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Dear Conference Participants,
Welcome to 4th International Conference on Food, Agriculture and Animal Sciences.
On behalf of the Organizing Committee, I am very happy to open 4th 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, as the COVID-19 pandemic is gaining an ever tighter grip on our daily lives, as in many events, we also are forced to organize our conference as online meeting. 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. The conference is going to be held with bigbluebutton platform for participants. All presentations will be virtual presentation. 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 92 participants from 17 countries (Algeria, Jordan, Thailand, Lithuania, Iran, United Arab Emirates, Pakistan, Azerbaijan, Switzerland, Moldova, Tunisia, Russia, Ukraine, Egypt, Germany, Korea, Turkey), out of which 83 are contributing to the meeting with oral online presentations and with 9 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, 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. Tolga KARAKÖY
Chair of ICAFOAS 2023

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ORAL PRESENTATIONS

POSTER PRESENTATIONS

Agrisharing on the Basis of Bioeconomy on The Way to Achieving Sustainable Development Goals

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ABSTRACT: The study reveals the importance of Ukraine in the global food system and the need for an urgent innovative approach to solving the global food problem associated with the introduction the state of war in Ukraine. The aim of the study was to determine the effectiveness of the development of agrisharing platform based on bioeconomy, its advantages and disadvantages, as well as its contribution to the Sustainable Development Goals. As a way to solve the global food problem, the study proposes the introduction of an appropriate platform as a tool for the sustainable development of goods, natural resources, material and technical base and intellectual capital in the system of mutual education, business and science in the agricultural sector. The authors present the prerequisites and advantages of the proposed system, as well as the contribution to the Sustainable Development Goals, subject to the implementation of the agrisharing system based on the bioeconomy. Possible problems and difficulties in the implementation of the system, which are envisaged to be overcome through the introduction of regulatory measures, are considered. It is envisaged that agrisharing based on the bioeconomy will contribute to the implementation of more than 50% of the Sustainable Development Goals.

Keywords: Sustainable Development Goals, Agrisharing, Bioeconomy, Global food problem

Enhancing Environmental Stress Tolerance in Plants: Physiological Approaches

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ABSTRACT: Plants growth and development are affected by changing environments that induce abiotic stresses. This study provides insight into how stress affects growth, yield qualities, and plan yield. Understanding the mechanisms underlying this stress is necessary to comprehend how plants can more effectively adapt to a changing climate that causes stress. Applying innovative tactics, including creating transgenic crops with increased stress tolerance, can result in better performance and yield in response to stress. Stress can be reduced with the help of effective management techniques, such as the use of compatible antioxidants like proline or glycinebetaine, in conjunction with a better nutrient balance or the use of organic fertilizers. This increases yield and other growth-related quality parameters in plants.

Keywords: Environmental stresses, Tolerance, Physiology, Adaptation

Agricultural Land Remediation: Addressing the Toxic Remnants of the War in Ukraine

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ABSTRACT: The war in Ukraine caused the depletion and deterioration of natural resources, primarily agricultural land, as the basis of the country's food security. Today 30% of the country's farmland is occupied or unsafe. This situation can ultimately undermine our already weakened economy. Before the war agricultural sector contributed up to 12% of the GDP and was a vital revenue that Ukrainians could historically rely on. Since the Russian's invasion in 2014, Ukraine's agriculture has shown consistent improvement and was the only part of the country's economy to buck the recession. Ukraine was one of the leading world exporters of grains as well as vegetable oils. And now we are witnessing the destruction and damage of agricultural lands due to mine pollution; pollution caused by burning and spilling petroleum products; pollution from emissions of substances deposited on the ground; pollution by military waste, and other hazardous substances due to military actions. From February 24, more than 190 000 explosives have been neutralized in the territory of Ukraine. However, more than 176,000 square km remain contaminated. In addition, mine explosions led to soil contamination with heavy metals – lead, strontium, titanium, cadmium, and nickel that make the soil dangerous and, in some cases, unsuitable for further agricultural use. Complete demining of areas contaminated with explosives will take 5-10 years and more than $70 billion. The war has set the Ukrainian agriculture sector back by years, especially after gains made in developing healthier and organic crops. Moreover, it is fuelling a global food crisis. FAO estimated 400 million people worldwide rely on Ukraine for their food supply. The longer the Russia-Ukraine conflict lasts, the more insecurity about food supplies it may bring to the whole world. Compared to 2021, farmers planted 30% less and harvested 40% less acreage in 2022. In addition, the war raises food and energy prices and contributes to economic and food instability in several world regions. Building capacity for mapping, monitoring and managing environmental risks from critically affected by the war Ukraine agricultural lands is crucial for now. For that, we should create a network of science and business to provide a comprehensive approach by using all modern technologies such as geospatial data, UAV surveys, and soil sample analyses to safeguard sustainable farming and food security. But, developing plans for land restoration, we should think with a long-term perspective and consider time demands and global challenges. Hence, Ukrainian farmers should be offered an action plan or to-do-list: to start with recommendations on land decontamination, remediation and proceed to a sustainable farming strategy to meet the requirements of the organic world market, SDG, Green Deal, Paris Agreement. It is generally assumed that armed conflicts lead to reductions in the emissions that contribute to climate change because it often halts or reverses economic development. In fact, armed forces are one of the biggest emitters of greenhouse gases in the world. Agriculture can offer reasonable solutions to reducing the number of greenhouse gases trapped in the atmosphere. Reducing tillage, expanding crop rotations, planting cover crops and reintegrating livestock into crop production systems have proven to reduce agriculture's footprint and capture the excess carbon generated by other industries. This captured carbon is then converted into plant material and/or soil organic matter, improving soil health and increasing the ability to produce food on the land in the future. The recovery plans for the local farms and agribusiness must ensure the implementation of environmental policy following the European integration aspiration of Ukraine and the European Green Deal provisions, as well as the integration climate-compatible decisions into all aspects of land treatment.

Features of the Development of the Leaf Surface European Grape Clones in the Conditions Southern Region the Republic of Moldova*

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ABSTRACT: Currently, certified virus-free clones of classic European varieties are being introduced in the Republic of Moldova. In this connection, the study of introduced clones of grapes in specific environmental conditions of the area is relevant. The article presents the results of long-term of research on monitoring changes in the development of the Leaf Apparatus of the introduced clones R5 Cabernet Sauvignon and 348 Merlot in the agro-ecological conditions of the South of the Republic of Moldova (ATU Gagauzia). The experimental vineyard plot planted in 2006 to the scheme 2.5m x 1.35m (2963 vines per hectare). It has been established that the studied clones are characterized by different parameters of the Leaf Surface. These indicators are higher in clone 348 Merlot for all years of the research. Monitoring of vineyards made it possible to reveal the relationship between the parameters of the Leaf Surface and meteorological conditions. In favorable meteorological years (2017), the Area of Leaf Blades of clone R5 Cabernet Sauvignon is 151.8 cm^2/leaf, the Leaf Surface is 53.7 dm^2/shoot and 17.0 m^2/vine; clone 348 Merlot – 212.5 cm^2/leaf, 66.3 dm^2/shoot and 18.6 m^2/vine, respectively. In unfavorable meteorological years (2020), there is a sharp decrease in these indicators by 1.6-1.7 times.

Keywords: Cabernet Sauvignon, Clone, Grapes, Leaf Area Index, Merlot, Moldova

SARS-CoV-2 Main Protease a Potential Target for Treatment of COVID-19 by Phytochemicals

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ABSTRACT: Over the past years, the world has suffered from the emergence of the disease COVID-19, one of the most difficult pandemics in human history. It is a new coronavirus that has been confirmed as a human coronavirus by sequencing and analysis of its genome. Millions of confirmed deaths from this pandemic have been reported worldwide. To date an enormous amount of knowledge has been gained about the causative agent of this disease the SARS-CoV-2 virus, including its molecular structure, life cycle and interactions with the host cell. This information has led to the development of several vaccines. The global pandemic caused by SARS-CoV-2 has become a challenge for humans. However, there is still no effective antiviral approved for the treatment of COVID-19. The development of new effective antiviral agents to fight the disease is a reported urgency. 3CLpro protease is the major cysteine protease composed of approximately 300 amino acids and three domains. This protease, in its active form, is a homodimer with a Cys-His catalytic dyad. This enzyme plays a central role in viral genome replication, transcription and other important viral life processes. Inhibition of 3CLpro can effectively block viral RNA transcription and replication. As humans do not have a homologous protease, 3CLpro is a very specific antiviral target. Therefore, 3CLpro is a promising target for the development of potential anti-coronavirus drugs. Various studies have shown that natural compounds have effective antiviral properties against coronaviruses by inhibiting several viral targets. in our studies conducted many different phytochemicals of plants showed that the main Protease is a potential target for SARS Cov-2 inhibition. This inhibitory effect could be beneficial in the treatment of coronavirus infection to prevent it from multiplying in the host cells of the human body.

Keywords: SARS-CoV-2, 3CLpro, Inhibition, Phytochemicals

Biochar Technology for Sustainable Organic Farming

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ABSTRACT: Biochar is a solid product obtained by heating of biomass or organic waste in the total or partial absence of oxygen and is applied to improve soil properties, or considered a means of carbon sequestration. Several positive effects of biochar on soil chemical, physical, and biological properties have already been demonstrated. Biochar amendment has also been repeatedly discussed as an effective means to restore saline lands and increase plant tolerance to salt stress. Especially, improved soil cation exchange capacity, water holding capacity, soil nutrient retention, and increased soil enzyme activities and diversity of microbial communities, were reported. Biochar application into the soil may positively affect the soil microbiome and its activities in soil nutrient cycling. Soil bacterial community structure and diversity were shown to be positively correlated with biochar amendment and soil organic matter conten. The improved microbial diversity in the root system indicates potential beneficial effects of root-associated bacteria, which help plants to absorb more nutrients from the soil and tolerate abiotic stresses. Moreover, biochar application effectively increases salt stress tolerance in plants by modulating physiological processes, biochemical properties, photosynthetic performance, and mitigation of oxidative and salt stress through alterations of antioxidant enzymes. Biochar amendment can be a reliable approach to improve soil properties and plant growth and development on salt-affected soils, thus promoting long-term field sustainability. However, more in-depth studies are needed to understand biochar interactions with soil organisms under extreme environments, which will help achieve maximum benefits of biochar under saline soil conditions.

