weaned Piglets." *Animal* 11 (2). https://doi.org/10.1017/S1751731116001543.

Igreja, Willen Silva, Felipe de Andrade Maia, Alessandra Santos Lopes, and Renan Campos Chisté. 2021. "Biotechnological Production of Carotenoids Using Low Cost-Substrates Is Influenced by Cultivation Parameters: A Review." *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms22168819.

Kang, Juhyon, and Zhiyou Wen. 2015. "Use of Microalgae for Mitigating Ammonia and CO2 Emissions from Animal Production Operations — Evaluation of Gas Removal Efficiency and Algal Biomass Composition." *Algal Research* 11 (September): 204–10. https://doi.org/10.1016/J.ALGAL.2015.06.020.

Lamminen, Marjukka. 2021. "Nutritional Value of Microalgae for Ruminants and Implications from Microalgae Production." *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 2021 (2021). https://doi.org/10.1079/PAVSNNR202116054.

Lum, Krystal K., Jonggun Kim, and Xin G. Lei. 2013. "Dual Potential of Microalgae as a Sustainable Biofuel Feedstock and Animal Feed." *Journal of Animal Science and Biotechnology* 4 (1): 1–7. https://doi.org/10.1186/2049-1891-4-53/TABLES/3.

Machmudah, Siti, Wahyudiono, Hideki Kanda, and Motonobu Goto. 2018. "Supercritical Fluids Extraction of Valuable Compounds from Algae: Future Perspectives and Challenges." *Engineering Journal*. https://doi.org/10.4186/ej.2018.22.5.13.

Madeira, Marta S., Carlos Cardoso, Paula A. Lopes, Diogo Coelho, Cláudia Afonso, Narcisa M. Bandarra, and José A.M. Prates. 2017. "Microalgae as Feed Ingredients for Livestock Production and Meat Quality: A Review." *Livestock Science* 205 (November): 111–21. https://doi.org/10.1016/J.LIVSCI.2017.09.020.

Maoka, Takashi. 2011. "Carotenoids in Marine Animals." *Marine Drugs*.

https://doi.org/10.3390/md9020278.

Mata, Teresa M., António A. Martins, and Nidia S. Caetano. 2010. "Microalgae for Biodiesel Production and Other Applications: A Review." *Renewable and Sustainable Energy Reviews*. https://doi.org/10.1016/j.rser.2009.07.020.

Mikami, Koji, and Masashi Hosokawa. 2013. "Biosynthetic Pathway and Health Benefits of Fucoxanthin, an Algae-Specific Xanthophyll in Brown Seaweeds." *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms140713763.

Mobin, Saleh, and Firoz Alam. 2017. "Some Promising Microalgal Species for Commercial Applications: A Review." *Energy Procedia* 110 (March): 510–17. https://doi.org/10.1016/J.EGYPRO.2017.03.177.

Morais, Michele Greque De, Bruna Da Silva Vaz, Etiele Greque De Morais, and Jorge Alberto Vieira Costa. 2015. "Biologically Active Metabolites Synthesized by Microalgae." *BioMed Research International* 2015. https://doi.org/10.1155/2015/835761.

Nzayisenga, Jean Claude, Xavier Farge, Sophia Leticia Groll, and Anita Sellstedt. 2020. "Effects of Light Intensity on Growth and Lipid Production in Microalgae Grown in Wastewater." *Biotechnology for Biofuels* 13 (1). https://doi.org/10.1186/s13068-019-1646-x.

Ogbonna, I.O., J. Ikwebe, J.C. Ogbonna, C.N. Eze, and J.B. Ndrimbula. 2022. "Effects of Light Intensity and Photoperiod on Growth, Lipid Accumulation and Fatty Acid Composition of Desmodesmus Subspicatus LC172266 under Photoautotrophic Cultivation." *Nigerian Journal of Biotechnology* 38 (2). https://doi.org/10.4314/njb.v38i2.1.

Osabutey, Augustina, Noor Haleem, Seyit Uguz, Kyungnan Min, Karlee Albert, Gary Anderson, and Xufei Yang. 2023. "Growth of Scenedesmus Dimorphus in Swine Wastewater with versus without Solid–Liquid Separation Pretreatment." *Bioresource Technology* 369 (February): 128434. https://doi.org/10.1016/J.BIORTECH.2022.128434.

Oxley D, Currie G C, Bacic A. 2004. *Analysis of Carbohydrate from Glycoproteins. In: Purifying Proteins for Proteomics: Purifying Proteins for Proteomics: A Laboratory Manual*. Edited by R J Simpson. New York, USA: Cold Spring Harbor Laboratory Press.

Parjikolaei, Behnaz Razi, Annette Bruhn, Karin Loft Eybye, Martin Mørk Larsen, Michael Bo Rasmussen, Knud Villy Christensen, and Xavier C. Fretté. 2016. "Valuable Biomolecules from Nine North Atlantic Red Macroalgae: Amino Acids, Fatty Acids, Carotenoids, Minerals and Metals." *Natural Resources* 07 (04). https://doi.org/10.4236/nr.2016.74016.

Patel, Anil Kumar, Jae Min Joun, Min Eui Hong, and Sang Jun Sim. 2019. "Effect of Light Conditions on Mixotrophic Cultivation of Green Microalgae." *Bioresource Technology* 282 (June): 245–53. https://doi.org/10.1016/J.BIORTECH.2019.03.024.

Pierce, Emily C., Peter R. LaFayette, María A. Ortega, Blake L. Joyce, Dean A. Kopsell, and Wayne A. Parrott. 2015. "Ketocarotenoid Production in Soybean Seeds through Metabolic Engineering." *PLoS ONE* 10 (9). https://doi.org/10.1371/journal.pone.0138196.

Sedkova, Natalia, Luan Tao, Pierre E. Rouvière, and Qiong Cheng. 2005. "Diversity of Carotenoid Synthesis Gene Clusters from Environmental Enterobacteriaceae Strains." *Applied and Environmental Microbiology* 71 (12). https://doi.org/10.1128/AEM.71.12.8141-8146.2005.

Spolaore, Pauline, Claire Joannis-Cassan, Elie Duran, and Arsène Isambert. 2006. "Commercial Applications of Microalgae." *Journal of Bioscience and Bioengineering* 101 (2): 87–96. https://doi.org/10.1263/JBB.101.87.

Sui, Yixing, and Patricia J. Harvey. 2021. "Effect of Light Intensity and Wavelength on Biomass Growth and Protein and Amino Acid Composition of Dunaliella Salina." *Foods* 10 (5). https://doi.org/10.3390/foods10051018.

Takaichi, Shinichi. 2011. "Carotenoids in Algae: Distributions, Biosyntheses and Functions." *Marine Drugs*. https://doi.org/10.3390/md9061101.

Uguz, Seyit, Gary Anderson, Xufei Yang, Ercan Simsek, and Augustina Osabutey. 2022. "Cultivation of Scenedesmus Dimorphus with Air Contaminants from a Pig Confinement Building." *Journal of Environmental Management* 314 (July): 115129. https://doi.org/10.1016/J.JENVMAN.2022.115129.

Yaakob, Zahira, Ehsan Ali, Afifi Zainal, Masita Mohamad, and Mohd S. Takriff. 2014. "An Overview: Biomolecules from Microalgae for Animal Feed and Aquaculture." *Journal of Biological Research (Greece)* 21 (1): 1–10. https://doi.org/10.1186/2241-5793-21-6/METRICS.

Zainan, Nur Hidayah Binti, Razif Harun, Siti Mazlina Mustapa Kamal, Shamsul Izhar Siajam, Mohd Azan Mohammed Sapardi, and Yanti Maslina Mohd Jusoh. 2022. "Profile of Amino Acids Production from Microalgae Nannochloropsis Sp. Biomass Using Subcritical Water Technology." *ASEAN Journal of Chemical Engineering* 22 (1). https://doi.org/10.22146/ajche.69517.

Study of Intensive Cultivation Technology That Ensures High Yield from Cereal Crops in Short Rotation Cropping Cycle

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ABSTRACT: The article mainly talks about the effect of predecessors on aboveground dry matter biomass, grain yield and main economic indicators of cereal crops in winter forage pea, winter wheat, barley crop rotation in the research conducted in 2022. The obtained results revealed that the amount of aboveground dry biomass at the end of the spring tillering of the winter wheat variety "Gobustan" was 14.7-16.5 s (1.4 per hectare) with the same nitrogen fertilizer (N) applied in both crops depending on the crops (crop rotation and continuous cropping) due to the effect of the predecessor. s increase) has been. At the end of the tube emergence phase, compared to continuous planting, this increase is 1.6 s per hectare, and in the full ripening phase, the increase in the total above-ground dry biomass is 5.3 s, and the "Jalilabad 19" variety of barley is 1.4, respectively; It is set at 1.7 and 4.8 s. The productivity and economic indicators of the plants cultivated in the crop rotation option were higher than those in continuous cultivation. In this variant, the grain yield of winter wheat and barley was 44.6-37.7 s per hectare, and 42.5-35.4 s in continuous cultivation, respectively.

Keywords: Cultwation technology, Plant rotation, Kontinuous cropping, Predecessor, Plant

INTRODUCTION

In order to obtain a high-quality, economically efficient crop from winter wheat, it is important that the amount of the main nutrients in the soil is normal, depending on the soil-climatic conditions [5,3,11].

The efficiency of cultivation technologies and other factors applied to the cultivation of agricultural plants are characterized by the amount of productivity. Wheat crops make up 60% of cereals in the country's crop structure. Currently, although there are wheat varieties with high potential productivity per hectare, the average productivity indicators in the regions are much lower. According to the data of 2021, the cultivated area of winter barley was 373757 hectares, and the average yield per hectare was 30.7 centners. Therefore, in order to increase productivity and its quality, it is necessary to achieve the application of cultivation technology with high adaptability in accordance with each region, taking into account the resistance to drought, the transformation of plant residues entering the soil, the maintenance of the humus balance, and the directions of recovery [4,10].

Diversification of plants that serve to increase the income of peasant-farmers, efficient use of natural resources, prevention of unjustified removal of lands from agricultural circulation, and their protection from harmful anthropogenic effects are important issues.Favorable conditions for collecting the highest amount of mineral nitrogen resources in the soil during the vegetation period are the application of organic fertilizers.It has been confirmed by researchers that the organic and organic-mineral fertilization system leads to an increase in the amount of water-soluble, mobile and exchangeable forms of phosphorus [6,8].

According to the information of researchers (2,9), the efficiency of fertilizers depends on the availability of easily assimilated forms of basic nutrients in the soil, soil-climatic conditions, norms and ratios of mineral fertilizers.According to Z.R. Movsumov, the yield of winter wheat increased by 4-5 seconds with the application of nitrogen-phosphorus-potassium (N90P60K60) fertilizers, compared to the variant where only nitrogen fertilizer (N90) was given to the soil. Also, accumulation of nitrogen in above-ground biomass crop of winter wheat depends on organic and mineral fertilizers. Thus, when only nitrogen fertilizer was given in irrigated gray-brown soils, at the end of the spring flowering of the plant, at the end of the tube emergence phase, as well as in the wax and full maturity phases, the amount of nitrogen carried by the above-ground biomass product was higher than in the full fertilizer norm (N120P60K60). According to other researchers, nitrogen uptake by aboveground dry biomass production of winter wheat plant also depends on the predecessor plant [1,7].

The change of total nitrogen in the soil during the growing season is very weak. Also, it is generally not possible to determine the change of nitrogen in a short period of time. However, in several rotational crops, fertile conditions are created for the accumulation of nitrogen in the soil. Clover and especially khasha and raps (green, when chopped and applied to the field as fertilizer) cause a noticeable increase in total nitrogen in the soil. When inter-row crops are cultivated for a long time, the amount of total nitrogen and also humus in the soil decreases [1,4].

As it is known, legumes play an important role in restoring soil fertility as an irreplaceable predecessor for cereals. Leguminous plants absorb nitrogen from the air and enrich the soil with nitrogen [3,6]. These plants play an important role in changing the chemical composition of the soil in addition to organizing the food and fodder base. More humus, total nitrogen and other nutrients accumulate in the soil than in cereals [11].

With the symbiotic fixation of air nitrogen by plants, the amount of nitrogen in the soil is normalized and the cost of the product obtained from a unit area is significantly reduced [2,4].

The main three importance of cereals and legumes are known: to increase the fertility of the soil, to provide industry with grain and protein. The branches, leaves, and pods of these plants contain a large amount of protein, which means that it is a powerful feed for animals and a protein deficiency in human nutrition. Compared to spiked plants, legumes have 2-3 times more protein, and 3-5 times more protein in their stubble [8].

MATERIALS AND METHODS

The main purpose of the conducted researches is to use fodder pea in winter fodder pea-winter wheat-barley type, taking into account the importance of using Absheron as a siderate (green fertilizer) and a good precursor for grain crops in crop rotation, which has both a valuable feed value under irrigation conditions. was to study the effect of short rotation crop rotation on the productivity and economic indicators of plants.

Researches were carried out in two schemes (crop rotation and continuous cropping) and three replications on the territory of the Auxiliary Experimental Farm of the Agricultural Research Institute. 30% of the annual norm of nitrogen (N90) was given in pre-sowing cultivation, and the remaining 70% in the form of feeding in early spring. As an object of study of fodder peas "Azerbaijan-1508", wheat "Gobustan" and barley "Jalilabad-19" varieties were used.

In the field experiments, agrochemical analyzes of the soil, phenological observations on plants, structural elements, aboveground dry and green biomass yield, grain yield were determined based on general methodical instructions.

With a Ph-ı, pH-meter (in water solution), which is an indicator of soil acidity, neutrality, alkalinity; calcium carbonate (CaCO3) - with a calcimeter using the Scheibler method; by the common humus-Tyurin method; total nitrogen (N)-Keldal method; activated phosphorus (P2O5)-Machigin's method; exchangeable potassium (K2O5) was determined by the Machigin method in a flame photometer.

RESULTS AND DISCUSSION.

In order to regulate the balance of the main nutrients that are easily assimilated in the soil for the normal nutrition of plants during the growing season, importance should be paid to predecessor plants along with mineral fertilizers, and predecessors should be taken into account when applying fertilizer norms [1,5].

At the beginning of vegetation, agrochemical parameters were determined by taking soil samples from different parts of the experimental field in two-sided diagonal direction (0-20; 20-40 cm deep).

Table 1. Basic agrochemical indicators

Depth, cm	pH (in water)	Calcium carbonate (CaCO3), %	Total humus, %	Total nitrogen, %	Phosphorus (P2O5), %	Potassium (K2O) mg/kg
0-20	8.38	12.4	1.287	0.10	8.7	339
20-40	8.41	18.9	0.806	0.063	2.5	179

As can be seen from the table, the level of soil nutrient supply is very low. The amount of total humus and total nitrogen is higher in the 0-20 cm depth than in the lower layers. The pH value is 8.38 in the upper layer and 8.41 in the lower layer compared to the upper layer. The amount of calcium carbonate was determined as 12.4% at the depth of 0-20 cm and 18.9% at the depth of 0-40 cm. Predecessor plants significantly affect the productivity and quality indicators of plants to be planted in the future by changing soil fertility, nutrients and its moisture supply [4].

In the conducted study, the effect of the predecessors on the above-ground dry biomass productivity and the main economic indicators in the crop rotation variant was determined and compared with continuous cultivation. Depending on the cropping schemes and development phases, the above-ground dry biomass yield per hectare was different (table 2).

Table 2. Collection of above-ground dry biomass of winter soft wheat and barley by developmental stages, s/ha (2022)

Phases of development	Winter wheat "Gobustan" variety		Barley "Jalilabad-19"	
	Continuous planting	Crop rotation	Continuous planting	Crop rotation
End of spring cleaning	14.7	16.1	12.3	13.7
The end of the tube	38.7	40.	34.5	36.2
Full ripening	98.6	103.9	93.7	98.5

Note: For determination of biomass yield, plants of 2 rows 66.7 cm long (30 cm wide) from 5 locations of I-III repetitions of each variant were cut from the root throat ($66.7x30x5=1m^2$**).**

As can be seen from the table, at the end of the spring tillage of winter wheat, the amount of aboveground dry biomass yield was 14.7-16.1 s (increase of 1.4 s) by applying the same nitrogen fertilizer in both crops depending on the crops due to the influence of the predecessor

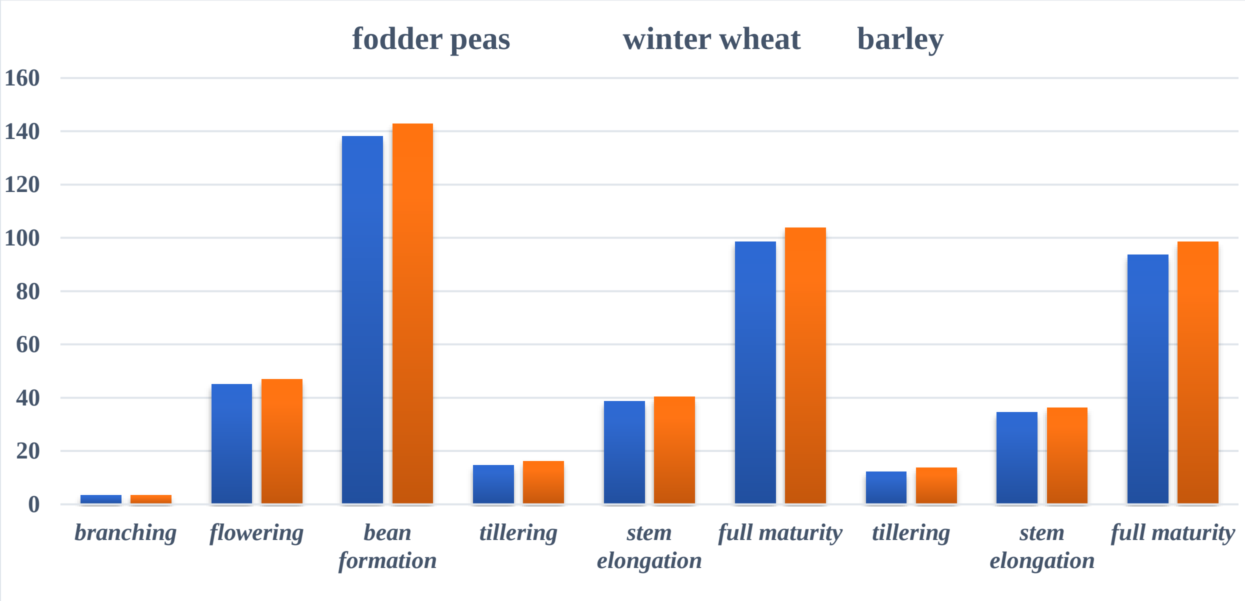

Figure 1. Above-ground dry biomass accumulation, s/ha, depending on cropping schemes

Compared to continuous planting at the end of the stem elongation, this increase was 1.6 s per hectare, and at the full maturity phase, the increase in total aboveground dry biomass was 5.3 s (figure). The increase in the collection of above-ground dry biomass of barley is 1.4 according to the hectare (by development phases); It is set at 1.7 and 4.8 s. Green and above-ground dry biomass of fodder pea in the phase of bean formation was 559.4 and 142.8 s, respectively, in the crop rotation option, and 537.6 and 138.1 s in the continuous crop option.

Depending on the cropping schemes, the change in above-ground dry biomass also caused a change in grain yield per hectare. In the second year of the study, a significant difference was obtained in the productivity of wheat and barley in the crop rotation option with the same nutrient (N90) ratio compared to continuous cropping. This means that it is possible to increase the grain yield per hectare in the irrigated gray-brown soils of Absheron by reducing the costs incurred per hectare, which creates difficulties for farmers in the cultivation of cereal crops (using salifs and siderates instead of high fertilizer rates).

The productivity and efficiency indicators of crops cultivated in the crop rotation option were higher than those cultivated in continuous cultivation. In this variant, the grain yield of winter wheat and barley was 44.6 and 37.7 s per hectare, and 42.5 and 35.4 s in continuous cultivation, respectively. According to the obtained yield, the net income per hectare for the "Gobustan" variety of winter wheat is 1115.5 manats, the level of economic profitability is 243.3%, and for the "Jalilabad-19" variety of barley, these indicators are 686.2 manats, respectively; 162.4% received. In the option of continuous cultivation, the net income from one hectare for winter wheat is 1041.6 manats, the level of profitability is 227.1%, and for barley it is 618.6 manats; 146.4% was calculated. In the case of continuous cropping, the cost of one centner of grain was higher compared to crop rotation. The highest net income for both plants was obtained in the crop rotation variant. The increase per hectare was 73.9 manats for winter wheat and 67.6 manats for barley compared to continuous cultivation. The cost of one centner of product is low, and the level of profitability has increased.

CONCLUSION

Any cultivation technology that is economically efficient is considered suitable for peasant-farmers with a small area and is widespread. Based on the results of the research, it can be said that the introduction of plants (cereal legumes) that differ in their agrobiological characteristics into the crop rotation has led to an increase in the above-ground dry biomass and grain yield of cereals, as well as economic indicators.

REFERENCES

Butteru B.R. Effects of variation in leaf area index on growth of maize and soybeans. Crop Science, 1970, 10, №1, p.9-13.

Donald C.M. Competition among crop and pasture plants. Adv.in Argon, 1963, 15, p.1-114.

Gay S. physiological aspects of yield improvement in soybeans. “Agr.J.”, 1980, 72, №2, p. 587-591.

T.H. Tamrazov, 2022. Drought effects on physiological parameters of durum and bread wheat. SABRAO Journal of Breeding and Genetics, http://doi.org/---http://sabraojournal.org/ pISSN 1029-7073; eISSN 2224-8978 http://doi.org/10.54910/sabrao2021

T. H. Tamrazov, Z. M. Abdullaeva.2022. The effect of plant diversification on the productivity of different plant samples under the same agrotechnical maintenance conditions . «Бюллетень науки и практики» https://doi.org/10.33619/2414-2948/71/0/

Tamrazov T.H., Abdullayeva Z.M., Bakhshaliyeva S.S. Effect of diversification in irrigation conditions on plant productivity. Institute of Soil Science and Agrochemistry, Khazar University, International scientific-practical conference, December 5-6, 2022, pp. 176-178.

Hajimammadov I.M., Talai C.M., Kosayev E.M. Methods of agrochemical analysis of soil plants and fertilizers. "Teacher", publishing house 2016, 130 p.

Беденко В.П. Фотосинтез и продуктивность пщеницы на Юговостоке Казахстана. Алма-Ата, 1980, с.9-12.

Балакай Г.Т. Пищевой режим почвы и вынос основных элементов питания различными сортами сои. Режим орошения, водопотребления и агротехника с.-х. культур на Сев. Кавказе, 1984, с.43-46.

Ничипорович А.А. и др. Фотосинтетическая деятельность растений в посевах. М., Изд-во АН СССР, 1961,133с.

Устенко Г.П. Фотосинтетическая деятельность растений в посевах как основа формирования высоких урожаев. В кн.: Фотосинтез и вопросы продуктивности растений. М., АН СССР, 1963,с.37-40.

Changes The Assimilation Surface Area and Photosynthetic Potential of Different Assimilating Organs in Wheat Cultivars Differing by Maturity due to the Effect of Drought

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ABSTRACT: The article mainly talks about the dynamics of changes in the assimilation surface area and photosynthetic potential of different assimilating organs in Absheron conditions of Azerbaijan in different high quality and productive wheat varieties as a result of drought. In the 2022-2023 research year, various morphophysiological studies were conducted on diferent samples of durum and bread wheat genotypes, grouped into 3 groups (early, medium, late ripening) by maturity, with an area of 10 m2 in the Absheron Experimental Base Station, of Research Institute of Crop Husbandry in Azerbaijan. The subject of the reserarch was determination of a drought effect on different physiological parameters and yield indexes of cultivated in different soil - climatic conditions and differ on maturing bread and durum wheat genotypes, study of photosynthesis and transpiration intensity, daily and ontogeny respiration intensity dynamics of different level leaves of the wheat varieties in various water supply conditions. According to the results of physiological measurements, the value of photosynthetic potential of leaves and other assimilating organs in different periods of vegetation showed that the photosynthetic potential of leaves and other assimilating organs is higher in Gyrmyzy bugda, and Giymatli-2/17 varieties compared to solid Garagylchyg-2 and Azametli 95 varieties. The maximum value of photosynthetic potential also corresponds to the maximum value of leaves in ontogeny. Drought, as a negative external factor, strongly affects various morpho-physiological indicators and reduces the assimilation activity of plants, which affects the final product. Thus, drought affects the assimilation surface area of plants as a negative factor, and as a result, noticeable changes occur in the dynamics of assimilation surface area and photosynthetic potential. This ultimately leads to a decrease in yield.