Key words: Biochar, Plant, Microbes, Nutrients, Legumes, Crops, Organic farming

Tea cultivation in Turkey: A historical perspective

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ABSTRACT: Tea is one of the most important beverage in the world and it is popular throughout the world. Tea is a beverage as old as human history. Türkiye is one of the 30 countries in the world where tea plants are grown and tea is produced economically. The country improves knowledge and and cultivation of tea year by year and as of 2021, it exports tea to more than 30 countries. Tea cultivation in Türkiye is carried out mainly in the Eastern Black Sea Region, from the Georgian border to the Fatsa district of Ordu province. Rize alone meets 80-85% of Türkiye's tea production; Other provinces where tea is grown are Ordu, Giresun, Trabzon and Artvin. Unlike tropical and equatorial regions, where tea cultivation can be done for 12 months, tea is produced only for 6 months of the year in Türkiye and the harvest period, which starts in May every year, is completed in October or November. Since Türkiye is one of the newest countries for tea cultivation, it does not experience many diseases and pests. In addition, it is the one of the country in the world where snows cover tea plantations during winter months, which in this case poses an obstacle for diseases and pests.

Keywords: Tea, cultivation, unique characteristics, harvest period

ABSTRACT

ORAL PRESENTATIONS

Study of the Relationship between Household Food Insecurity with Nutritional Status of Children Under the Age of 5 Years in Irbid Municipality

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ABSTRACT: This study aimed at determining the level of food security for families with children under the age of five years in Bani Kenana District in Irbid, Jordan; identifying prevalence of malnutrition among these children; and examining association of malnutrition among these children with food insecurity in their families. The study used the descriptive analytical and correlative research approach. The appropriate data were collected using the Household Food Insecurity Scale (HFIS), which is a questionnaire consisting of three sections. The first section of the HFIS covers demographic, social, and economic information about the family members who reside together with the head of the family. The second section is concerned with family food insecurity. It consists of eight paragraphs that use a binary Likert scale. The third section, however, is related to the children under study. It targets information about them such as gender, date of birth, and date of taking anthropometric measurements (child's height, weight, and Body Mass Index (BMI)). The study adopted three measures of malnutrition, which are the body mass index (BMI) for age (BAZ), height to age (HAZ), and body mass to age (WAZ). The study population consisted of all children under the age of five years in Bani Kenana District, who were 18,947 boys and girls according to statistics of the Department of General Statistics, Jordan, for the year 2020. The study sample, however, comprised 386 children who were chosen by the researcher following the simple random sampling approach. They consisted of 197 boys and 189 girls. The results of statistical analysis revealed that 41.7% of the children in the study sample were enjoying food security, about 38.3% of the sample members had severe level of food insecurity, and 19.9% of the sample children had a moderate level of food insecurity. Furthermore, the results uncovered a general prevalence of wasting and malnutrition characterized by underweight, severe underweight, stunting, and severe stunting. Moreover, statistical analysis disclosed that there is a statistically-significant negative correlation between the values of the WHZ, HAZ, and BAZ for the children and household food insecurity. The study recommends implementation of educational programs for pregnant women within health centers related to their nutrition and child nutrition in order to raise their awareness of the best nutritional practices that contribute to normal growth of children. The study also recommends periodic anthropometric examination of children from birth so as to track their growth and detect any malnutrition at an early time in order to remedy it.

Keywords: Household Food Insecurity, Malnutrition, Anthropometry, Pre-school children, Anthro&AnthroPlus

Foot-and-Mouth Disease Virus Detection in Asymptomatic Dairy Calves without FMD Outbreak*

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ABSTRACT: Foot-and-mouth disease (FMD) is a highly contagious viral disease in cattle and causes economic losses directly and indirectly. Monitoring the FMD virus is useful management for the prevention of an FMD outbreak. Therefore, the objectives of this study were to determine the FMD virus in calves using nasal swab samples in the absence of an FMD outbreak. Nasal mucosal swab collections were performed using 185 dairy calves with asymptomatic FMD in Chiang Mai Province, Thailand, and then the presence of the FMD virus was evaluated using the real-time rt-PCR assay. In total, 4.9% of calves detected FMD virus, including 2 farms in Mae-on district (8 samples; 9.6%) and 1 farm in Chai-Prakan district (1 sample; 1.2%). Interestingly, 6 months after this detection, both farms in Mae-on had an outbreak of FMD. This indicated that the FMD virus presented in asymptomatic cattle might relate to the subsequent outbreak of FMD. The outbreak demonstrates the presence of the virus in the environment. In conclusion, monitoring of FMD can be performed by nasal swab collection. Further investigation is needed to show whether the FMD virus presented in asymptomatic FMD cattle could be the cause of the subsequent FMD outbreak or not.

Keywords: Cattle, Foot-and-mouth disease, nasal swab, real-time rt-PCR assay

Molecular Docking and ADMET of β-Elemonic Acid from *Boswellia carterii* Resin against the Main Protease Mpro of SARS CoV-2

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ABSTRACT: COVID-19 caused by SARS-CoV-2 has become a global epidemic since late 2019. Mpro protease is the major cysteine protease. This enzyme plays a central role in viral genome replication, transcription and other important viral life processes. Its inhibition can effectively block viral RNA transcription and replication. Therefore, it is a promising target for the development of potential anti-coronavirus drugs. Here we evaluate the inhibitory effect of β-elemonic acid (β-EA) as a natural triterpene from *B.carterii* against the Mpro. Docking results indicated that β-EA inhibit the 3CLpro with binding energy of -7.17 Kcal/mol. It exhibited two donor hydrogen bonds with Cys145. Its inhibitory effect was revealed by its score energy and its interactions with one of the amino acid residues of catalytic dyad of the active site, His41 and Cys145. β-EA obeyed to Lipinski's rules with one violation regarding to its Moriguchi Log P (MLOGP>4.15). The oral bioavailability radar plots showed that this compound was in the optimal range for the physicochemical properties (polarity: 20 $Å^2$<TPSA (54.73)<130 $Å^2$, flexibility: 0<Num. of rotatable bonds(5)< 9), saturation: 0.25< Fraction Csp3 (0.80)<1, size: 150 g/mol <MW (454.68)<500 g/ mol, with the exception of lipophilicity: −0.7<XLOGP3 (7.15)<5.0 and slightly solubility: -6< Log S (ESOL) (-6.83)<0). β-EA exhibiting good drug-likeness properties and orally bioavailable. However, ADMET revealed that β-EA exhibited negative results on Ames mutagenesis. In addition, no hepatotoxicity and skin sensitization, which allows it to be good safety drugs.

Keywords: ADMET, β-elemonic acid, Molecular docking, 3Mpro, SARS-CoV-2

Salt in Terms of Food Safety

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ABSTRACT: Excessive salt consumption is a factor in the formation of cardiovascular diseases, hypertension, kidney diseases, osteoporosis, stomach diseases, obesity and some other diseases. For this reason, health institutions are working to reduce salt consumption. On the other hand, salt can contain important risks such as radioactives, heavy metals, microplastics, dynamite residues and exhaust in terms of food safety. In this study, salt was evaluated in terms of food safety.

Keywords: Salt Residue, Salt, Heavy Metal, Microplastic, Radioactivity

Title of The Manuscript Role of Marination, Essential Oils, and Packaging on Microflora During Storage of Chicken Tawook*

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ABSTRACT: The aim of this study was to investigate the antimicrobial effect of marination, essential oils (EOs) and packaging atmosphere on the microbial population of chicken tawook during storage at 4°C. Chicken meat was cut into 10 g cubes and marinated. The chicken was then mixed individually with 0.5% or 1% (w/v) vanillin (VA), β-resorcylic acid (BR), or eugenol (EU), and stored under aerobic (AP) or vacuum (VP) packing at 4°C for 7 d. The marinade decreased microbial growth as monitored by total plate count, yeast and mould, lactic acid bacteria, and *Pseudomonas* spp. by about 1 log cfu/g under AP. The combination of the marinade and EOs under AP and VP decreased the growth of spoilage-causing microorganisms by 1.5 – 4.8 and 2.3 – 4.6 log cfu/g, respectively. Overall acceptability was the highest for marinated samples with 0.5% BR. The change in pH in VP meat was less than 0.5 in all treated samples including the control.

Keywords: Aerobic packaging, Vacuum packaging, β-Resorcylic acid, Eugenol, Vanillin

Salt in Meat and Meat Products

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ABSTRACT: Meat and meat products are considered essential components of the human diet, as they are a source of very high quality protein, essential amino acids, B-group vitamins and important minerals such as iron and zinc. Meat and meat products are among the products in which salt is used most intensively. Salt; It is an indispensable additive because it limits microbial growth, forms emulsion by dissolving proteins, increases water holding capacity, and formation of characteristic structure, taste, flavor and odor. On the other hand, excessive salt consumption brings many health risks. For this reason, there has been pressure on the meat industry to produce less salty and/or unsalted products. In this study, the functions and reduction strategies of salt in meat and meat products were evaluated.

Keywords: Meat, Meat Products , Salt, Salt Reduction, Salt Replacement

Investigating The Amount of Essential oils in Coriander (*Coriandrum Sativum* L.)

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ABSTRACT: Essential oils have been used for thousands of years for various purposes. Coriander, a herbaceous plant, was harvested, and its EOs were measured using a GC-MS. Essential oils evaluation was done in three replicates. A total of 24 essential oils were measured in Coriander. Linalool (3.46) was identified as the main component of coriander essential oil. The results also showed that the essential oils content of corriander contained more than 20% Germacrene. The amount of Decanal, Cyclodecane, 2-dococen-1-ol, Tetradecanal, Neophetadiene, Oleic acid, and phytol was considrale. The mentioned compounds were the main compounds amoung identified compounds in the coriander. The characteristics of the essential oils of plants, in general, are affected by many things, such as the plant's location, agricultural practices and environmental conditions, all of which significantly affect the composition of coriander chemical compounds. In this regard, more research on the changes in the essential oils of coriander is recommended.

Keywords: Coriander, Essential Oils, GC/MS, Food Chemistry, Plant Science

Investigating The Amount of Chlorophyll Changes in Coriander *(Coriandrum Sativum L.)* During The Spring Season

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ABSTRACT: Many studies have been conducted on the biological activity related to the content of chlorophyll derivatives, which show that these compounds are potential cancer prevention agents and have antioxidant and anti-mutagenic activities. It is also used as a color in food under the name of Natural Green 3 or E141. Considering the nutritional importance of chlorophyll, the amount in coriander was measured in three consecutive months of spring 2021. The results of chlorophyll contents that were measured by spectrophotometric application in coriander indicated an increasing rate in all the stages. The amount of chlorophyll increased significantly from 0.017±0.0033 mg.kg^{-1} in the first harvest to 0.74±0.00 mg.kg^{-1} in the last harvest in June.

Keywords: Coriander, Chlorophyll, Spectrophotometer, Food Chemistry, Plant Science

Optimization of Pheromone Dose and Trap Density for Mating Disruption of *Chilo suppressalis* (Lepidoptera; Pyriladae)

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ABSTRACT: *Chilo suppressalis* is an oligophagous pest that mainly feeds on rice and currently is largely controlled through the chemicals, leading to several ecological problems and often low control efficiency due to the larvae's obscure feeding habits. The alternative techniques are urgently needed to be developed, among which the use of sex pheromones is perspective, via means of large scale of trap and kill or mating disruption. The main purpose of the present research was aimed to optimize the pheromone dose and trap density for mating disruption of *C. suppressalis*. In first year of trials, we tested blend mixture of two pheromones (Z11-16: Ald, Z9-16:Ald) with equal ratio (50:50) applied at different doses i.e, 50, 100, 200, 300 and 500µg/20µl with various trap heights of; 3, 4.5 and 6 feet. Results revealed that, 200 and 300µg/20µl caught significantly more numbers of *C. suppressalis* and resulted in lowest damage percentage (white ear and dead hearts) as compared to other doses and insecticide treatment of Fipronal G 2 %. The second year´s trial was targeting to optimizing the trap density in which 8, 12, 18 and 25 traps/ha were tested with a median dosage 250µg/20µl, based on first year's results. It exhibited that 18 traps/ha captured maximum number of moths and reduced the significant damage symptoms followed by other 25, 12 and 8 traps/ha and Fipronal G 2% ($P < 0.001$). Therefore, 18 traps/ha with a dose of 250µg/20µl, could achieve better results for control the *C. suppressalis* in rice-growing areas and consequently minimum costs of applications as compared to the synthetic insecticide.

Keywords: Rice crop, *Chilo suppressalis,* Pheromones, Traps density and IPM

Evaluation of Rainfall Variations According To The Growth and Age of The Woody Ring

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ABSTRACT: This study integrates the data of the periodic radial increases carried out on samples of carrots and sections of Aleppo pine wood resulting from artificial and homogeneous reforestation and the corresponding rainfall slices at the same periods. The ratios of the relative deviations of successive tree rings (ERC) show a clear regressive trend in young trees. Thus the average sensitivities (SM) and the inter-dating coefficients (SR) respectively for young and older trees confirm the fairly strong dependence of the former on climatic factors, particularly rainfall. The results of this work show a fairly strong correlation between the periodic radial increase over 5 years and the rainfall section corresponding to the same period. Thus, from the analysis of the results obtained, it is established that the rainfall variations have a more marked influence on the young subjects. These various observations led us to ask ourselves a certain number of questions relating to the interpretation of the values of the average sensitivities and the inter-dating coefficients. Indeed, if on the one hand, all the samples of the same age have equivalent average sensitivities; however, it remains to be seen whether there is a definite relationship between mean annual rainfall and annual radial increment. On the other hand, the relationship between the periodic rainfall over 5 years with that of the current radial increase of the same periods remains established. On these bases, it seems that it is possible that from these results confirmed by the values of the correlation coefficients to think of placing the dendroclimatology of the Aleppo pine of this region of the Maghreb in a valid approach in the knowledge and the determination of the local Mediterranean climate, particularly the periodic five-year rainfall.

Keywords: Rainfall varations, Radial growth, Inter-dating coefficients (SR)

Evaluating the Effect of LED Regimes on Mizuna Red Plants in a Hydroponic Tier Module

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ABSTRACT: Growth rate, plant biomass and concentration of useful compounds largely depend on the quality and intensity of light. Plants of 'Mizuna Red' variety were grown according to low-volume technology in a regulated agroecosystem of tier hydroponic module produced by VIM (Russia). Plants were illuminated by specially designed LED lamps (VIM, Russia) with dynamically adjustable spectral composition on 4 channels. For experimental studies several modes of illumination have been included: continuous, pulsed and scanning with 15000 lx radiation intensity and total PAR of 321 mmol/m²s: blue - 97 mmol/m²s; green - 84 mmol/m²s; red - 122 mmol/m²s; far red - 18 mmol/m²s (Proportion B:G:R ~ 30:26:44). The aim of the study was to evaluate the effect of different irradiation modes on productivity, physico-chemical parameters and to develop technological methods of obtaining high quality commercial products of Japanese cabbage 'Mizuna Red' grown in the tier hydroponic module. The use of pulsed radiation regime allowed to increase the photosynthetic activity of plants 'Mizuna Red', which eventually influenced the growth of the above-ground mass and indicators of its quality with a strong correlation between these indicators. The concentration of photosynthetic pigments was dependent on the radiation regime. As a result of evaluating the effectiveness of using different sources of light radiation to increase the productivity of Japanese cabbage variety 'Mizuna Red' in artificial agro-ecosystems, the technological method of using pulsed radiation was determined.

Anti-arthritic Effect of *Ammodaucus leuchotricus In vivo*

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ABSTRACT: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation of the synovium, which outlines the inner cavity of synovial joints except for cartilage surfaces. It is a heterogeneous disease spanning several disease subsets with potential distinct pathogenic pathways. Some plant-derived agents are known to be immunomodulators that can affect both innate and adaptive immunity. Traditional medicine interventions have been used in the treatment of rheumatoid arthritis (RA) due to the limitations of conventional drugs. The present study aimed to evaluate the antiarthritic potential of methanolic extract of *Ammodaucus leuchotricus* at the concentrations of 150, 300 and 600 mg/kg in type 2 collagen induced rheumatoid arthritis in rats. RA was induced by intradermal injection of type 2 collagen (3 mg/ml) at the base of the tail and hind limbs of each rat. Methanol extract and methotrexate (MTX) were used as oral treatments from day 21 to day 42. Changes in body weight, lipid peroxidation markers and malondialdehyde (MDA), and histological sections were monitored to evaluate the severity of rheumatoid arthritis (RA). Results showed that RA induction caused a marked increase ($p < 0.001$) in MDA and MPO, and significant reductions ($p < 0.001$) in body weight and GSH levels. In addition, remarkable destruction of cartilage of limb articulation was observed compared to the normal control. Treatment with methanolic extract of *Ammodaucus leuchotricus* at different MTX-like doses significantly ($p < 0.001$) reduced the level of oxidative stress parameters MDA and MPO, increased the level of GSH and induced weight gain as well as restoration of histological pattern and cartilage compared to the negative control. In conclusion, these results suggest that the methanolic extract of *Ammodaucus leuchotricus* has anti-arthritic potential and therefore could be used as an alternative therapy for rheumatoid arthritis

Keywords: *Ammodaucus leuchotricus*, Anti-arthritic, Rheumatoid arthritis, MDA, GSH, MPO.

Design and Construction of Carrot Plug-cutting Machine for Optimal Processing

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ABSTRACT: In the most developing countries, some process in the food industry is done as non-mechanized. Theie automation is essential such as carrot plug-cutting operation that done fully hand work in carrot jam production. By automation of this operation through design an automatic carrot plug-cutter machine, the production costs and labour would decrease significantly and the quality of jam and the capacity of plant would increase. In this study, a new carrot plug-cutting system was introduced. This system consisted of a carrot lifter, vibratory mechanism, cutter unit and delivery unit with capacity of 1 ton per hour. The proper materials in construction were selected based on the food equipment and hygienic standards. The intended machine has the ability of transfer the carrots by roller and V-belts. Plug-cutting system has used flat disc blades with the speed of 2000 rpm and it was made from stainless steel. Plug-cutting of each carrot would be carried out in two stages. First, one side of carrot is plug-cutted, the transfer system rotates the carrots 180 degree and the other side of carrot will be plug-cutted. The developed system is easy to use with simple mechanism, inexpensive, low cost of repair and maintenance and adjustable.

Keywords: Carrot Jam, Carrot plug-cutting Machine, Design, Product processing

Design, Construction, and Evaluation of Saffron Flower Harvest Machine with Electro-Mechanical Mechanism

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ABSTRACT: Saffron is used as a medicinal product. More than 90% of Saffron is produced in Iran. Its manual harvesting is an expensive, time consuming and labor expensive operation. Therefore, Saffron harvester was designed, constructed, and evaluated. Width of cutting head was 15 cm and was consisted of 6 cutting units and every unit was made from rotational motion, two rotary ridged blades with diameter of 15 mm and rotational speed of 2570 rpm. A vacuum system was used to collect the flowers. Its field capacity was about 0.11 ha/h. The machine was equipped with selective system to recognize the flowers from other parts of plant. Therefore, the best detection and diagnostic system was chosen based on the machine vision system. Image segmentation was used to locate flowers in the image. System was able to detect the flower within the farm in less than 0.2 s. The system was coupled with mechanical part of machine and tested in Saffron farm. The detection performance of flower in field was 100% and the system was capable to operate the special blades according to flowers location by performance of 90%.

Keywords: Saffron, Harvesting machine, Cutting head, Selective system, Electro-Mechanical mechanism

Use Of Agricultural Saline Drainage Water For Irrigation Plants in ZAB EL GHARBI BISKRA Province

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ABSTRACT: The problems of salty soil and irrigation with salty water are not specific to any particular region of the world. In arid or semi-arid areas, the main purpose of agricultural drainage is often to provide runoff to control erosion, or to remove salt-laden percolating water from irrigated land to avoid the risk of soil salinization. The main objective of this study is to make a diagnosis of the quality of water drained to determine their suitability for agricultural use. Our site methodological approach adopted is to make a spatial study of the salinity and Sodicity of the waters, as well as and Sodicity of the waters. According to the C.E. and the S.A.R. They allowed us to detect water class as follow: Class C5-S2: contains water of excessive salinity with a medium danger of alkalinization. And according to the Wilcox diagram, we notice that all the waters of the samples are grouped in the bad class. Also Piper's diagram, it can be deduced that the waters analyzed from the different sites studied have a great similarity in terms of hydro chemical characteristics. The chemistry of the analyzed waters is characterized only by the chloride and sulphate facies calcium and magnesium.