Keywords: Drought, Photosynthetic pigment, Assimilation surface area, Photosynthetic potential, Bread wheat, Durum wheat

INTRODUCTION

The restriction (limiting) of photosynthesis intensification at a drought and the methods of changing the various morphphysiological indexes among which it would be the extension of leaf blade, the accumulation of a drybiomass, SSD of leaves, photosynthetic potential and also photosynthetic pigment contents are the physiological bases of increasing the drought resistance of agricultural crops. The very complex soil and climate conditions of Azerbaijan require the creation of wheat and other plant genotypes resistant to biotic and abiotic factors. The existence of 9 out of 11 natural-climatic zones in the world in the territory of the Republic is of great theoretical and practical importance in the purposeful conduct of regional selection works in the selection and application of high-yielding wheat varieties suitable for local conditions. The most important of the measures to increase wheat production in regions with insufficient supply of moisture is the creation of new drought-resistant and high-quality varieties and their introduction into production. In recent years, various new varieties of wheat have been created at the Scientific Research Institute of Crop Husbandry, and because of ecological tests, new high-yielding and high-quality varieties resistant to adverse factors of the external environment (diseases, drought, etc.) One of the important conditions is the study of resistance to stresses at the molecular level. For this, the physiological, biochemical, and molecular-genetic characteristics of the plant's resistance to biotic and abiotic stresses should be clarified and the existing selection methods of selection should be improved to direct it in the desired direction.

It is known that as a result of the impact of stress factors (high and low temperature, drought, salt, UV-rays, etc.) on plant organisms, the metabolism inside the cell is disturbed, the natural conformation of protein molecules and nucleic acids changes, and the activity of enzymes decreases (and some increase) occurs. The light factor plays an important role in the formation and functioning of the photosynthetic apparatus in green plants by influencing the expression of species and chloroplast genes [1;11; 13].

In most scientific works dedicated to the research of the productivity process in plants, especially wheat, under drought conditions, the main attention is paid only to the study of changes in physiological and biochemical indicators because of the effect of drought, based on which the degree of drought resistance of plants is characterized.

In some studies, the effect of drought on productivity is evaluated only by the final result of the crop. Thus, it remains unclear which physiological process plays a leading role in fertility to one degree or another [2;5; 12]. Cultivation of grain crops in the country's ecological regions with complex soil-climate conditions, periodic spring-summer droughts lead to a fractional harvest. In this regard, one of the most important tasks facing agrarian science is the creation of drought-resistant, high-yielding and high-quality wheat varieties for regions with little moisture. The successful solution of this problem in breeding depends primarily on the detection of morphological characteristics of drought-resistant wheat varieties. It is known that the yield depends mainly on assimilation measures and its long-term functional state as a result of photosynthetic activity in the plant. From this point of view, monitoring the dynamics of these indicators in ontogeny in various assimilating organs of wheat varieties in drought conditions is of great practical importance. Drought resistance of plants is mostly based on water regime studies, and is associated with their ability and ability to adapt to drought and heat under natural conditions [1;13]. The feature of resistance to drought of plants is of practical importance in addition to scientific interest. So it is directly related to the final product.

MATERIALS AND METHODS

The water demand of cereal plants was different in different periods of ontogenesis. At this time, the level of product depression is related to the fact that various assimilative organs are in a functionally active state for a long time. Some authors have shown that the photosynthetic potential in drought-resistant plants remains at a higher level than in non-resistant plants [2; 8; 12;]. In the scientific literature, information about the effect of drought on the main integral indicators of photosynthesis is almost insufficient. Leaf surface specific density (LSD) and optimal dimensions of assimilation surfaces are the amount of photosynthetic pigments, photosynthetic potential (FP), economic efficiency and net productivity of photosynthesis (NPP), etc. is considered one of the factors that determine the productivity of wheat [3; 9].

As is known, drought primarily affects the development process, which leads to a decrease in photosynthetic potential and assimilation surface area in leaves [1; 2; 8; 13]. Therefore, reductions in the size of the leaf surface during the drought period are one of the main reasons for the change in productivity from year to year. Drought limits the development of the leaf surface during the growing season in the Southern and Eastern regions, and it rarely reaches the optimal size to absorb the photosynthetically active rays (PSR) falling on the crop here [3; 6; 7]. The productivity of plants is determined by the dimensions of the assimilation surface and the continuity of its surface. Therefore, it is important not only to achieve the optimal dimensions of the leaf surface faster, but also to keep it active for a long time [2;5; 11].

The leaf surface equal to 40-50 thousand m^2/ha is considered optimal. Field experiments conducted with wheat, including other crops, suggest that a higher yield can be obtained with optimal cultivation conditions and a leaf surface of 50-70 thousand m^2/ha [2; 3; 4; 11].

RESULTS AND DISCUSSION

An excessive increase in leaf cover surface does not lead to an increase in economic and biological productivity. The increase of the leaf cover beyond the optimal dimensions worsens the light regime inside the plant, and as a result, the possibility of assimilation of the lower tier leaves decreases sharply. To study the required parameters, different durum and bread wheat varieties were taken (Garagylchyg-2, Gyrmyzy bugda, Giymatli-2/17 and Azametli - 95). The results of measuring the area of the leaf surface and other organs are given in table 1.

Table 1. Dynamics of assimilation surface area (thous. m^2/ha)

The name of the variety organs	organs	March			April			May			June
			II	III	I	II	III	I	II	III	I
Irrigated											
Garagylchyg-2	Leaf		36,2	41,3	55,2	60,4	62,8	58,5	54,2	40,6	38,2
	Stems		18,8	31,2	34,4	48,1	53,2	58,6	70,1	63,5	55,8
	Spike							20,2	28,7	25,4	25,0
Gyrmyzy bugda	Leaf		32,7	45,2	50,0	56,4	60,2	72,4	68,9	43,8	36,1
	Stems		6,5	16,4	20,4	35,8	71,4	74,5	76,8	75,2	60,5
	Spike								17,5	25,6	22,3
Giymatli-2/17	Leaf		36,2	40,2	43,1	54,3	57,2	60,3	56,3	40,2	36,4
	Stems		10,5	16,3	24,5	53,5	62,8	65,8	67,4	60,4	58,2
	Spike							20,2	21,4	22,5	19,6
Azametli - 95	Leaf		32,0	34,3	37,5	46,8	50,8	55,4	48,2	34,6	28,2
	Stems		10,6	16,8	21,3	45,3	50,6	53,5	61,5	54,6	50,3
	Spike							15,2	17,4	18,2	15,3
Drought											
Garagylchyg-2	Leaf		35,6	39,4	45,4	48,5	45,8	41,5	32,8	28,5	18,5
	Stems		16,4	25,2	32,7	41,8	50,2	54,5	55,0	52,0	41,8
	Spike							18,2	19,5	18,3	17,2
Gyrmyzy bugda,	Leaf		30,3	37,4	45,8	48,3	56,4	55,8	43,5	30,8	22,5
	Stems		3,2	13,5	19,4	22,8	55,3	58,2	60,5	57,5	50,4

	Spike								15,5	19,4	17,9
Giymatli-2/17	Leaf		30,1	34,5	36,3	41,7	45,8	46,3	40,5	30,3	25,8
	Stems		10,3	12,6	20,3	38,3	50,3	52,8	55,4	53,8	42,4
	Spike							15,2	20,0	21,2	19,2
Azametli - 95	Leaf		24,2	32,3	35,4	40,6	44,5	50,2	40,8	28,3	24,2
	Stems		10,1	12,6	18,3	34,6	42,4	46,7	52,8	44,5	40,6
	Spike							13,2	15,4	14,8	13,2

As can be seen from the table, at the beginning of the ontogenesis, the leaf area of the leaves of Garagylchyg-2 and Gyrmyzy bugda varieties in the control and experimental variants increases suddenly, reaching a maximum at the end of the tube emergence phase in the second decade of April, 62 and 46 thousand m^2/ha in the Garagylchyg-2 variety, and 60 and 56 thousand m^2 in the Gyrmyzy bugda variety thousand m^2/ha, and the difference between the options is 26% and 6%. Starting from the spiking phase, the shortening of the leaf surface is observed, and at the end of the first decade of June, in the control and experimental options, it decreases to 38 and 18 thousand m^2/ha in the Garagylchyg-2 variety, and to 36 and 92 thousand m^2/ha in the Gyrmyzy bugda variety, the difference between the options and it is 53% and 38%.

Bread wheat varieties Giymatli-2/17 and Azametli-95 have 54 and 41 thousand m^2\ha, 47 and 41 thousand m^2/ha at the beginning of ontogenesis, and the difference between the variants is 24% and 12%. At the end of ontogeny, in the first decade of June, there were 36 and 25 thousand m^2 /ha in the Giymatli 2/17 variety, and 28 and 24 thousand m^2 /ha in the Azametli-95 variety. The difference between the options was 30% and 14%. Correspondingly, the leaf surface area is reduced by more than half at the end of ontogeny due to drought effects and leaf senescence.

The increase of the stem surface in the control and experimental options continues until the grain formation phase and reaches the maximum, 70 and 55 thousand m^2/ha in the Garagylchyg-2 variety, 79 and 60 thousand m^2 /ha in the Gyrmyzy bugda variety, the difference between the options is 21% and 24%, and at the end of ontogenesis, it is 24% in Karagilchik-2 variety and 17% in Gyrmyzy bugda variety. In the Giymatli-2/17 and Azametli -95 varieties, the dynamics of the stem area shows that its growth rate is higher than that of the leaves in the tube emergence phase. Apparently, this is due to the fact that in the tuber phase, basically all the leaves complete their development, while the stems continue to develop. The maximum area of stems, as in other varieties, is observed in the phase of grain formation. At the end of ontogeny, the difference between variants in the parameters of stem area is 32% in Giymatli 2/17 variety and 20% in Azametli 95 variety. Spike surface area in all mentioned cultivars increases faster in the control variant than in the experimental variant and reaches a maximum in May, from the complete formation of the spike then reaches a maximum. 28 and 19 thousand m^2 /ha in Karagilchik-2 variety, 25 and 19 thousand m^2/ha in Gyrmyzy bugda, variety, where the difference between variants was 32% and 24%.

Bread wheat varieties Giymatli 2/17 and Azametli - 95and 22; 21; and 18; It is 16 thousand m2/ha. Here, the difference between the options was 5% and 11%. Comparison of these data allows us to note that as a result of drought, the leaf surface area decreases more than the stem and spike surface area.

Photosynthetic potential (FP) is used as an index that combines both leaf surface area and leaf (LSD) to estimate the optimal dimensions of leaf surface area and different (LSD) of leaves. Table 2 shows the values of the photosynthetic potential of leaves and other assimilating organs in different periods of vegetation. Mainly in 2 periods: pre-flowering and post-flowering period.

Table 2. Photosynthetic potential of different wheat cultivars under cultivation

Variety After Flowering F P mln.m^2\ha Leaves	Variant	Before Flowering			After flowering		
		Number of days	FP mln.m^2\ha		Number of days	FP mln.m^2\ha	
			Leaves	Additional assimilating organs		Leaves	Additional assimilating organs
Garagylchyg-2	1	189	2,67	2,20	36	1,45	2,52
	2	184	2,13	2,01	31	0,84	2,07
Gyrmyzy bugda	1	199	3,37	2,67	31	0,94	1,86
	2	196	2,76	2,22	27	0,62	1,42
Giymatli-2/17	1	197	3,28	3,12	31	0,91	1,78
	2	195	2,50	2,46	26	0,58	1,36
Azametli - 95	1	198	1,85	1,68	31	1,85	1,76
	2	197	1,53	1,26	25	0,44	1,33

Note: 1-control variant, 2-experimental variant.