Keywords: Agricultural, drainage water, irrigation, quality, saline, ZAB ELGHARBI

The Analysis of Chemical Hazards in Food Using Two-Dimensional Photonic Crystal

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ABSTRACT: Photonic crystal materials have unique properties, including a surface periodic structure, structural color, a large surface area, and easy integration with photoelectronic and magnetic devices. These properties make them ideal for the development of rapid, low-cost, and highly sensitive analytical methods for the detection of chemical hazards in food samples. The use of suspension array based on two-dimensional PC microspheres by droplet-based microfluidic assembly is a promising and powerful platform for food safety detection fields. Additionally, biological antibodies, aptamers, and molecularly imprinted polymers (MIPs) can be modified for specific recognition of target substances, particularly MIPs because of their low-cost and easy mass production. Based on these functional PCs, various toxic and hazardous substances can be selectively enriched or recognized in real samples and further quantified in combination with liquid chromatography method or optical detection methods including fluorescence, chemiluminescence, and Raman spectroscopy. The development of these PC-based detection methods for food safety is important for ensuring the safety of our food supply and protecting public health.

Keywords: Photonic crystal , Chemical hazards, Food, Microfluidic, Biological antibodies, Safety

Groundwater Contamination By Pesticides: Fuzzy Analysis

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ABSTRACT: Groundwater consumption of the inhabitants of agricultural areas is characterized by contamination in pesticides and various harmful chemicals produced human health. The runoffs of rainwater through the underground layers induce toxic residues in groundwater infiltration. The soil, the thickness, the nature of toxic products, the quantity of infiltrated water ...etc., and their effects on human health are uncertain parameters, complicated and imprecise. In this study, we propose a model based on the techniques of artificial intelligence including the principles of fuzzy logic in the analysis of such data. Based on field data, we established a predictive model relating the input parameters to their effect on public health as the system output variable. This allows acting upstream on the quality and quantity of pesticides and chemicals used in agriculture in order to preserve public health of the inhabitants of these areas.

Keywwords: Groundwater, pesticides, public health, fuzzy logic

Pesticides Toxiciyty: Fuzzy Analysis

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ABSTRACT: The importance of pesticides used in public health is used for the prevention and control of disease to vector transmission in humans. However, the long-term sustainability of the control of vector-borne diseases in the region is threatened by the development of resistance to insecticides and by the resulting exhaustion of the arsenal of both hazardous and dangerous insecticides cost-effective. Many factors can influence the soil contamination. The problem of the harmful effects of pesticides is the long-term consequences. Surface water has a direct influence on the degree of contamination by pesticides in groundwater. These are contaminated by significant infiltrations. Some factors are related to climatic conditions, others to the nature of the soil and others to the nature of the pesticide used. In this study, we propose a model based on the techniques of artificial intelligence including the principles of fuzzy logic in the analysis of such data. In addition to other factors involved in the process and are poorly understood or completely unknown. We are then into focus, which justifies the application of a fuzzy system in the data analysis. Based on field data, we established a predictive model relating the input parameters to their effect on public health as the system output variable. This is then to connect the inputs-output spaces through a database compiled from recorded data. This allows acting upstream on the quality and quantity of pesticides and chemicals used in agriculture in order to preserve public health of the inhabitants of these areas.

Keywords: Groundwater, pesticides, public health, fuzzy logic

The Effects of Different Concentrations of Cadmium on Germination and Physiological Parameters in Tomato (*Solanum lycopersicum* Lam.)*

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ABSTRACT: The aim of this study was to investigate to determine different concentrations of cadmium (Cd) on germination parameters and physiological effects in early developmental stage of tomato plant (*Solanum lycopersicum* Lam.). Cd concentrations were organized as Control (0 ppm Cd), Cd5 (5 ppm Cd), Cd10 (10 ppm Cd), Cd20 (20 ppm Cd) and Cd40 (40 ppm Cd). For this purpose, 10 tomato seeds were germinated in petri plates with bi-layered filter paper. Germinating rate and Vigor Index were determined. Tomato seedlings were cultured in hydroponic Hoagland's medium to explore physiological parameters such as length, fresh and dry weights and relative water content (RWC). Additionally, the effects of Cd on pigment and total protein contents of tomato seedlings were measured at the end of the experimental stage. 20 and 40 ppm applications of Cd resulted with significant decrease in germination rate compared to Control. Additionally, Cd concentrations caused significantly decrease in Vigor Index according to Control plants. Significant decreases in length, fresh and dry weight and RWC in shoot of Cd treated tomato seedlings were determined compared to Control. Root tissue was negatively affected from Cd treatments. Fluctuated results were determined for pigment and protein contents. As a conclusion, tomato seedlings were negatively affected from Cd stress.

Keywords: Cadmium, Vigor Index, Germination, Tomato, Heavy metal

Experimental Study of the Efficacy of the Extract of a Medicinal Plant *Thymus vulgaris* in the Fight against Onion Thrips

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ABSTRACT: This work aims to evaluate bioinsecticidal activity of extract of plant species, *Thymus vulgaris* growing in our arid region Biskra,Algeria, was evaluated on a predatory culture of onion *Thrips Tabaci*. With concentrations D1 = 25g / L, D2 = 50g / L and D3 = 100g / L, by the infusion method. These extracts were tested against *Thrips Tabaci* by contact-inhalation into petri dishes. The study of the bio-insecticidal activity of the plant species on Thrips tabaci showed an LD50 of 25.4 g / L.The results of our experiment D3 after 1 hour,

Keywords: Bionsecticidal, Extracts, Contact-inhalation, *Thymus vulgaris*, *Thrips tabaci*

Experimental Study of the Efficacy of Extracts of Medicinal Plants *Artemisia herba alba Asso* and *Peganum Harmala* in the Fight against Onion Thrips

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ABSTRACT : This work aims to evaluate bioinsecticidal activity of extracts of two plant species, *Artemisia herba alba Asso* and *Peganum Harmala* growing in our arid region Biskra, Algeria, was evaluated on a predatory culture of onion *Thrips Tabaci*. With concentrations D1 = 8g / L, D2 = 16g / L and D3 = 32g / L for *Artemisia herba alba* by the maceration method, and for the other plant D1 = 25g / L, D2 = 50g / L and D3 = 100g / L, by the infusion method. These extracts were tested against *Thrips Tabaci* by contact-inhalation into petri dishes. The study of the bio-insecticidal activity of the two plant species on Thrips tabaci showed an LD50 of 18.84 g / L for *A, herba alba* and 33.52 g / L for *P, Harmala*. The results of our experiment can be summarized as follows: The most effective plant is *A, herba alba*, (D3 after 1 hour), then *P, Harmala* after 1 hour and with dose D3. The results direct us to further work in order to use the extracts of these plants as an alternative to chemical pesticides.

Keywords: Bionsecticidal, Extracts, Contact-inhalation, *Artemisia herba alba Asso, Peganum Harmala, Thrips tabaci*

Investigation of the Effects of Three Different Generations of Fluoroquinolone Derivatives on Erythrocyte Fragility and Hematological Parameters in Rats*

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ABSTRACT: Fluoroquinolones (FQs) are synthetic broad-spectrum antimicrobial agents derived from nalidixic acid. They are used in the treatment of urinary tract infections, respiratory tract infections, skin infections, digestive system infections, genital infections and other bacterial infections. Although the relationship between FQs and oxidative stress has been demonstrated, their effects on erythrocyte fragility have not been adequately studied. In this study, the effects of three different FQ derivatives (ciprofloxacin (CIP), levofloxacin (LVX) and moxifloxacin (MXF)) on erythrocyte fragility and hematological parameters were investigated in rats at the end of the 1st, 7th and 14th day treatments. 72 *Wistar albino* male rats were divided into 4 groups with 18 rats in each group and sacrificed at three different time points (1st, 7th and 14th days). A significant increase in 0.2% sodium chloride (NaCl) concentration erythrocyte fragility value was found in the MXF group on 1st day compared to the control group on 1st day. While the FQ derivatives used in the study did not cause a general change on the erythrocyte and leukocyte parameters, they caused various fluctuations on the platelet parameters such as PCT, LPCR, PDW, MPW and PLT. As a further study, it would be beneficial to reveal the reasons for these effects on platelet parameters with more detailed studies.

Keywords: Erythrocyte fragility, Fluoroquinolone, Hematological parameters, Levofloxacin, Moxifloxacin, Ciprofloxacin

Comparative Study Between The Two States of Banana Peel and Fruit

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ABSTRACT: Banana fruit is widely consumed globally. Their peels represents around 30% of the weight of fresh fruit. It is an interesting source of bioactive compounds like polyphenols, carotenoids, and others. Thissecondary metabolites contribute to human health and have multiple biological effects such as antioxidant. The aim of this work is to extract the phenolic compounds from the different states of Algerian banana peels of *musa cavendish and the fruit* with a ternary solvent system composed of methanol / acetone / water; 7/7/6 (V/V). Polyphenols, flavonoids, and condensed tannins were evaluated. The results revealed that the fruits, yellow and green peels are rich in polyphenols with respectively contents of 365.51 mg GAE / 100 g ; 301.21 mg GAE / 100 g; 157.98 mg GAE / 100 g; in flavonoids (130.31, 78.31, 56.48 mg EC / g MS), and in condensed tannins with contents of97.14 mg / 100 g MS , 160.14 mg / 100 g MS , 70.31 mg / 100 g MS respectively.This result confirms that the yellow peels have the most polyphenols contents explain its use in traditional medicine.

Keywords: Banana peels, Banana fruit, Polyphenols, Flavonoids, Tannins

The Monogenea (Plathеminthes, Metazoan) Branchial Parasites of Triglidae Teleosts of the Genus *Chelidonichthys* (Kaup, 1873) from the Algiers Coast

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ABSTARCT: Examination of the gills of 663 specimens of Triglidae fish belonging to 3 species of the genus *Chelidonichthys*: *C.lucerna* (Linnaeus,1758), *C.lastoviza* (Bonnaterre,1788) and *C. obscurus* (Walbaum, 1792) fished along the Algiers coast allowed us to collect 211 parasites belonging to the Monogenea class. The morpho-anatomical study revealed the presence of 5 parasitic species attached to 2 subclasses: Monopisthocotylea (Odhner, 1912) and Polyopisthocotylea (Odhner, 1912) represented by the Capsalidae (Bosc, 1811) family and the Plectanocotylidae(Poche, 1925) family respectively. The Plectanoctylidae family includes 4 species of Monogenea: *Plectanocotyle gurnardi* (Van Beneden & Hesse, 1863), *P. lastovisae* (Ayadi, Tazerouti, Gey & Justine, 2022), *P. major* (Boudaya, Neifar & Euzet, 2006) and *Plectanocotyloides obscurum* (Euzet & Suriano, 1974). The Capsalidae family represented only by *Trochopus pini* (Van Beneden & Hesse, 1863). This work allowed us to know more about the biodiversity of Monogenea parasites in Algeria and in the Mediterranean.

Keywords: Monogenea, Parasites, Triglidae, Capsalidae, Plectanocotylidae, Algeria

Partial Purification and Some Optimization Studies of Phytase from *Pantoea Agglomerans* RK-79

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ABSTRACT: Bacterial phytase improves plant growth by breaking down phytic acid and increasing mineral availability, especially in phosphorus-poor soils. It also reduces environmental pollution by reducing the amount of phosphorus fertilizer needed. Overall, it is an important enzyme for plant growth and reducing the environmental impact of agriculture. In this study, phytase was first partially purified from Pantoea agglomerans RK-79 using the method of ammonium sulfate precipitation. For this purpose, the bacterial homogenate was precipitated by ammonium sulfate at ranges of 0-80%, and the phytase activity of the precipitate and the supernatant was examined to identify the range with the highest activity. The enzyme's ammonium sulfate precipitation range was determined, and its optimum pH was identified in the pH range of 2-11. Additionally, the optimum temperature range of the enzyme was found to be between 10-90°C. As a result of ammonium sulfate precipitation, the highest phytase activity was found to be 51.5 EU/ml in the supernatant after precipitation at the 60-80% range. The optimum pH of the partially purified bacterial phytase was determined to be 4, and the optimum temperature was determined to be 50°C. The study has results that can direct many fields such as optimizing the activity of the partially purified phytase enzyme, industrial production and laboratory research.

Keywords: *Pantoea agglomerans*, Phytase, Partial purification, Microbial enzyme

Experimental *in vitro* Contamination of Comistible Fish by an Antiparasitic Product: Consequences for *Alburnus alburnus* (1758)

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ABSTRACT: Thiamethoxam is a second generation neonicotinoid that presents a significant risk to aquatic ecosystems and human health due to its resistance to biological treatment. The objective of this study is to evaluate the effects of Actara (active ingredient thiamethoxam) that is widely used in the North-East of Algeria, on the fry of a freshwater fish, Alburnus alburnus. The effects of this insecticide were tested on fry growth, morpho-organometric indices, and macronutrient levels . Actara was added to the rearing water of the fry at a final concentration of 78 mg active matter/L, the previous parameters were determined at different times up to one monthe of treatment. The results obtained show that Actara has no significant effect on the hepatosomatic ratio of this fish and that this experimental period is insufficient to determine the alteration of growth induced by this compound. On the other hand, Actara significantly reduces the condition index from three weeks of treatment. On the other hand, and in our experimental conditions. The content of the hepatopancreas is also influenced by this pesticide. So this chemical stress may be affecting the food intake, the efficiency of conversion of food into nutrients and the energy metabolism in this non-target species.

Keywords: *Alburnus alburnus*, Neonicotinoid, Growth, Morpho-organometric indices, Marconutrients

New Insights for The Treatment of Diabetes*

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ABSTRACT: Diabetes mellitus (DM) is one of the most common diseases that people suffer from today as it was in the past. DM affected 171 million people in the 2000s, 425 million people in 2019, and is expected to affect 643 million people in 2030 and 783 million people in 2045 if required precaution are not taken. While worldwide DM health expenditures were 760 billion US dollars in 2019, this amount is expected to rise to 825 billion US dollars in 2030 and 845 billion US dollars in 2045. Since there is no definitive treatment for DM, studies on treatment methods of DM are increasing day by day. Diet, sedentary life, environmental toxins, stress, insomnia, and genetic predisposition cause to the development of DM. Oral antidiabetics such as sulphonylureas, α-glucosidase inhibitors, biguanides and insulin analogues are used commonly for the treatment of DM. Some monoclonal antibodies including teplizumab, peptides, and surgery are the treatment approaches used for the treatment of DM recently. We aimed to summarize the recent treatment approaches which are used in the treatment of DM in the present study.

Keywords:.Diabetes, Treatment approaches, Monoclonal antibodies, Peptides

In Vivo Evaluation of Biological Activity of Eriobotrya Japonica Leaves Extract

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ABSTRACT: The present study was planned to investigate the influence of Eriobotrya japonica extract on obesity induced by High-fat diet (HFD). 25 rats were divided randomly into five groups: Treatment groups were defined as follows: Group 1 (C): control rats fed with normal diet**,** Group 2 (HFD): rats fed with a high-fat diet, Group 3 (ND +EJ Extract)**,** Group 4 (HFD+ EJ extract), Group 5 (HFD+ orlistat): rats fed with a high-fat diet and treated with standard drug orlistat for 6 weeks. Body weight was measured every week during the experimental period. Plasma triglyceride and total cholesterol levels were measured. organs were collected for histopathological sections. It was demonstrated that loquat was effective in ameliorating the HFD-induced anti-obesity as well as in decreasing the levels of body weight (BW). HFD treated group showed decreased levels on TC, TGs, Low density lipoprotein (LDL), while HDL levels increased as compared to the normal control group after 6 weeks of treatment. The drop levels were 59%, 40%, 13% in TC, TGs and LDL respectively. Histopathological studies of tissues showed no pathological changes. It showed that EJ extract and Orlistat treatment reduced fat cell sizes in the treated HFD group. We concluded that Eriobotrya japonica leaves has potential as an anti-obesity agent which could be an important source of natural compounds for development of new drugs.

Keys words: Obesity, Eriobotrya *japonica*, High fat diet, Plasma, Histopathological

Ethnobotanical Study of Anticancer Medicinal Plants Used in The Region of Setif (Algeria)

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ABSTRACT: Cancer is a major public health problem. Modern treatments are based primarily on chemotherapy, surgery, and radiotherapy, with variable but significant and distressing symptoms and side effects. The use of complementary and alternative medicines, namely phytotherapy, is clearly increasing in all pathologies, in particular cancer. In this sense, a survey of three-month (February, March and April 2023) was conducted among herbalists, in order to contribute to the knowledge of medicinal plants used used for the treatment of cancer in the region of Sétif (Algeria). Respondents were mostly men, middle-aged (41-50 years old) and primary level. The analysis of the results obtained from the questionnaire sheets, allowed us to identify 39 medicinal plants belonging to 33 botanical families. The most represented family is Rosaceae, followed by Asteraceae; Lamiaceae; Fabaceae; Apiaceae; and Zingiberaceae. Leaves are the most parts of plants used, followed by seeds, roots and stems. The other parts (flowers, bark, fruits) are rarely used. Plants are mostly prepared in powder form. However, some plants are used in the form of infusion, or decoction. Finally, these results must benefit from scientific studies evaluating the benefits of the listed plants, and also their possible interactions with anti-cancer drugs.

Keywords: Algeria, Ehnobotany, Medicinal plants, Phytotherapy, Herbal medicine, Cancer

Antiobesity And Anti-inflammatory Activities Of S our Cherry Leaves Extract

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ABSTRACT: Obesity is a risk factor for several diseases which is associated with a state of chronic systemic low-grade inflammation. The objective of this study was the valorization of Sour cherries *Prunus cerasus* leaves with an evaluation of anti obesity and anti inflammatory activities (*in vivo)*. The aqueous extract was administered to rats on HFD at the end of the week 14 and continued until the end of treatment (20 weeks). The animals received Orlistat (30 mg/kg bw) along with diet. Carrageenan-induced rat paw edema model was used to investigate the in vivo anti-inflammatory action. Wistar rats were pre-treated with this extract orally before 1 h of injection of 1% carrageenan. The extract of *Prunus cerasus* decrease the body weight about 20 to 30% compared to HFD treated rats. Pre-treatment with sour cherry extract could inhibit the paw inflammation significantly which was elevated due to carrageenan induction. The results of blood parameters and histological sections of groups receiving the extract show a remarkable efficacy of this extract. Based on these results, the extract of sour cherry leaves can exerts an interesting anti obesity activity and significant anti inflammatory activity.

Keywords: *Prunus cerasus*, Anti obesity activity, Anti inflammatory activity, Leaves, Aqueous extract

A Study of Genetic Parameters of Growth in Some Tunisian Sheep Breeds

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ABSTRACT: This study aims to evaluate the growth performance of lambs of Tunisian sheep breeds. It involved 45780 lambs from 134 flocks on 99 farms. The zootechnical data, weights at standard ages (WAS), and average daily gains (ADG) for the period 2011-2016. The results obtained showed a significant difference in weights and daily gains at typical ages. Lambs from single births were heavier than lambs from double births (1.05±0.14kg and 1±00). Daily gains were influenced by the sex of birth ($P<0.05$). Single-born lambs showed higher GMQ followed by double births. The breed factor significantly influenced lamb growth ($P<0.05$); indeed, performance (weight growth and average daily gain) was better in the QFO breed. The age of dams showed higher GMQ in lambs from dams aged between 3 and 4 years, while the lowest was recorded in lambs from dams aged 5 years and over. The heritability coefficients for weights at different typical ages were estimated to be 0.16 ± 0.04 for GMQ3060 and 0.48 ± 0.03 for P10. The lowest values are observed for GMQ3060 (0.16± 0.021). The highest value is obtained for P10. Estimates of the genetic correlation for P10 with other parameters (P60, GMQ0030, GMQ3060) were in the range of 14-42%.

Keywords: Growth, Genetics, Performance, Sheep

The Validation of a Simplified Animal Welfare Indicator Protocol for the Assessment of Tunisian Dairy Sheep Welfare

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ABSTRACT: This study aimed to develop a simplified tool to assess sheep welfare from existing methods. For this reason, a survey was conducted in 10 Tunisian dairy sheep farms. Nine indicators were chosen depending on their validity, reliability, and feasibility (lean animals, bad fleece condition, fleece dirtiness, skin lesions, docked tail, lameness, hoof overgrowth, mastitis, and access to pasture), and their prevalence was evaluated according to the AWIN protocol. The farmers were then asked to rate the relevance of these 9 indicators on an unstructured scale ranging from 0 (not relevant) to 100 (extremely relevant). Results showed that the average percentage of lean animals was 26.98%. The percentage of animals with bad fleece condition and dirty fleece was 9.99 and 18.27%, respectively. A low percentage of animals with skin lesions was observed (1.657%). The relevance given to the indicators by the farmers was higher for mastitis, leanness, and lameness, with scores of 83.7, 80.0 and 77.5%, respectively. Our data allowed us to rank the farms based on the following formula: $\sum P1*R1 + \dots + P9*R9$. Different rankings may be obtained if a larger number of farmers or other stakeholder categories are interviewed.