As can be seen from the table, the FP of the leaves in the pre-flowering period is 2.67 in the control version of Karagilchik-2 and Gyrmyzy bugda varieties; 3.37 thousand m^2/ha day, 2.32 in the experimental version; It is 2.76 thousand m^2/ha day. 2.20 in the control option in accordance with other assimilatory organs; 2.67 thousand m^2/ha day, 2.01 in the experimental version; It is 2.22 thousand m^2/ha day. Bread wheat varieties Giymatli-2/17 and Azametli – 95 and 3.28 in the control options; 1.85 thousand m^2/ha day, 2.50 in the experimental version; It is 1.53 thousand m^2/ha day. 3.12 in the control option in accordance with other assimilatory organs; 1.68 thousand m^2/ha day, and 2.46 in the experimental version; It is 1.26 thousand m^2/ha day. In the post-flowering period, FP in durum wheat varieties is 1.45 in the control variant; 0.94, 0.84 in the experimental version; 0.62 thousand m^2\ha day, correspondingly 2.52 in other assimilating bodies; 1.86 and 2.07; It is 1.42 thousand m^2\ha day. Indeed, the FP of the leaves and the growth dynamics of the leaf area according to the phases show that the maximum values of the indicators are observed in the same phases in Karagilchik-2 and Gyrmyzy bugda varieties.

The value of FP of leaves and other assimilating organs in different periods of vegetation shows that the FP of leaves and other assimilating organs is higher in Gyrmyzy bugda and Giymatli-2/17 varieties compared to Karagilchik - 2 and Azametli 95 varieties.

CONCLUSION

Apparently, these figures are due to the excessive increase in leaf area in tall and broad-leaved varieties. The maximum value of photosynthetic potential also corresponds to the maximum value of leaves in ontogeny. Drought, as a negative external factor, strongly affects various morpho-physiological indicators and reduces the assimilation activity of plants, which affects the final product. Thus, drought affects the assimilation surface area of plants as a negative factor, and as a result, noticeable changes occur in the dynamics of assimilation surface area and photosynthetic potential. This ultimately leads to a decrease in yield.

REFERENCES

Abdoli M, Saeidi M (2013). Evaluation of water deficiency at the post anthesis and source limitation during grain filling on grain yield, yield formation, some morphological and phonological traits and gas exchange of bread wheat cultivar. *Albanian J. Agric. Sci.* 12: 255-265.

Ahmadizadeh M, Nori A, Shahbaziand H, Aharizad S (2011). Correlated response of morpho-physiological traits of grain yield in durum wheat under normal irrigation and drought stress conditions in greenhouse. *Afr. J. Biotechnol.* 10(85): 19771-19779.

Aliyev JA (2000). Physiological leaves of what breeding tolerant to water stress wheat in global environment. Proceedings of the 6th International Wheat Conference, Budapest, 5-9 June 2000. pp. 693-698.

Almeselmani M, Abdullah F, Hareri F, Naaesan M, Ammar MA, Kanbar OZ, Saud ABD (2011). Effect of drought on different physiological characters and yield components in different Syrian durum wheat varieties. *J. Agric. Sci*. 3: 127-133.

Blum A, Jordan WR (1985). Breeding crop varieties for stress environments. *Crit. Rev. in Plant Sci*. 2: 199-238.

Fischer RA, Maurer R (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. *Aust. J. Agric. Res*. 29: 897-912.

Gholamin R, Khayatnezhad M (2010). Study of some physiological responses of drought stress in hexaploid and tetraploid wheat genotypes in Iran. *J. Sci. Res*. 6: 246-250.

Gürel A, Avcıoğlu R (2001). Bitkilerde Abiyotik Stres Faktörlerine Dayanıklılık Mekanizmaları. pp.288-326. In: S Özcan , E Gürel & M Babaoğlu (eds.), Bitki Biyoteknolojisi, Genetik Mühendisliği, S.Ü. Vakfı Yayınları, Izmir, Turkey.

Kuzmin MS (1986). Formation of assimilation surface and productivity of photosynthesis in soybean plants. biology, genetics and breeding of soybeans, Siberia, pp.125-134.

Moral GLF, Rharrabti Y, Villegas D, Royo C (2002). Evaluation of grain yield and its components in durum wheat under Mediterranean conditions: An ontogenic approach. *Agron. J*. 95: 266-274.

Tamrazov TH (2016). The research on drought influences the development dynamics of wheat plant and the change of morpho-physiological indicators. International Conference on New Approaches in Biotechnology and Biosciences "NABB-2016"- Feb (18-20.2016), pp. 11.

Tamrazov TH, Javanshir M, Atif T, Zamanov A (2016). Formation of assimilating surface areas and photosynthetic potential of various assimilating parts of wheat species under drought conditions. *Am. J. Plant Sci.* 7(06): 824-827.

Tamrazov TH, Khudayev FA (2020). Morpho-physiological parameters of late maturing wheat genotypes with various yield and dry resistance. *Agrar. Sci*. 4: 56-60.

Technological Methods in The Cultivation of Field Plants

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ABSTRACT: According to the article, the volume of production in agriculture is increased by improving the cultivation technology of plants, switching to the cultivation of more productive varieties, and developing and implementing more effective measures to combat the unfavorable living conditions of plants. One of the most important issues in crop production is the management of yield and crop quality. Also, the manufactured product should be as cheap as possible. To achieve this, more productive varieties and hybrids should be created and cultivated. It is also possible to increase the yield and improve the quality of the crop by cultivating after favorable predecessors in crop rotation. In order to increase the quantity and quality of the product, the most favorable options of soil cultivation, fertilization, irrigation, sowing and planting care should be used. It is known that it is impossible to get products from plants when life factors are in minimum and maximum amount. The greatest yield is obtained when the life factor is in optimal amounts. According to this law (optimum law), the potential yield of the plant can be realized only when each nutrient element is provided in an optimal amount. In the event that soil types differ significantly from each other in terms of their chemical composition (in terms of pH, the amount of individual mineral nutrients, the amount of nutrients that can be assimilated, etc.), then the formed plant with different pH and individual elements needs to be provided. Each plant or group of plants has its own optimum pH level to realize its potential yield.

Keywords: Cultwation technology, Technological methods, Fertilization, Environmental factors, Reutilization

INTRODUCTION

Land resources are considered as one of the main elements of its existence and potential for the development of all countries. It is for this reason that throughout history, there has been a struggle to own land both between individuals and administrative regions within the country, as well as between states [1,7]. During drought years, the reduction of surface water resources, as a result of the lack of regulation of the water flow of internal rivers, flood waters flow into the sea without full use, making it difficult to provide irrigation water to agricultural fields. From this point of view, the role of efficient use of available land and water resources in increasing the production of agricultural products is very important [4,10]. Protection of soil fertility and its effective use is of great importance for the development of the agricultural sector. Maintaining and increasing the fertility of the land creates great opportunities for the future generation, in addition to ensuring the living conditions and health of the population. In order to effectively use the land, its water, food, air and heat regimes should be properly regulated, depending on the phenological and biological development characteristics of agricultural plants, an optimal agrophone should be created. However, if the soil is not used properly, its beneficial properties gradually disappear and become unsuitable for growing crops [1,4, 8]. Any improper operation on the land leads not only to a decrease in productivity and quality indicators, but also to a violation of the ecological balance, long-term loss of fertility, etc. leads to deficiencies [2,9].

The productivity of field plants depends on the nature of plant growth and development. This is related to the biological properties of the plant. Therefore, during cultivation, it is necessary to implement technological measures according to the characteristics of the plant.

MATERIALS AND METHODS

Management of plant growth and productivity, research methods and methods of plant breeding

In order to control the development of plants, it is necessary to know their biological requirements. As we know, the development of plants depends mostly on the thermal regime. Each plant requires a certain active temperature limit during its growing season. Based on meteorological data, it is possible to predict the period of transition of any plant to development phases in any region. It is also possible to determine whether plants or varieties can grow in the intended region according to the active temperature conclusion requirement. This is an important economic issue [3,6,11].

The moisture requirement of plants is determined by their transpiration coefficient. The transpiration coefficient of winter wheat is between 400-500, that of corn is between 230-370, and that of sugar beet is between 240-500. In order to meet the moisture requirement of the plant, it is necessary to know the moisture requirement in different periods of its development. The period when the plant shows more demand for moisture is called the crisis period. If the plant's demand for moisture is not met during this period, its productivity decreases sharply [5,7,9]. One of the most important issues in increasing productivity is increasing the solar energy utilization ratio of plants. Currently, plants use 1.0-1.5% of solar radiation. It is planned to increase it to 5-6%. One of the most important issues in crop production is the management of yield and crop quality. Also, the manufactured product should be as cheap as possible. To achieve this, more productive varieties and hybrids should be created and cultivated. İt is also possible to increase the yield and improve the quality of the crop by cultivating after favorable predecessors in crop rotation. In order to increase the quantity and quality of the product, the most favorable options of soil cultivation, fertilization, irrigation, sowing and planting care should be used [3,8].

In order to deeply analyze the results obtained in the field and vegetation experiments, the chemical composition of the product, soil moisture, and the amount of nutrients in the soil are analyzed in the laboratory [4,7,10]. Thus, the type of plant (its genotype) reflects the ecological conditions of the region in which it was formed. In the process of evolution, cultured natural selection has adapted to the biological needs of the species, the parameters of the main environmental factors of the region where it was formed. The more harsh the species has been, the less demanding it is on growing conditions. The further a species is cultivated from its zone of origin, the more resources are spent on the production of a single product of this species, the more time it falls on man to correct the timing of the main environmental factors with agrotechnical measures. As an alternative to this situation, a variety can be created, which completely changes its biological requirements compared to the initial form and can meet the parameters of the main environmental factors of a particular zone. Thus, in order to find out what kind of demand the plant has for cultivation conditions, it is necessary to know the ecological conditions of the zone where the species is formed [5,10].

It is known that it is impossible to get products from plants when life factors are in minimum and maximum amount. The greatest yield is obtained when the life factor is in optimal amounts. According to this law (optimum law), the potential yield of the plant can be realized only when each nutrient element is provided in an optimal amount. In the event that soil types differ significantly from each other in terms of their chemical composition (in terms of pH, the amount of individual mineral nutrients, the amount of nutrients that can be assimilated, etc.), then the formed plant with different pH and individual elements needs to be provided. Each plant or group of plants has its own optimum pH level to realize its potential yield. During the development of the fertilization system in crop rotation, liming of acidic soils increases the efficiency of mineral fertilizers. During liming, the soil is enriched with calcium and magnesium, the degree of saturation of ASC (absorbing soil complex) with bases increases, the mobility and easy assimilation of some nutrient elements for plants (phosphorus, molybdenum, nitrogen) increases, the biological activity of the soil is strengthened and its physical and chemical properties are improved, the coefficient of use of nutrients from mineral fertilizers increases.

Nutrient requirements of plants

It is known that some nutrients are used by the plant in a large amount of 100-300 kg per hectare (macroelements), and others in a small amount, a few grams per hectare (microelements). Micronutrients are absolutely necessary for normal physiological processes. Growth, plant development, yield and its quality are often limited by the lack of macronutrients such as phosphorus and potassium.

RESULTS AND DISCUSSION.

Uptake of nutrients by plants and maximum demand

In the development of the fertilization system for individual plants, the uptake of mineral nutrients by plants and the indicators of maximum demand are used. Maximum use - the maximum amount of nutrients involved in the creation of a single commodity product. Harvesting is the self-absorption of nutrients from the field with 1 ton of main and by-products (stem, leaves). It is always less than the maximum demand (table 9). The difference between maximum demand and output equals the amount of nutrients that the plant leaves behind in the field with root and shoot residues and plant waste. The maximum demand and indicators produced by plants depend on the biological characteristics of the plant. So, on average, the plant spends 23 kg of potassium oxide for the formation of 1 ton of wheat grain and the corresponding amount of other organic mass, 50, and 186 kg of potassium oxide for the formation of 1 ton of yellow lupin seeds. There is no significant difference between plants in using nitrogen and phosphorus per unit yield. Indicators are determined during the harvest period, but the maximum demand is determined during the phase of maximum accumulation of nutrients and dry matter.