Keywords: Sheep, Animal welfare, On-farm assessment, Simplification

Evaluation the Effect of Antibiofilm Activity of Bioactives Molecules Extracted from Plants

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ABSTRACT: The resistance of bacterial biofilms to host antibiotics has led to the search for alternative approaches for bioactive molecules of plant origin capable of destruction of biofilm. Essential oils are natural compound considered antibiofilm and antioxidant. This work aims to evaluate the antibiofilm activity effect of 3 extracts plants originating from Souk Al-Ahras against 5 clinical isolated pathogens. *Rosmarinus officinalis*, *Aloysia citrodora* and *Artemisia herba alba Asso* oils provided a yield of (0.93%), (0.24%) and (0.87%) respectively. These essential oils were extracted by hydrodistillation. The study of the antibacterial power by the micro-dilution method has confirmed certain properties possessed by these essential oils. Indeed, the latter showed an antibiofilm effect against both Gram-positive and Gram-negative strains in a dose-dependent manner. The highest antibiofilm activity was observed in *Aloysia Citrodora* extract oils against all tests pathogen bacterials. The results obtained in this study represent the high potency of natural products from medicinal plants as a source of biologically-active compounds for the development of future phytotherapeutic antibiofilms agents.

Keywords: *Rosmarinus officinalis*, *Aloysia citrodora*, *Artemisia herba* alba *Asso*, Bioactive molecules, Essential oil, Phytotherapeutic, Antibiofilm

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Bioactive compounds from medicinal plant belonging to the *Asteraceae* family, and evaluation of antioxidant activities

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ABSTRACT: Our study aimed to characterize the composition of ethyl acetate extract obtained from Asteraceae family, and evaluation of antioxidant activities. The secondary metabolites composition was established by high-performance liquid chromatography combined with mass spectrometry. The antioxidant activity was assessed using four different assays including DPPH, β-carotene, CUPRAC and reducing power assays. The HPLC-TOF / MS analysis identified for the first time the presence of 19 bioactive compounds. Rosmarinic acid and 4-hydroxy benzoic were detected as major components.caffeic, cichoric, gentisic, p-Coumaric, Protocatechuic, ferulic, vanilic, Protocatechuic and gallic acids as well as apigenin-7-glucoside, catechin, kaempferol and quercetin were also present. Furthermore, in DPPH, β-carotene, CUPRAC and reducing power assays, the ethyl acetate extract of Asteraceae family showed a strong antioxidant activity compared to antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) , tannic and ascorbic acids, in which the activities were almost equal to the standards.

Keywords: Asteraceae, Phenolic acids, Flavonoids, Antioxidant activity

Technological Properties of Lactic Acid Bacteria Isolated from Raw Milk

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ABSTRACT: Lactic acid bacteria (LAB) play an essential role in the fermentation and bio-preservation of food, which stimulates the search of new strains with technological properties. In this study, different raw milk samples were used in order to isolate lactic acid bacteria such us caw milk and goat milk. Preliminary identification revealed that isolated strains were lactobacilli, moreover, isolated strains were tested for their acidifying activity by measuring pH and Dornic acidity, proteolytic activity on MRS broth with peptone replaced by milk casein, lipolytic activity on MRS agar supplemented with olive oil, flavoring activity by testing acetoin production and antimicrobial activity by the agar well diffusion assay. Results showed that all isolates were able to produce lactic acid, able to hydrolyze milk casein, do not have a lipolytic activity, some of the strains produce flavors and all strains were able to inhibit the growth of the test organisms with the largest zone of inhibition by the viable cells being 21.60 mm against *Escherichia coli*. The results acquired in this study showed that the isolated strains have different technological properties which can be exploited in the dairy industry. These strains are therefore good candidates for further studies to elucidate their full potential.

Keywords: *Lactobacillus*, Lactic acid bacteria, Milk, Technological properties

Anti-Inflammatory and Anti-Obesity Properties of the Mixture of Two Leaves from the Rosaceae Family

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ABSTRACT: The prevalence of inflammatory diseases is on the rise around the world. The need for adjunctive therapies for weight loss and reduce inflammation has enhanced the progress in the pharmaceutical industry. *Eriobotrya japonica* and *Prunus cerasus* (Rosaceae family) leaves are popularly used against mouth sores and digestives disorders respectively. In this study, we investigated the anti-inflammatory and anti-obesity effects of aqueous extract obtained from the mixture of leaves of these two plants in wistar rats. Diclofenac sodium and Orlistat were used as standard for anti-inflammatory and anti-obesity treatments, respectively. The mixture of plants (*E. japonica* and *P. cerasus* leaves) was prepared with 1g of plant (50/50)/ 100 mL of water. Once obesity was established, the animals were treated with the aqueous extract during 6 weeks. Edema was induced with 1% carrageenan to *Wistar* rats after one hour of the oral treatments. Paw thickness was measured every hour (during 4h). The results showed a decrease in body weight in obese rats and a reduction in carrageenan-induced inflammation of the rats' paws upon treatment with the aqueous extract. The blood parameters and histological sections support these results. Our preliminary findings support herbal formulation for the prevention and treatment of obesity and inflammation.

Keywords: *Eriobotrya japonica, Prunus cerasus*, Leaves, Anti-inflammatory, Anti-obesity

Anti-Inflammatory and Anti-Obesity Properties of the Mixture of Two Leaves from the Rosaceae Family

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ABSTRACT: The prevalence of inflammatory diseases is on the rise around the world. The need for adjunctive therapies for weight loss and reduce inflammation has enhanced the progress in the pharmaceutical industry. *Eriobotrya japonica* and *Prunus cerasus* (Rosaceae family) leaves are popularly used against mouth sores and digestives disorders respectively. In this study, we investigated the anti-inflammatory and anti-obesity effects of aqueous extract obtained from the mixture of leaves of these two plants in wistar rats. Diclofenac sodium and Orlistat were used as standard for anti-inflammatory and anti-obesity treatments, respectively. The mixture of plants (*E. japonica* and *P. cerasus* leaves) was prepared with 1g of plant (50/50)/ 100 mL of water. Once obesity was established, the animals were treated with the aqueous extract during 6 weeks. Edema was induced with 1% carrageenan to *Wistar* rats after one hour of the oral treatments. Paw thickness was measured every hour (during 4h). The results showed a decrease in body weight in obese rats and a reduction in carrageenan-induced inflammation of the rats' paws upon treatment with the aqueous extract. The blood parameters and histological sections support these results. Our preliminary findings support herbal formulation for the prevention and treatment of obesity and inflammation.

Keywords: *Eriobotrya japonica, Prunus cerasus*, Leaves, Anti-inflammatory, Anti-obesity

Antibiotic Residues in Broiler Meat Marketed in Algeria: Public Health Risk*

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ABSTRACT: The objective of the present study is the establishment of an information basis on the contamination level of commercialized of white meat of broiler chicken by antibiotic residues; using the microbiological method of diffusion in agar, in particular to search for the residues of: Enrofloxacin, Tetracyclines, Macrolides and Colistine.This analysis based on the use of three bacterial strains sensitive to antibiotics which are Staphylococcus aureus, Pseudomonas auruginosa and Escherichia coli.

results from this study, shwon that 49 of the samples out of a total of 50 are positive in antibiotic residues; however, the different molecules sought during this study were detected with rates of the order of 96% Tetracyclines, 98% Macrolides, 46% Enrofloxacin and 28% Colistin. The presence of antibiotic residues in meat can be the cause of allergies, cancers, changes in the intestinal flora, bacterial resistance. So measures must be taken at several levels to preserve public health.

Keywords: Residues, Antibiotics, Broiler chicken, Public health, Algeria

Identification and Prediction of Allergenic Proteins in Lentils (*Lens culinaris* subsp) in Silico approach

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ABSTRACT: Allergy is one of the most common diseases in the world, with WHO having ranked it fourth among global diseases. This continues to be an open problem that affects also other areas of nutrition. Legumes are healthy foods, but cause allergies because they contain allergenic proteins, including lentils, which are consumed in most countries of the world. Research is discovering allergenic proteins in lentils such as Len c1; Len c2 and Len c agglutin, which are described in the Allergome database. Thus, the goal of the present study is to search for allergenic proteins of lentils (Lens culinaris subsp). The first step in this process was extracting all protein sequences of this lentil from the UniProt database, after testing many sequence alignment by Clustal Omega, Following epitope prediction by Bepipred Linear Epitope Prediction 2.0 and prediction of the allergenicity potential of the proteins by AllerCatPro 2.0. The principal theoretical implication of this study is that there in silico results show an identity of two proteins (sp|Q93WH6 t; tr|A0A173G7D5) with the allergen Lenc agglutin (P02870) is 99.64%; 99.62% . The epitope residues of these two proteins are almost the same as Lenc agglutin. Here, the present study suggests that both proteins of Lens culinaris subsp have allergenic power, thus provoking allergy.

Keywords: Allergy, Lentils, Healthy foods, In silico, Epitope prediction

Growth Analysis of Various Onion Genotypes for Yield and Pharmaceutical Traits

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ABSTRACT: Onion (*Allium cepa*) is an important crop at the global level and ranks fourth in terms of consumption. It has many uses, including cooking, as a dry powder, for medicinal purposes, and even in ice creams. Although it is a biennial crop, it is grown as an annual crop worldwide. Onions grow best in low temperatures, but bulb formation requires higher temperatures. The interaction between genotype and environment is crucial for growth and development and plays a significant role in growth analysis studies. The aim of this study was to determine the sensitive stage of different genotypes and their growth pattern at specific time intervals. The study was conducted at PMAS Arid Agriculture University Rawalpindi, Pakistan. Data for growth analysis was collected at seven-day intervals. Data on different yield-contributing and pharmaceutical traits were collected and analyzed. The results showed that early-maturing genotypes like NARC-1 completed their vegetative growth at 83-90 days after transplanting (DAT), while late-maturing genotypes like SWAT-1 continued to grow until 139-146 DAT. Most of the genotypes showed maximum growth at 104 DAT, which then declined after this stage. This study concludes that the SWAT-1 genotype is late-maturing but has high yield and pharmaceutical value. Furthermore, at 104 DAT, plants are more affected by temperature, which impacts each trait of all genotypes. The study also found that pharmaceutical traits were at their maximum during vegetative growth and before entering the bulbing stage. Crop growth rate and net assimilation rate were maximum in the early stages due to a higher number of leaves and plant height, which then reduced and decreased the photosynthetic rate. Finally, this study provides information on the growth pattern of different genotypes for various traits, which will be used in future breeding programs.