The maximum consumption of all nutrients and the accumulation of organic matter in cereal crops occurs at the beginning of the wax ripening phase, but in the case of legumes, at the full grain filling phase, when the beans in the lower layer begin to turn yellow, the upper ones become fuller, but the leaves are no longer shed. This is a better time to harvest legumes for green mass. Then the leaves begin to fall (first the lower ones, then the upper ones), the destruction of small rhizomes and the shedding of immature generative organs. This process continues until the seed is fully ripe, as a result, the plant loses some of its nutrients. On average, cereal plants carry 29 kg of nitrogen, 10 kg of phosphorus and 19 kg of potassium (total 58 kg) with 1 ton of grain and a corresponding amount of other organic mass. On average, 58 kg of nitrogen, 19 kg of phosphorus and 33 kg of potassium are carried by cereal-legume plants with

1 ton of seeds. As a result, the increase in the share of cereal-leguminous plants and leguminous grasses in the structure of the arable land not only leads to an increase in the production of plant protein, but also allows more efficient use of the natural fertility of the soil and mineral fertilizers, and increases the yield of agricultural products at the expense of a single mineral fertilizer.

Fertilization of cereals and legumes. The following factors must be taken into account when calculating the mineral fertilizer rate under cereals and legumes.

1. In well-drained soils, sufficiently supplied with active phosphorus and exchangeable potassium (80-120 mg/kg in the soil), appropriate to the plant's biological needs (pH-salt 6.0-6.5 for most legumes), environmental response and the plant forms an active symbiotic apparatus during adequate supply of moisture. Due to the natural fertility of the soil and nitrogen from the air, peas, common beans, forage beans and broadleaf lupine can form a seed yield of 1 hectare to 2 tons.

2. During the optimization of phosphorus-potassium nutrition, the activity of symbiosis in these conditions increases and the productivity of the listed plants increases to 3 tons per hectare.

3. When there is a lack of moisture, the application of phosphorus-potassium fertilizer does not increase the productivity of grain-leguminous plants, because in this case, determination of air nitrogen does not occur.

4. In acid soils, even with normal moisture supply, most legumes do not produce eggs, and phosphorus-potassium fertilizers do not increase seed yield. Its level is limited by nitrogen deficiency. There are other methods of determining mineral fertilizer rates under individual plants, but all calculation methods are based on the amount of elements that can be used from the soil and the nutrient elements carried by the crop.

Fertilizer application period and methods. During the development of the fertilization system, it is necessary not only to choose a reasonable rate, but also the duration, as well as the methods of giving organic and mineral fertilizers under individual crops in rotation. Depending on the application time, the main (autumn plowing), additional, during sowing (row, band) and after sowing (feeding) fertilizers differ. The main fertilizer is pre-determined to provide the plants with nutrients only during the growing season, especially during intensive tillering and nutrient consumption under deep autumn plowing. Organic fertilizers, as well as phosphorus and potassium fertilizers are applied. The main fertilizer is applied to the deep moisture layer of the soil with the help of a front cot, so that it can be efficiently used by the plant during the growing season.

Studies conducted by the method of marked atoms show that the phosphorus contained in the superphosphate given at the bottom of the furrow is fully used by the plant 5 times, the amount of this element in them is 1.5 times higher than in shallow burial. This is explained by the fact that the mobility of the phosphorus compound is quite low. Soluble phosphates are adsorbed by UTK during interaction with the soil, undergo chemical precipitation, and even in rainy years, they move no more than 1.0-1.5 cm during the summer in medium-grained soils. Therefore, the phosphorus fertilizers applied to cultivation at a depth of 7-10 cm remain in this layer throughout the vegetation. When solid (solid) phosphorus fertilizer is given on the surface, even in wet years, 85-95% of phosphorus remains in a layer (layer) with a depth of up to 2 cm. Due to the rapid drying of this layer, the plant's roots do not spread there. Phosphorus fertilizer applied on the surface is practically not used by the plant. Liquid phosphorus fertilizers - orthophosphoric acid and ammonium phosphate have a very strong reaction. When they are supplied with irrigation water, up to 30% works to a depth of up to 10 cm, but at this time up to 55% of it is collected in the top layer of 1-2 cm. Compared to phosphorus, potassium is slightly more mobile in the soil. When in contact with the soil, most of it is attached to the absorbent soil complex (ASC), but a part of the free ions moves with capillaries and water flow and changes its location. When the soil moisture is low and at high temperature, the mobility of potassium decreases suddenly (sharply). During the summer, potassium can move 4-6 cm if the surface is given to the soil with normal moisture, but it remains at the top in dry weather. Mineral potash fertilizers should be applied under autumn plowing as the main fertilizer, in which case the plant will use them throughout the growing season.

They use crops of perennial herbs such as alfalfa, sedgeless bonfire grass, wormwood for 3-4 years or more. In order for these plants to use phosphorus fertilizers throughout the period, they put it under autumn plowing. In soils moderately supplied with potassium, if the rates of potassium fertilizers are not high, all of the potassium together with phosphorus is applied under the main plow. If the soil is poor in potassium, but a high yield is planned, then 50-70% of the norm is given as the main fertilizer, and the rest as feeding.

It has been determined by researches that when phosphorus is given to the rows under cereal plants, the demand of sprouts for it increases sharply, but complete mineral fertilizer increases the amount of NPK in sprouts several times. Accordingly, the height of the plant and the accumulation of dry matter in them increases. Reutilization of dry matter and mineral nutrients of the grain do not depend on the composition and availability of mineral nutrients in the environment. Two weeks after sowing (2-3 leaf phase), the nutrition of cereal plants is completely determined by the mineral nutrient elements of the environment. The dry weight of sprouts is 2 times more when full fertilizer is applied between the rows, but 2.5 times more on the 19th day compared to the rows without fertilizer. Similar differences were recorded in the utilization of nitrogen, phosphorus and potassium by sprouts. Cultivation technology of field plants is a complex agrotechnical measures aimed at satisfying the biological needs of the plant and carrying out planned high yield in a certain sequence. In order to develop the cultivation technology of plants on scientific basis, it is necessary to know varieties in specific soil-climate conditions, varieties and parameters of soil-climate conditions, biological requirements of plants.

Some agrotechnical methods - basic and pre-sowing soil preparation, application of fertilizers, preparation of seeds for sowing, sowing, crop care, harvest - are performed when any field crop is cultivated. The sum of these methods forms the "body" of field crop cultivation technology. Agrotechnical methods reflect "first-order branches" specific to the agrotechnics of a separate group of plants, having similar biological properties (spring sowing of autumn crops), from the same season (pre-sowing infection of legumes) or similar uses (soaking of flax fiber and hemp stubble), additional agrotechnical methods, during the cultivation of a specific plant and the characteristics of its agrotechnics, which constitute "second-order branches". All technological methods are aimed at providing favorable conditions for the growth and development of cultivated plants and their biological needs. It refers to the issues solved by technological methods: optimization of the water-air regime of the soil through cultivations for the normal functioning of the root system; optimization of the nutritional regime of cultivated plants by applying organic and mineral fertilizers; optimization of the reaction of the soil solution by liming or plastering the soil; reducing competition between cultivated plants and weeds as a measure to combat crop littering; bring planting and sowing materials to the highest sowing standard indicators; leveling for sowing seeds, compacting the top layer so that the seeds are located at the same depth and at the same distance from each other in the row; protection of plants from pathogens and pests; growth regulation, plant development and crop quality; reduction of quantity and quality loss during collection.

Methods	
Liming *Facing the curve* *Providing organic fertilizers* *Autumn plowing* *Re-ploughing (re-ploughing* *Spring plow* *Fall plowing in spring* *Nitrogen fertilization of fallow and cereal grasses in spring* *Mulching of deciduous and perennial grasses in spring* *Cultivation of the soil with a combined RVK-type unit* *Cultivation before sowing* *Soil compaction before sowing* *Preparing the seed for sowing* *Sprinkle* *Plowing the soil after sowing* *Trowel up to the exits* *Mulching after sprouts are obtained* *Cultivation between rows* *Root feeding* *Deep filling* *Foliar feeding* *Treatment of crops with herbicides* *Application of biologically active substances* *Harvesting*	*Lowering of soil acidity to the level corresponding to the plant's biological needs in crop rotation* *Mixing plant residues into the soil; destruction of capillaries in the upper layer - preventing moisture loss; creating conditions for the germination of weed seeds* Improvement of soil water-physical properties and plant nutrition Optimizing the regime of mineral nutrition of cultivated plants *Mixing of corner residues, organic and phosphorus-potassium fertilizers into the soil; improving the water-air regime of the soil, revitalizing its microbiological activity* *Improvement of soil water-physical properties and plant nutrition* *Optimizing the regime of mineral nutrition of cultivated plants* *Mixing of corner residues, organic and phosphorus-potassium fertilizers into the soil; improving the water-air regime of the soil, revitalizing its microbiological activity* *Softening of the planting layer of the soil with particles coming with irrigation water; conversion of organic and phosphorus-potassium fertilizers given in the spring into the soil* *Mixing plant residues, organic and mineral fertilizers into the soil, softening the soil layer (if fall plowing is not possible)* *Breakage of capillaries in topsoil – prevention of moisture loss for late spring sowing crops Providing the soil with weakened nitrogenous compounds at the beginning of plant growth Illumination of the height point during the transition of deciduous and perennial grasses in autumn* *Softening, leveling and leveling of the soil before seeding.* *Softening of the top layer of the soil, weed control* *Compaction of the upper layer of light soils, restoration of capillary connections* *Separation of seeds into fractions according to their size; bringing them to high planting standards; neutralization of pathogenic*

	microflora; of germination energy and amplification of outputs *Placement of seeds at the same depth (sowing and planting material), at an equal distance from each other* *Determination of the relationship between soil capillaries and small seeds* *Destruction of the root strands of germinating weed seeds, destruction of soil crust Destruction of weed sprouts Destruction of weeds between rows, softening of rows, fertilizing plants with mineral fertilizers* *To improve the mineral nutrition of the plant in accordance with the biological needs of the plant in different periods of ontogenesis* *Increasing the volume of soil inhabited by tubers and roots and improving aeration, fighting weeds* *Compensation of the missing nutrients of the plant from the root; improvement of product quality* *Prevent weeds when they appear, destroy mature weeds (with herbicide); reduce or prevent harmful disease (fungicides, bactericides); reduce plant damage by harmful insects (insecticides)* *Regulation of plant height and development; prevention of sleep (retardants); flow of plastic substances to reserve organs (senicants); termination of vegetation, drying of plants (desiccants); release of leaves from plants, preparation for collection (defoliants)* *Harvesting from the field with minimum loss of quantity and quality of the product*

The current situation requires scientific and research institutions operating in the field of agriculture to work more closely with farmers and on the basis of mutually beneficial contracts. Our farmers study the physiology, morphology, origin of the soil and plants, resistance to adverse factors of nature, etc. they should understand the essence of such problems and take practical measures in the field of environmental protection, as they say, they should sound the alarm. It is necessary to remember that the soil is also a living thing, the attitude towards it should be like the attitude towards other living things. Land improvement should be done today, delaying it until tomorrow or the day after will aggravate the situation and multiply the capital investment. Figuratively speaking, every land user today must learn to speak the "language" of the soil and plants and know how to deal with them. The saying "There is no such thing as bad soil, there is a bad farmer" should always be remembered and the cultivation of land should be approached from the point of view of strictly following these principles. Restoring and increasing the fertility of our land in order to improve its health, protect it, and use it efficiently, as well as produce an ecologically clean and high-quality, abundant product is a sacred and urgent task for all of us.

REFERENCES

Donald C.M. Competition among crop and pasture plants. Adv.in Argon, 1963, 15, p.1-114.

Gay S. physiological aspects of yield improvement in soybeans. “Agr.J.”, 1980, 72, №2, p. 587-591.

T.H. Tamrazov, 2022. Drought effects on physiological parameters of durum and bread wheat. SABRAO Journal of Breeding and Genetics, http://doi.org/---http://sabraojournal.org/ pISSN 1029-7073; eISSN 2224-8978 http://doi.org/10.54910/sabrao2021.

T. H. Tamrazov, Z. M. Abdullaeva.2022. The effect of plant diversification on the productivity of different plant samples under the same agrotechnical maintenance conditions . «Бюллетень науки и практики» https://doi.org/10.33619/2414-2948/71/0/

Tamrazov T.H., Abdullayeva Z.M., Bakhshaliyeva S.S. Effect of diversification in irrigation conditions on plant productivity. Institute of Soil Science and Agrochemistry, Khazar University, International scientific-practical conference, December 5-6, 2022, pp. 176-178.

Hajimammadov I.M., Talai C.M., Kosayev E.M. Methods of agrochemical analysis of soil plants and fertilizers. "Teacher", publishing house 2016, 130 p.

Беденко В.П. Фотосинтез и продуктивность пщеницы на Юговостоке Казахстана. Алма-Ата, 1980, с.9-12.

Балакай Г.Т. Пищевой режим почвы и вынос основных элементов питания различными сортами сои. Режим орошения, водопотребления и агротехника с.-х. культур на Сев. Кавказе, 1984, с.43-46.

Ничипорович А.А. и др. Фотосинтетическая деятельность растений в посевах. М., Изд-во АН СССР, 1961,133с.

Устенко Г.П. Фотосинтетическая деятельность растений в посевах как основа формирования высоких урожаев. В кн.: Фотосинтез и вопросы продуктивности растений. М., АН СССР, 1963,с.37-40.

Коренева Г. В. и др. Интенсивные технологии возделывания сельскохозяйственных культур. Москва, 1988, 300 с.

Косиниский В. С. и др. Основы земледелия и растениеводство. Москва, «Колос», 1980, 333 с.Можаев Н. И. Аринов К. К. и др. Растениеводство. Астана, 2002, 250 с.

Determination of University Students' Intercultural Communication Competence and Their Attitudes Towards New Foods: The Case of Kastamonu Province

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ABSTRACT: Nutritional plans to be created by determining the tolerance to cultural differences and food neophobia of students who go to a different city lead to healthier nutrition. The study is cross-sectional and conducted on volunteer students. The questionnaire form included sociodemographic characteristics, nutritional habits, anthropometric measurements, cultural effectiveness, and food neophobia levels of the students. 32.8% of the students allocate more than 50% of their general budget for nutrition, 76% smoke, 49.6% skip lunch, 64% have irregular meals, 40.8% have dinner as the most important meal, 48.8% have a good appetite and 43.2% eat their meals with their friends. There is a significant difference between the food neophobia scale scores according to the age. The group aged 24 years and over have significantly higher scores than the other two groups. There is a significant difference between the food neophobia scale scores according to appetite. The group with good appetite has a significant higher score than the group with moderate and poor appetite. A weak negative relationship is found between food neophobia and cultural effectiveness.

Keywords: Intercultural communication, New foods, Food neophobia, Nutrition habits, Intercultural effectiveness

INTRODUCTION

Today, nutrition is an important factor in the development or prevention of many chronic diseases including obesity, diabetes, cardiovascular diseases and cancer (Kavas and Kavas, 1985). Examples such as the recommendation of potassium-rich foods to lower blood pressure, whole grain foods for diabetic patients, and foods such as vegetables and fruits for cancer patients reveal the effect of nutrition on diseases (Kavas and Kavas, 1985; Koyu et al., 2016). Therefore, healthy nutrition is very important for protecting health and minimising the risk of developing diseases (Yücecan, 2008). Eating habits vary according to age groups. When the eating habits of young people were examined, it was observed that irregular meals, skipping meals, low consumption of dairy products, fruits and vegetables and fast-food style nutrition were common (Garibağaoğlu and Özgüne, 2008; Korkmaz, 2010). Most of the students have to leave their homes with their families during their university education and live in dormitories or student houses. This situation negatively affects the adequate and balanced nutrition levels of young people because they are in different environments (Ermiş et al., 2015). It has been found that most of the university students who do not live with their families do not consume sufficient amounts of vegetables, fruits and meat (El Ansari et al., 2012). Mobility in education has also been observed with increasing opportunities. The interest of students to receive education in places other than the cities where they were born and raised has increased (Güçlü, 1996). The diet and diversity of students who cannot adapt to the nutritional culture of the cities they go to are gradually deteriorating and becoming unhealthy. Apart from adaptation to school and environment, nutritional problems increase with economic inadequacies and interest in fast-food style nutrition (Ul Haq et al., 2018). This situation affects the health of young people negatively. This study aims to determine the favourable or unfavourable effects of cultural influence and food neophobia on the nutritional habits of students. It is thought that nutritional facilities to be prepared in the light of this information will positively affect the nutritional habits of students, contribute to the prevention of possible health problems and reduce the economic burden on public health.

MATERIAL AND METHOD

The study is a non-experimental, descriptive and cross-sectional research model in which the survey method is used. All individuals who voluntarily participated in the study and responded completely to the questions and scales that were validated and reliable in Turkish constituted the sample of the study.

Inclusion Criteria:

1- Being an university student

2- Volunteering to participate in the research and

3- Not having psychological or psychiatric illness.

At the beginning of the study, the sampled individuals were asked questions about their socio-demographic characteristics (age, gender, health status, income status, etc.) and dietary habits. Afterwards, anthropometric measurements (body weight and height) were taken. BMI was calculated from these measurements. The questionnaire form was administered face-to-face with the researchers or through the online online-based "Google Forms", depending on the students' request. In the study, "Intercultural Effectiveness Scale" was used to measure the intercultural communication competence of the students, and "Food Neophobia Scale" was used to determine the effect of fear of new foods on nutrition.

Intercultural Effectiveness Scale

This scale was developed by Portalla and Chen in 2010 to assess the intercultural effectiveness of university students (Portalla and Chen, 2010). The validity and reliability of the scale was translated into Turkish by Yakar et al. in 2017 (Yakar et al., 2017). This scale was developed to measure the intercultural communication competence of individuals. For the reliability analysis of Intercultural Effectiveness scales, the internal consistency coefficient is calculated using cronbach's alpha coefficient. Cronbach's alpha coefficient was determined as 0.85 for the Intercultural Effectiveness Scale. The Intercultural Effectiveness Scale consists of 24 items in total.

Food Neophobia Scale

The Food Neophobia Scale was developed as a psychometric tool by Pliner and Hobden in 1992 (Pliner and Hobden, 1992). Its validation and reliability in Turkish was performed by Uçar et al. in 2021 (Uçar et al., 2021). This 7-point Likert-type scale, consisting of ten items, is arranged in such a way that each option increases by 1 point from strongly disagree (1 point) to strongly agree (7 points) and has a value between 10-70 points (questions 1, 4, 6, 9 and 10 are reverse scored). High scores indicate fear of novel foods and low scores indicate liking of novel foods.

The scales and indices to be used in the study were used face-to-face or online. Students were informed about the purpose of the research and that it would be used only for scientific purposes. They voluntarily participated in the study and answered the scales and indices. For participants who completed the online questionnaire, the completion and submission of the questionnaire meant that the participant agreed to participate in this research. This survey was conducted only once with the same IP address.

Statistical Analyses

IBM SPSS 22.0 statistical package programme was used for entering, analysing and evaluating all data throughout the study. Frequency tables and descriptive statistics were used in the interpretation of the findings. The values of descriptive variables were expressed as number (n), percentage (%), arithmetic mean, standard deviation (SD), median (median), and lower and upper values. Frequency (n) and frequency percentages (%) were calculated for qualitative variables in the study. ANOVA and bonferroni tests were used to analyse the scale scores. Spearman test was used to analyse the relationship. P value < 0.05 was found to be significant.

RESULTS AND DISCUSSION

Demographic Data Examination

The total number of university students who volunteered and participated for the study was 125. 108 (86.4%) of the students were female and 17 (13.6%) were male. The students lived in Kastamonu and the mean age was 21.67±2.16 years. The departments of the students were 80 students (64%) in the faculty of health sciences, 4 students (3.2%) in the faculty of education, 7 students (5.6%) in the faculty of veterinary medicine, 3 students (2.4%) in the faculty of human and society, 2 students (1.6%) in the faculty of science, 4 students (3.2%) in the faculty of economics and administrative sciences, 14 students (11.2%) in the faculty of engineering and architecture, 2 students (1.6%) in the faculty of sport sciences and 9 students (7.2%) in other faculties. The percentages of those who allocated 5%, 10%, 20%, 20%, 30%, 30%, 40% and 50% or more budget for nutrition were determined as 3.2%, 7.2%, 8.8%, 24.8%, 23.2% and 32.8%, respectively. Those with health problems were 12.8% and those without health problems were 87.2%. Smokers were 76%, non-smokers 22.4% and former smokers who quit smoking 1.6%. While 21.6% of the students used alcohol, 78.4% did not. Vitamin and mineral supplements were used by 19.2% of the students, while 80.8% did not use them. 40.8% of the students live in student dormitories, 19.2% live in student houses, 39.2% live in family houses and 0.8% live in other options.

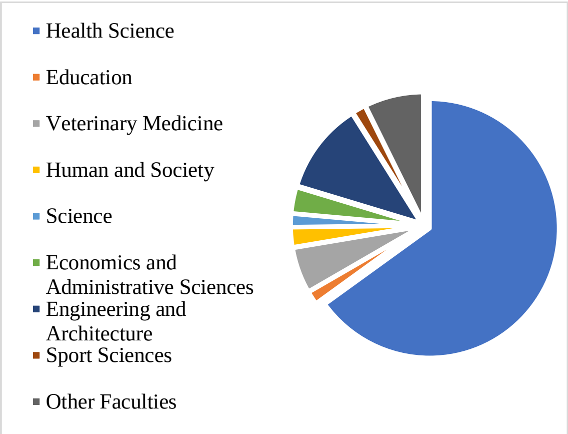

Figure 1. The departments of the students

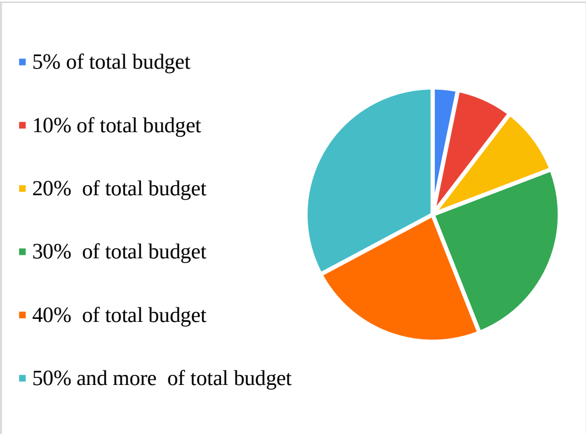

Figure 2. Percentage of nutrition expenditure

Nutrition Habits Investigation

The most important meal was 43.2% breakfast, 2.4% mid-morning meal, 9.6% lunch, 1.6% afternoon meal, 40.8% dinner and 2.4% night meal. Those who skipped meals were 38.4%, those who did not skip meals were 5.6% and those who sometimes skipped meals were 56%. The most skipped meals were found to be 28.8% breakfast, 8.8% mid-morning meal, 49.6%

lunch, 4% afternoon meal, 3.2% dinner, 2.4% night meal and 4.8% not skipped. While 36% of the students had regular meals, 64% did not have regular meals. The rate of those who stated their general appetite status as good was 48.8%, 42.4% as moderate and 8.8% as poor. 21.6% of the students stated that they ate their meals alone, 43.2% with their friends, 34.4% with their family and 0.8% with others.