Keywords: Days after transplanting, Pharmaceutical value, Crop growth rate, Net assimilation rate

Development of Stage-Specific Onion Hybrids Through Heterosis Analysis at Four Main Growth Stages

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ABSTRACT: *Allium cepa*, commonly known as onion, is a valuable crop worldwide for its medicinal and culinary uses. However, its growth is highly sensitive to temperature changes during early stages, limiting its yield and profitability. While hybrid onion varieties offer higher productivity, disease resistance, and stress tolerance, they are expensive and lack stage-specific characteristics. To address this challenge, our study aimed to identify heterosis for stage-specific hybrids in onion farming. Over the course of three years, we conducted experiments using three lines and four testers, observing growth at four stages (73, 83, 123, and 138 days after transplanting) and analyzing variance in traits critical to yield and profitability. Our results showed highly significant improvements in all stages of growth for parent and cross interactions. Specifically, we found that the ANOVA of replication showed non-significance for plant height, leaf length, and bulb diameter, while the ANOVA of parents, parents vs. crosses, and Line x interaction was highly significant. Notably, MKS-8823GO × Super-Sarhad and MKS-8823GO × MKS-TPSWP hybrids were the best performers at the early stage for traits such as leaf weight and diameter, while 28540 × VRIO-2 was ideal for the second stage for traits such as bulb weight and yield. For the third and fourth stages, 28540 × MKS-TPSWP was optimal for traits such as bulb weight, yield, and dry matter content. These findings offer a promising solution to the cost and productivity challenges of hybrid onion farming, providing farmers with stage-specific hybrids for profitable and high-yield onion production.

Keywords: Heterosis, Line × tester analysis; Days after transplanting

The Development of Materials for Sustainable Alternatives to Non-Biodegradable Plastics

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ABSTRACT: The authors have demonstrated the creation of micro- and nanoscale photonic and plasmonic structures using pure cellulose and a blend of cellulose with non-edible agro-wastes. These materials meet the ISO 17556 standard for biodegradability in soil and seawater, indicating their potential as eco-friendly alternatives to plastic. The pure cellulose films are transparent in the visible to near-infrared spectrum, and their refractive index is similar to that of glass. The microstructured photonic crystals exhibit high-quality diffractive properties that are preserved even after prolonged exposure to water, suggesting that cellulose-based materials are robust in humid environments. Additionally, the authors have demonstrated that nanostructuring cellulose produces a biodegradable metasurface that displays vivid structural colors. The application of Ag on the metasurface enhances its plasmonic properties, which can be utilized for surface-enhanced Raman scattering and the production of plasmonic colors. Overall, these results indicate that cellulose-based materials are a promising alternative to plastic for high-end photonics and plasmonics applications, while also being environmentally sustainable.

Keywords: Photonic crystal , ISO 17556, Biodegradability, Eco-friend, Plastic, cellulose films

The Use of Bacteriophages in the Vaccination Industry for Aquaculture*

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ABSTRACT: Bacteriophages, more commonly known as phages, are viruses capable of infecting and replicating bacterial cells. They are assembled from two main types of biomolecules, nucleic acids and proteins, the latter forming a capsid and the former being encapsulated. In eukaryotic hosts, phages are inert particulate antigens and cannot cause pathogenesis. Bacteriophages have attracted great interest in vaccine design. This includes the use of phage display technology to select antigens, the use of engineered phages that display target antigens in vaccine formulations, and phage DNA vaccines. However, the development of these approaches has been limited in part by uncertainty about the underlying mechanisms by which phages induce immunity. This has hindered the clinical development of this technology. Research has shown that the use of phages can soon replace vaccines and control diseases.

Keywords: Bacteriophage, Vaccine, Fish, Viruses, Replacing, Control disease

Prevention of *Vibrio vulnificus*: a Threatening agent to Human Health

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ABSTRACT: Vibrio species are the most common bacteria in the marine environment that can infect humans through seafood consumption or direct contact. Nowadays increasing in aquaculture has caused a growing trend of bacterial diseases. Therefore, prevention, monitoring, early culture and diagnosis are crucial. The present study has provided to familiar with *V. Vulnifcus*, clinical symptoms and investigation of preventive ways to keep human health. To begin, *V. vulnificus* is one of the most important pathogens which has been detected in water and damaged aquatic tissues. According to the growing aquaculture industry that has made some jobs for people in coastal areas, Vibriosis should be considered a threatening issue for people and communities. Moreover, *V. vulnificus* has known as an opportunistic and transmissible bacterium to others. Hence, monitoring seawater and aquatics is an excellent idea to prevent any problems or infections, especially for humans at risk. On the other hand, increasing people's awareness about this pathogen due to its significant impacts is vitally important. To conclude, *V. vulnificus* not only harm the marine environment but also creates problems for humans. As a consequence, any bacterial agent, especially zoonosis is considerable due to its transmission to humans, and it requires compliance with the principles of prevention and rapid identification of the bacterial pathogen.

Keywords: *V. vulnificus*, prevention, zoonosis, Bacteria, Human, Sea food, Vibriosis

Molecular Characterization of Cotton Leaf Curl Multan Betasatellite Causing Leaf Curl Diseases on Chilli Plants in Multan, Pakistan*

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ABSTRACT: Cotton leaf curl disease on the Chilli (*Capsicum* spp.) plant acts as a limiting factor for its production and has become a widespread burning issue in Pakistan. An investigation of a chilli field in 2018 showed that the few chilli plants were showing severe leaf curling and yellowing and appeared stunted. A total of 39 infected samples with observed symptoms were collected from five different locations (Qasba Marla, Lar, 5-Faiz, Makhdoom Ali, and Rattan Hatti) in district Multan, Punjab, Pakistan. The DNA of infected samples was extracted in the plant virology laboratory at the Department of Plant Pathology, Bahauddin Zakariya University, Multan. Molecular characterization of extracted viral DNA through polymerase chain reaction (PCR) using Av/Ac Core, Beta 01/02, and CLCuMuBF11/R33 primers was done, and the positive amplification with Av/Ac Core, containing the amplicon of 579 bp, with Beta 01/02, the amplicon of 1.4 kb, and with CLCuMuBF11/R33, the amplicon of 481 bp, showed the confirmation of begomovirus infection of an infected Chilli plant with 71.79% of infection. The sequencing result of betasatellite in amplified positive samples revealed the association of Cotton leaf curl Multan betasatellite (**MT668934**). Thus, the prevalence of betasatellite in important agricultural crops, especially in Chilli fields, is a major concern, and the successful identification and management of these begomoviruses is necessary to stop the further spread of this virus.

Keywords: *Cotton leaf curl virus*, Begomovirus, Geminiviridae, Stunded growth, Phylogenetic analysis

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Chemical and Lipid Fraction Characterization of Algerian Traditional Meat Product: Khliaa Ezir During Ripening

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ABSTRACT: Khliaa ezir is a typical Algerian traditionnal cured cooked meat product, ripened in a mixture of olive oil and melted bovine fat, and preserved in earthenware jar (Ezir) at room tempertaure for nemerous months. The latter is a quite similiar to Kavurma a traditionnal turkish meat product. The aim of this study is to follow in kinetics the changes of the physicochemical properties (pH, moisture, color) of *Khliaa Ezir* as well as the lipidic fraction of the fat (animal fat and olive oil) in terms of : peroxyde value, acid value,during repining and that affects the quality attributes (tenderness, color,flavor) and prevents the oxydation. The results showed that :The variations of the pH during the 4 months of conservation are ranging between [5.47 - 5,73] ± 0,1 which favor a set of reactions mainly lipolysis, proteolysis and oxidation of pigments. For the myoglobin concentration, a destabilization was observed which seems to be affected by the different steps of the preparation. However, a development of a red-purple color to pink is remarkable following the formation of a pseudo oxy- myoglobin during the repning.The evolution of the humidity level showed a progressive fall during the preparation and marinating however during the four months of maturing, the humidity was stable. The lipidic characterization of Khliaa Ezir showed the effect of the fat on the organoleptic properties. Thus, we noticed the decrease of peroxide index to 5 meq/kg due to the condiments of the marinade which play the role of antioxidants and delay rancidity. For the free acidity rate we observed an increase during the four months of conservation due to the hydrolysis of triglycerides during lipolysis and the release of free fatty acids which improves the tenderness and the aroma of Khliaa Ezir. It could be concluded that fat fraction (olive oil, animal fat) reduce the oxydation of khliaa Ezir during repining and it contributes to the elaboration of the preservative and sensory characteristics of the final product in terms of (color, tendreness,flavor).

Keywords: Lipids, Meat product, Khliaa Ezir, Oxydation, Olive oil, Animal fat

Chromatographic Profile of Phenolic Compounds in Organic Fractions from *Hertia cheirifolia* Root Extract

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ABSTRACT: *Hertia cheirifolia* L. is one of the aromatic medicinal plants that have been used for a long time in folk Mediterranean medicine. The present study aimed to analyze the phenolic composition of different organic fractions prepared from the *Hertia cheirifolia* root extract (HC-RE). First, the HC-RE was délipidated using n-hexane solvent. Then, the delipidated phase was fractionated using organic solvent in a decreasing polarity order (chloroform then ethyl acetate). The obtained fractions were subsequently analyzed by a high-performance liquid chromatography-diode array detector (HPLC-DAD) system. The organic fractionation resulted in three different fractions (chloroform, ethyl acetate and aqueous) with different yields. Furthermore, the HPLC-DAD analysis led to the identification of six phenolic components. The highest total phenolic content (TPC) was found in the chloroform and aqueous fractions (7.92 ± 0.31 mg/g dw and 6.38 ± 0.32 mg/g dw, respectively). Our results demonstrated that the phenolic profile of the organic fractions from *H cheirifolia* root extract is probably related to the diverse chemical structures' behaviour according to the fractionation solvent's polarity. Therefore, the quality and quantity of bioactive compounds in *Hertia cheirifolia* root extract could be determinant for its medicinal properties.