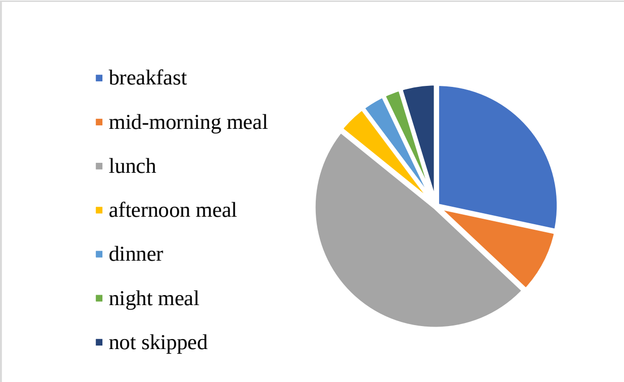

Figure 3. The most skipped meals

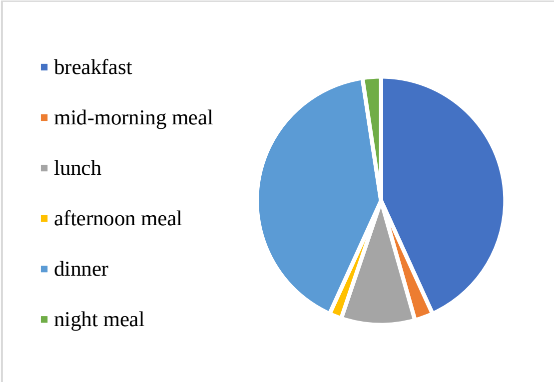

Figure 4. The most important meal

Food Neophobia and Intercultural Effectiveness Investigation

Table 1. Food neophobia scale and intercultural effectiveness scale scores according to age range

	Age	N	Mean	SD	
Food	18-20 age[a]	36	72.84	12.08	P=0.014*
Neophobia	21-23 age[a]	77	70.23	10.36	Test statistic=4.40
Scale	24 and + age[b]	12	75.35	10.44	
Scores	Total	125	71.47	10.93	
Intercultural	18-20 age	36	36.28	11.27	P=0.217
Effectiveness	21-23 age	77	37.18	9.56	Test statistic=1.55
Scale	24 and + age	12	27.92	9.52	
Scores	Total	125	36.03	10.35	

One-way variance analysis (ANOVA), Bonferroni test

A statistically significant difference was found between food neophobia scale scores according to the age range. The group aged 24 years and over is statistically significantly higher than the other two groups. No statistically significant difference was found between the cultural effectiveness scale scores.

Table 2. Food neophobia scale and intercultural effectiveness scale scores according to BMI categories

	BMI categories	N	Mean	SD	
Food	Underweight	13	69.55	11.05	P=0.29
Neophobia	Healthy weight	96	71.25	10.58	Test statistic =2.26
Scale	Overweight	12	71.75	13.47	
Scores	Obesity	4	82.11	8.72	

	Total	125	71.47	10.93	
Intercultural Effectiveness Scale	Underweight	13	38.69	9.31	P=0.24
	Healthy weight	96	36.01	9.89	Test statistic =1.43
	Overweight	12	36.25	14.94	
	Obesity	4	27.25	5.62	
	Total	125	36.03	10.35	

One-way variance analysis (ANOVA), Bonferroni test

No statistically significant difference was found between food neophobia scale scores and intercultural effectiveness scale scores according to BMI categories.

Table 3. Food neophobia scale and intercultural effectiveness scale scores according to appetite

	Appetite	N	Mean	SD	
Food Neophobia Scale Scores	Good[a]	61	72.34	10,52	P=0.000*
	Moderate[b]	53	71,74	10,52	Test statistic= 12.72
	Poor[b]	11	65,36	14,02	
	Total	125	71,47	10,93	
Intercultural Effectiveness Scale Scores	Good	61	31,67	9,22	P=0.146
	Moderate	53	39,89	9,82	Test statistic =1.96
	Poor	11	41,64	9,42	
	Total	125	36,03	10,35	

One-way variance analysis (ANOVA), Bonferroni test

A statistically significant difference was found between food neophobia scale scores according to appetite. The group with good appetite is statistically significantly higher than the group with moderate and poor appetite. No statistically significant difference was found between the intercultural effectiveness scale scores.

Relationship Between Food Neophobia Scale, Intercultural Effectiveness Scale and BMI Investigation

Table 4. Relationship between food neophobia scale, ıntercultural effectiveness scale and BMI

	Food Neophobia Scale Scores	
	r	p
Intercultural Effectiveness Scale Scores	-0.312	0.000*
BMI	-0.091	0.315

r: Spearman correlation

A weak negative relationship was found between food neophobia and intercultural effectiveness (r:-0.318; p=0.000). No significant relationship was found between food neophobia and BMI.

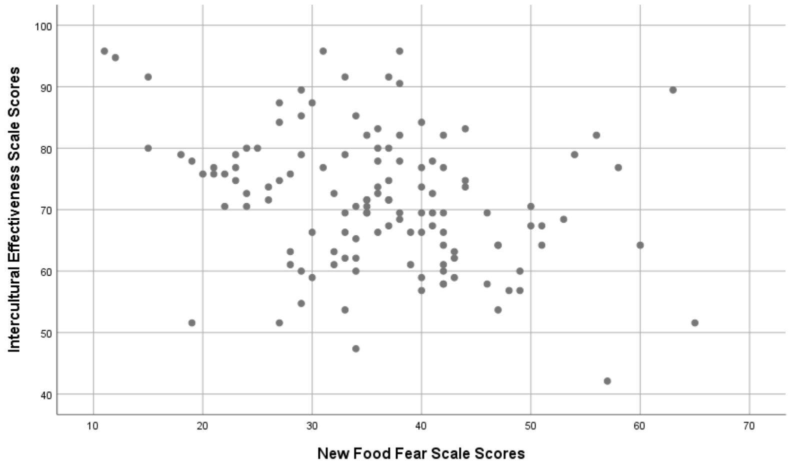

Figure 5. The scatter plot of relationship between food neophobia and intercultural effectiveness

In this study, university students' dietary habits, cultural influence and food neophobia were evaluated and the relationship between them was analysed. Healthy nutrition is indispensable for protecting public health and increasing the quality of life of individuals. Food choice is based on social, cultural and individual factors. Today's consumers are increasingly exposed to a wider variety of food choices as a result of advances in food production, marketing and transport. While new and different foods are often of interest to individuals, some individuals may approach these new and different foods with caution (Palamutoğlu et al., 2022). Food neophobia has been defined as a personal reluctance to accept and/or enjoy new or unfamiliar foods. The reluctance of some individuals to accept the addition of new ingredients or the application of new food production processes limits the variety of foods in their diets (Rabadán & Bernabéu, 2021). Inadequate and unbalanced nutrition, which is one of Turkey's social problems, is among the problems frequently experienced by university students. It has been found that students generally do not pay attention to meals, skip meals, prefer snacks such as sandwiches and bagels more, their economic situation causes them to have inadequate and unbalanced nutrition, and due to the poor conditions of the dormitories where they stay, the nutrition of the students residing here is not good, and they consume only to fill their bellies (Onurlubaş et al., 2015).

In our study, the mean score of the food neophobia scale was 71.47. In a study, the average food neophobia score of individuals working in the defence industry was determined as 36.5±10.66 points (Gümüş et al., 2022). The results of the studies conducted on this subject in the literature vary. In a study conducted in Italy with children aged 6-9 years, it was found that children in this age group had a high desire to try new and different foods (Laureati et al., 2015). In a food neophobia study conducted in South Korea, the food neophobia of the elderly was found to be higher than that of young people (Choe and Cho, 2011). According to the study conducted by Dovey et al. (2007), it is stated that food neophobia in individuals starts during the weaning period and the level of neophobia is highest in this age period. Another view is that the level of food neophobia continues at the same level from childhood to adulthood (Henriques et al., 2009). It is seen that different results were obtained in each of the studies examining the relationship between food neophobia and age. In a study conducted in Finland, the level of food neophobia of women was higher than that of men, and the level of food neophobia of the elderly was higher than that of the young (Tuorila et al., 2001).

In our study, intercultural effectiveness score was found to be 36.03±10.35. In a study, the mean intercultural effectiveness scale score of nursing students was determined as 66.98±8.34 (Uyanık and Tanrıverdi, 2021). In another study conducted on midwifery students, the mean total score of intercultural effectiveness was determined as 74±8.

The behavioural dimension of intercultural communication competence refers to the individual's ability to put the knowledge and attitude towards intercultural differences into life practice. Studies show that students' high cultural intelligence enables them to perceive differences more quickly and exhibit appropriate behaviours, ensure social adaptation and thus prevent culture-based conflicts, and be more effective in cultural environments by using communication skills effectively (Pawlicka et al., 2019). Therefore, it is thought that these individuals may exhibit an easier and more harmonious attitude in their transition to a healthy diet in new environments.

CONCLUSION

Being open to different cultures is one of the factors that will eliminate communication difficulties in daily life and facilitate adaptation to new environments. In this context, people whose communication with different cultures is harmonious will develop less neophobia towards different foods and exhibit a harmonious attitude towards healthier nutrition. It is thought that the geography where individuals live, the culinary culture, eating habits, experiences and country conditions affect the level of food neophobia and cause changes. The lack of diversity among the foods consumed by neophobic individuals may lead to nutritional and health problems. Therefore, it is important to determine the levels of food neophobia, factors associated with neophobia and the effects of neophobia on nutrition and health. In this direction, conducting similar studies in different regions of our country and in different age groups may contribute to researchers in the field and food production enterprises.

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Statement of Conflict of Interest

The author declares no conflict of interest.

REFERENCES

Choe, J. Y., Cho, M. S., 2011. "Food Neophobia and Willingness to Try Non – Traditional Foods for Koreans". Food Qualitiy and Preference, 22: 671-673.

Dovey, T. M., 2007. "Food Neophobia and 'Picky/Fussy' Eating İn Children: A Rewiew". Appetite, 50: 181-193.

El Ansari, W., Stock, C., Mikolajczyk, R.T. 2012. Relationships between food consumption and living arrangements among university students in four European countries—A cross-sectional study. Nutr. J., 11, 28.

Ermiş, E., Doğan, E., Erilli, N., & Satıcı, A. 2015. Üniversite öğrencilerinin beslenme alişkanliklarinin incelenmesi: Ondokuz Mayis Üniversitesi örneği. Spor ve Performans Araştırmaları Dergisi, 6(1), 30-40.

Garibağaoğlu, M., Özgüne G.N., 2008. Okullarda beslenme uygulamaları. Çocuk Dergisi,8 (3), 152-159.

Güçlü, N., 1996. Yabancı öğrencilerin uyum problemleri. Hacettepe Üniversitesi Eğitim Fakültesi Dergisi, 12(12).

Gümüş, D., Topal, G.G., Sevim, S., Kızıl, M., 2022. Savunma Sanayinde Çalışan Bireylerin Stres Düzeyleri, Besin Neofobisi ve Akdeniz Diyetine Uyumlarının İncelenmesi. Celal Bayar Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi, 9(1), 50-57.

Henriques, A.S., King, S.C., Meiselman, H.L., 2009. "Consumer Segmentation Based on Food Neophobia and Its Application to Product Development". Food Quality and Preference, 20: 83-91.

Kavas, A, Kavas, A., 1985. Üniversite öğrencilerinin beslenme bilgi düzeyi beslenmeye karşı tutumları ve beslenme durumları üzerine bir araştırma. Beslenme ve Diyet dergisi, 14, 63-73.

Korkmaz, N.H., 2010. Uludağ Üniversitesi Öğrencilerinin Spor Yapma ve Beslenme Alışkanlıklarının İncelenmesi. Uludağ Üniversitesi Eğitim Dergisi, 23 (2), 399-413.

Koyu, E. B., Kaner, G., Yıldız, E. A., 2016. İnme ve beslenmenin inme üzerine etkisi. Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi, 5(4), 112-118.

Laureati, M., Bergamaschi, V., Pagliarini, E., 2015. "Assessing Childhood Food Neophobia: Validation of a Scale in Italian Primary School Children". Food Qyality and Preference, 40: 8-15.

Onurlubaş, E., Doğan, H.G., Demirkıran, S. 2015. Üniversite öğrencilerinin beslenme alışkanlıkları. Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi, 32(3), 61–69.

Palamutoğlu, R., Palamutoğlu, M.İ., Kantar, A.G., Çorapçı, B., Kazak, M., Kasnak, C., 2022. Üniversite Öğrencilerinin Et Tüketimi ve Gıda Neofobisinin Değerlendirilmesi. Kırşehir Ahi Evran Üniversitesi Sağlık Bilimleri Dergisi, 6(3), 144-153.

Pliner, P., Hobden, K., 1992. Development of a scale to measure the trait of food neophobia in humans. Appetite.19:105-120. doi:10.1016/0195-6663(92)90014-W.

Portalla, T., Chen, G.M., 2010. The development and validation of the intercultural effectiveness scale. Intercultural Communication Studies, 19(3), 21-37.

Rabadán, A., Bernabéu, R., 2021. A systematic review of studies using the Food Neophobia Scale: Conclusions from thirty years of studies. Food Quality and Preference, 93, 1–18. https://doi.org/10.1016/j.foodqual.2021.104241

Tuorilla, H., Lahteenmaki, L., Pohjalainen, L., Lotti, L., (2001). “Food Neophobia Among The Finns and Related Responses to Familiar and Unfamiliar Foods”. Food Quality and Preference, 12: 29-37.

Ul Haq, I., Mariyam, Z., Li, M., Huang, X., Jiang, P., Zeb, F., Zhou, M., 2018. A comparative study of nutritional status, knowledge attitude and practices (KAP) and dietary intake between international and Chinese students in Nanjing, China. International journal of environmental research and public health, 15(9), 1910.

Uçar, E.M., Gümüş, D., Karabulut, E., Kızıl, M., 2021. Adaptation of the Food Neophobia Scale to Turkish and Determination of Appropriate Factor Structure. Turkiye Klinikleri J Health Sci. 6(3):393-400 doi: 10.5336/healthsci.2020-76664

Yakar, H. K., Alpar, Ş. E., 2017. Determination of the reliability and validity of intercultural awareness and intercultural effectiveness scales Kültürlerarası farkındalık ve kültürlerarası etkililik ölçeklerinin güvenirlik ve geçerliğinin belirlenmesi. Journal of Human Sciences, 14(3), 2748-2761.

Yücecan, S., 2008. Optimal beslenme. Sağlık Bakanlığı Yayınları, 726.

POSTER PRESENTATIONS

Valorization of by-product of Bean Agriculture (*Faba beans*) İntended for Feeding Broiler Chicken

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ABSTRACT: The broiler chicken is an animal that requires food very rich in protein; fat and vitamin. For this, the composition and formulation of the basic food is essential in the nutrition of chicks. These are crumbs of complete foods composed of cereals, such as wheat, corn and oilcake, to which are added proteins, fats and vitamins. The main objective of our research is to valorize by-products of agriculture of bean (*Faba beans*) intended for feeding broiler chicks.

Keywords: Bean, Chicken, *Faba bean*

INTRODUCTION

Broiler chicken breeding methods in Algeria have seen a remarkable improvement due to new techniques (Morinière 2014). but they still remain dependent on an imported and expensive feed based on soybean meal which constitutes a quarter of the rations distributed to broilers as a protein source and corn as an energy source (Dronne et al. 2018).in this work we tried to study the nutritional value of the by-product of the bean (*Faba beans*) and valorize it in order to incorporate them into the food ration of meat poultry following its richness in protein. (Martineau-Côté et al . 2022).

MATERIAL AND METHOD

A. Collection of samples: 95.00kg of agricultural by-products of faba bean plants (*Faba beans*); (remainder of the plant after harvest) were collected in the fields of the Stidia area, Wilaya of Mostaganem, Algeria during the months of March, April and May 2023.

B. Drying: broad bean plants (*Faba beans*) were cleaned of weeds and dried in the open air at an ambient temperature ≈ 37° for 02 and a half months, in order to eliminate humidity.

C. Grinding: After the drying operation, the dried plants were weighed and crushed using a professional seed grinder. After grinding, the Product was stored in glass boxes away from humidity to introduce them into the diet of broiler chicks.

D. Determination of the dry matter (DM) content of the bean by-product (*Faba beans*) (Thermogravimetric methods): The dry matter (DM) content is conventionally determined by the weight of the food after drying at 105°C in an oven for 24 hours according to (AFNOR, 1985).

E. The percentage of water content: it is calculated following the mathematical model:

H2O%= 100 – DM (%)

F.ash dosage (MM) (M.S.D.A 2004): The ash content of the sample is calculated on the basis of the weighing of the incinerated sample and the test portion (expressed in g / 100 g).

G. Organic Matter (OM) dosage: Organic Matter is the fraction burned during the combustion of the sample. Organic matter is composed of the chemical constituents contained in the cell walls or parietal constituents plus the cytoplasmic constituents, OM=dm-mm.

H. determination of total lipids: The lipid extracts were extracted with a chloroform-methanol mixture according to the method of Folch et al. (1957).

Briefly, a known mass of test sample is crushed and homogenized in the presence of 60 ml of Folch reagent (chloroform/methanol 2v/1) using a homogenizer (ultra Thurax type) for 3 minutes. The ground material is filtered through sintered glass (porosity: 1) under vacuum. The filtrate is added with a 0.73% NaCl solution at the rate of one volume of NaCl for 4 volumes of filtrate then allowed to decant for 2 hours. The lower phase (chloroform + lipids) is filtered through anhydrous sodium sulfate and collected in a previously weighed ground-neck flask. The upper phase (methanol-water) is rinsed using 50 ml of a mixture of 20% NaCl concentrated at 0.58% and 80% methanol+chloroform in order to extract the remainder of the lipids appearing at the end of this operation. After stirring, it is left to settle again for about ½ hour. The lower phase (chloroform + residual lipids) is thus recovered and added to the first filtrate. The chloroform-lipid mixture is then evaporated under vacuum. After evaporation of the chloroform, the dry lipid residues are weighed. The lipid content is given by the difference between the weight of the evaporation flasks empty and dry + lipid residues. It is expressed as a percentage of the weight of the dry tissue.




Figure1. Drying the plant. **Figure 2.** Grinding the dried plant. **Figure 3**. Semi finished products. **Figure 3.** Physicochemical analyzes.

% TL = (Full flask weight – empty flask weight) x 100 / sample weight.

RESULTS AND DISCUSSION

Drying the plants at an ambient temperature around 38° and not exceeding 40° allowed us on the one hand to reduce the volume and the weight of the plant; as well as preserving the nutritional values of the plant. During the drying period, the condition of the plants was checked two to three times a day. It will then be necessary to move certain parts to bring them closer to or further from the heat. in order to have good heat distribution and avoid rot.

The results obtained are mentioned in the table below:

Table 1. Weight of plants before and after drying

Name of plant	Weight before drying (kg)	Weight after drying (kg)	Reduction*
fève *(Faba beans)*	95.00	46.18	2.06

** Weight before drying (kg)/ Weight after drying (kg).

Drying the plants eliminated as much water as possible and reduced the humidity level of the plants as well as the weight by around half. With a reduction rate of 2.05 and this amounts to the evaporation of water due to temperature. The plants are removed from the soil as they have reached the desired degree of drying. . The plants are then weighed to determine their dry weight.

Grinding dried plants allows the size of the plants to be reduced to powder (depending on use), Storage in boxes allows the products to be preserved for a long time (summer period) for later use.the results of the dosage of dry matter, mineral matter, organic matter ,% H2O and total lipids are mentioned in table n°02 below:

Table 2. Nutritional value of the bean plant (*Faba beans*)

plant of bean (*Faba beans*)* %/100g dry matter	
DM	93.46 %
MM	18.68%
OM	81.32%
% H_2O	6.54%
Total Lipids	1.3%

* The value are compared to total dry matter

DISCUSSION

The results of the analyzes carried out on the faba bean plant samples (*fab bean* by-product) show that the plant is Faba beans are an important food rich in nutrients. in particular, lipids high amount of proteins rich in lysine, complex carbohydrates, dietary fiber, non-nutritive and bioactive secondary metabolites compounds (antioxidants, phenols and γ-aminobutyric acid), which have several health benefits (Khazaei et al., 2019; Liu et al., 2022). Additionally, also it is a good source of many macro- and microelements, including minerals (Hacısefero gulları et al., 2003; Rahate et al., 2020).

CONCLUSION

The goal of this work is to promote the by-product of faba bean agriculture, an agronomically reliable alternative for the nutrition of broiler chicks following its noted nutritional values (high protein levels, etc.).

Faba bean, is an important nutrient-rich legume, especially, high amount of lysine-rich protein, complex carbohydrates, dietary fiber, non-nutrient secondary metabolites, and bioactive compounds (antioxidants, phenols, and γ-aminobutyric acid), (Khazaei et al., 2019; Liu et al., 2022) which can give good results in vivo on broiler chicks on a zootechnical as well as nutritional level.

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Statement of Conflict of Interest

I declare that they are no conflict of interest.

Authors' Contributions

HEDIA Nacera, Pr. Meziane AHMED Malika and Dr.BOUDOUR Khedidja designed and analyzed the research, HEDIA Nacera studies arranged. HEDIA worked on the preparation of all pictures and tables. All authors contributed to the writing of the article and took part in the process of publication of the article and read and approved it.

REFERENCES

De Angelis, Davide et al. 2021. “Data on the Proximate Composition, Bioactive Compounds, Physicochemical and Functional Properties of a Collection of Faba Beans (Vicia Faba L.) and Lentils (Lens Culinaris Medik.).” *Data in Brief* 34: 106660.

Crépon, Katell et al. 2010. “Nutritional Value of Faba Bean (Vicia Faba L.) Seeds for Feed and Food.” *Field Crops Research* 115(3): 329–39.

Demay, Fanny, and B T S Bioanalyses. “TP7 Matiere Seche.” : 1–5.

Etienne, M., P. H. Duee, and B. Pastuszewska. 1975. “Nitrogen Balance in Lactating Sows Fed on Diets Containing Soybean Oil Meal or Horsebean (Vicia Faba) as a Protein Concentrate.” *Livestock Production Science* 2(2): 147–56.

Felix, Manuel, Alejandra Lopez-Osorio, Alberto Romero, and Antonio Guerrero. 2018. “Faba Bean Protein Flour Obtained by Densification: A Sustainable Method to Develop Protein Concentrates with Food Applications.” *LWT* 93: 563–69.

Frühbeck, Gema, Ignacio Monreal, and Santiago Santidrián. 1997. “Hormonal Implications of the Hypocholesterolemic Effect of Intake of Field Beans (Vicia Faba L.) by Young Men with Hypercholesterolemia.” *The American Journal of Clinical Nutrition* 66(6): 1452–60.

Jansman, A. J.M., J. Huisman, and A. F.B. van der Poel. 1993. “Ileal and Faecal Digestibility in Piglets of Field Beans (Vicia Faba L.) Varying in Tannin Content.” *Animal Feed Science and Technology* 42(1–2): 83–96.

Kadi, Si Ammar et al. 2018. “La Graine de Fève Sèche (Vicia Faba Major L) En Alimentation Cunicole : Effets Sur Les Performances de Croissance et d ’ Abattage . To Cite This Version : HAL Id : Hal-01742645 The Dry Seeds of Broad Bean (Vicia Faba Major L) for Fattening Rabbits : E.”

Lacassagne, L., M. Francesch, B. Carré, and J. P. Melcion. 1988. “Utilization of Tannin-Containing and Tannin-Free Faba Beans (Vicia Faba) by Young Chicks: Effects of Pelleting Feeds on Energy, Protein and Starch Digestibility.” *Animal Feed Science and Technology* 20(1): 59–68.

Lacassagne, L., J. P. Melcion, F. de Monredon, and B. Carré. 1991. “The Nutritional Values of Faba Bean Flours Varying in Their Mean Particle Size in Young Chickens.” *Animal Feed Science and Technology* 34(1–2): 11–19.

Mohammed, Belhamra et al. 2018. “Evaluation de La Résistance Naturelle de Quelques Cultivars de Fève (Vicia Faba L .) Propres à La Région de Biskra à l ’ Égard Des Thrips (Thysanoptera : Thripidae).

U.S. Department of Agriculture, Agricultural Research Service (2014). USDA National Nutrient Database for Standard Reference, Release 27. Nutrient Data Laboratory Home Page, Http://www.ars.usda.gov/ba/bhnrc/ndl. “Val Nut de Feve Seche.