Keywords: Hertia cheirifolia, Root extract, Organic fractions, Polyphenols, HPLC-DAD

Enhancing of the Drying Process Using Ultrasound Technology

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ABSTRACT: Because of enormous demand, shrimp processing is a large sector. The shrimp processing business wastes could attend 60% of the total volume. These wastes are high in bioactive substances such as chitin, protein, fat, carotene, and minerals. Bioactive chemicals derived from shrimp processing waste have a wide range of bioactivities and may be utilized as food, feed, and a component in functional food preparation. Shrimp waste utilization is now focusing on the bioremediation and energy conversion sectors. This work presents the application of ultrasound technology in the drying of a shrimp waste, by ultrasound assisted drying. The effect of main parameters and ultrasound technology on the drying kinetics was discussed. The temperature in the hot air dryer was fixed at 40, 50 and 60 °C, with an applied vibration frequency of 40 kHz. Results showed that for ultrasound assisted drying, the ultrasound power always gave a positive effect on the drying process, however, the magnitude of ultrasound improvement was largely dependent on the process variables, such as air temperature. The application of ultrasound technology will somehow affect the product quality, including the physical and chemical ones. Generally, the ultrasound application could be a promised process to treat large quantities of shrimp wastes.

Keywords: Drying, Ultrasound, Hot air drying, Shrimp, Wastes

Biodegradation of the Endocrine Disruptor, Dimethyl phthalate by *Enterobacter* spp.

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ABSTRACT: Mono-(2-ethylhexyl) phthalate (MEHP) is one of the Phthalates representatives; it is high-production-volume synthetic chemicals widely used in industrial applications, consumer products, food processing and medical applications.Mono-(2-ethylhexyl) phthalate (MEHP) is the active metabolite of di-(2-ethylhexyl) phthalate (DEHP), which is most commonly used as a plasticizer. MEHP has been proved to be harmful for human reproduction and affects human fertility. MEHP has been demonstrated to be a reproductive toxicant in rodent and human. MEHP fail to covalently link to the products containing them therefore it can easily leach from food packaging materials therefore MEHP and phthalates are found widespread in food and the environment due to their use as plasticizers in consumer products, food packaging materials, and biomedical devices. Intensive efforts have been made to develop methods to remove MEHP from the environment; the most common methods to remove pollutants from the environment included photo-degradation, photo-electrocatalytic oxidation, and oxidation, though bacterial degradation is a suitable method and promising way to remove these toxic compounds from the environment because of its safety and ecosystem friendly. The aim of this study was to isolate and identify promising bacterial degrading strains that can degrade high concentration of MEHP using the isolation by spread plate method and detection the degradation by Gas chromatography (GC). *Enterobacter* spp., a novel isolated strain was demonstrated to have a high efficiency to degrade the endocrine disruptor MEHP with (80%) completely degraded within 7 days of incubation.

Keywords: Mono-(2-ethylhexyl) phthalate, Pollution, Biodegradation, *Enterobacter* spp, Toxicity

Effect of The Cladode Mucilage of Prickly Pear Plant on Wastewater Treatment

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ABSTRACT: Prickly pear, whose scientific name is *Opuntia ficus-indica*, is a cactus species known for its many benefits for human health, its various food applications, and for his growing interest in wastewater treatment processes. Our objective is the extraction of the cladode mucilage to enhance electrocoagulation-electroflotation (EC-EF) wastewater treatment process: (i) for the removal of solution turbidity, mucilage was extracted using conventional and ultrasound-assisted extractions. The ultrasound extraction improved mucilage extraction yield and allowed a considerable time and energy saving: after 10 min of extraction time, the yield reached 60.77 ±1.07 %, while conventional treatment reached 36.71 ±1.07 % after 60 min at 80 ^{0}C. The addition of the mucilage to the treatment water process improved the turbidity removal efficiency by 30 %. (ii) For the removal of copper from wastewater, mucilage was extracted by microwave-assisted extraction. The copper reduction efficiency reached 100 % in less than 5 min with 30 mg/L mucilage, the sedimentation rate increases with mucilage by 15 %. These results encourage the use of mucilage as a natural coagulant to replace some chemical coagulants that are sometime added to the EC-EF process. Further studies should be done in order to understand the involved adsorption mechanism.

Keywords: *Opuntia ficus-indica*, Mucilage, Extraction, Electrocoagulation-electroflottation

Evaluation of Antihemolytic and Antioxidant Activities of *Santolina chameasyparissus* Extracts

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ABSTRACT: In recent years, much research has been directed towards the enhancement of traditional medicine in order to verify the safety and efficacy of medicinal plants and establish scientific rules for the use of these plants. In this context fits our work aims to verify the Antihemolytic and antioxidant activities of *Santolina chameasyparissus* extracts. Total phenolic content of the extracts were performed using Folin-Ciocalteu reagent and gallic acid as standard. As a result of the present study, Polyphenolic and aquouss extracts exhibited a significant antihemolitic activity. Polyphenolic extract exhibited a strong scavenging activity against DPPH radical and prevent β-Carotene bleaching in a concentration-dependent manner. This activity was higher than that obtained with the aqueous extract. The phytochemical screening reveals the presence of polyphenols in the crude extracts. These results demonstrated that there is a correlation between phenolic content of *Santolina chameasyparissus* extracts and antIhemolytic activity. The antihemolytic effect and the phenolic content of *Santolina chameasyparissus* extracts showed that this plant has a strong pharmacological power which supports its traditionally use.

Keywords: Antihemolytic activity, Antioxidant, Santolina chameasyparissus, Total phenolic content

Exopolysaccharide from *Rhodococcus pyridinivorans* ZZ47 Strain: Evaluation of Biological Activity and Toxicity

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ABSTRACT: Microbial polysaccharides are extracellular polymeric macromolecules excreted in microorganisms. These are widely used in food, cosmetic and pharmaceutical industries. One of them, exopolysaccharides (EPS), plays important role against the factors such as phage attack, antibiotics, toxic compounds or osmotic stress. Recently, this natural polymer has received great attention due to their therapeutic potential. The purpose of the study was to evaluate biological activity and potential toxicity of EPS from *Rhodococcus pyridinivorans* ZZ47 strain isolated from nature. The antioxidant properties of EPS were determined by DPPH free radical and hydroxyl radical scavenging activities methods and DPPH free radical removal was found to be 54% of EPS. It was not significantly changes cell viabilities of cancerous cell lines by MTT assay. EPS has no genotoxic effect on *Salmonella typhimurium* TA98, TA102, and TA1537 strains by Ames Test. No lethality was observed with single dose oral toxicity test of EPS and LD_{50} value of it is calculated by >2000 mg/kg in mice. In addition, EPS demonstrated dose-dependent anti-angiogenic properties by HET-CAM test. In conclusion, the isolated EPS has antioxidant activity with no genotoxicity and the biological activities of the polymer indicated that it may be suitable for use in different sectors.

Keywords: Exopolysaccharide, *Rhodococcus pyridinivorans*, cytotoxicity, genotoxicity, acute toxicity, anti-angiogenesis

A Contractual Agreement as a Type of Agricultural Contracts

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ABSTRACT: The formation of knowledge according to established principles is aimed at in-depth comprehension of the essence of the whole society. A systematic study of contracts, as well as the factor contributing to their emergence, allows you to more accurately understand their role, specificity, grounds of origin. The norms of the current Civil Code of the Republic of Moldova provide for the conclusion of unnamed (nameless) contracts, which represent the principle of freedom of contract, as a factor that presupposes the possibility of individuals to conclude contracts of various contents. This principle provides for the freedom to conclude contracts that are not provided for (contracts not named in the law), as well as mixed contracts.

Keywords: Analysis, Contractual essence, Specifics, Principle of freedom of contract

Development of Novel KASP (Kantitative Allele Specific PCR) Markers for Yellow Rust (*Puccinia striiformis* f.sp. *tritici*) Resistance Gene Yr5*

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ABSTRACT: Yellow Rust (*Puccinia striiformis* f.sp. *tritici*) is one of the most important fungal leaf diseases seen in wheat fields in our country. The most effective method of combating the disease is the use of resistant varieties. Endurance breeding studies are carried out all over the world for the control of the disease. The Yr5 resistance gene is still actively used in disease resilience breeding programs. Molecular markers are used safely to transfer resistance genes to new genotypes with high accuracy, regardless of environmental conditions. Within the scope of this study, PCR primers were designed by using the sequence information of a DNA region associated with the Yr5 gene. Whether the newly developed primers can be used to follow the Yr5 gene in known and unknown genotypes containing the endurance gene was tested by classical PCR analyzes. Among the genotypes studied, it was revealed by sequence analysis whether there was an SNP mutation in the replicated region that would allow the development of KASP markers. At the end of the study, no sequence differences were found in the replicated DNA region. However, it is understood that the developed primers form different DNA fragments in genotypes with and without the resistant allele of the Yr5 gene, even with classical PCR, and this difference can be used to follow the Yr5 gene in hybrid populations.

Keywords: Wheat, Yellow Rust (*Puccinia striiformis* f.sp. *tritici*), Yr5 Resistance Gene, Molecular Marker, Resistance Breeding.

POSTER PRESENTATIONS

Quality and Safety Assessment of Cheese Imported into The United Arab Emirates

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ABSTRACT: This study was performed to assess the quality and safety profile of different types of cheeses imported to the United Arab Emirates. The samples (n=3299) were analyzed for moisture content, acidity, fat content, the addition of an unpermitted colorant, addition of sorbic acid, and the presence of rust. The cheese samples were also subject to microbiological analysis to evaluate *E.coli*, *Salmonella*, *Listeria* and molds. Out of 3299 samples of cheese, 3014 comply with the cheese standards, and 285 were shown to be not conforming due to non-compliance with one parameter or a combination of quality parameters, thus indicating some levels of adulteration. The highest percentage of non-conformity (13%) was noted for processed cheese with 129 not-conform samples out of 1023. The quality assessment of cheeses has shown the highest percentage of moisture and fat non-conformity for processed cheese, with percentages of 65% and 17% out of the total number of non-conform processed cheese, respectively. The microbial assessment of cheese has shown the percentage of *E.coli* non-conformities for semi-hard cheese with followed by soft cheese, with percentages of 85.7% and 77.5%, respectively. Cheese sample non-conformities were grouped according to their country of origin. This showed the importance of food safety awareness to strengthen legislation in the Emirates dairy sector.

Keywords: Cheese, Fraud, Adulteration, Non-Conformity

Antioxidant Activity of Pomegranate Skin Grown in Ghardaïa (ALGERIA)

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ABSTRACT: The fruits of the pomegranate punica granatum as well as its flowers, seeds and bark are used by various ancient civilizations. According to modern research following a diet is the most obvious way to keep our body healthy and prevent various diseases. Pomegranate skin (Punica granatum L.) is an inedible part obtained during the processing of pomegranate juice. Pomegranate skin is a rich source of tannins, flavonoids and other phenolic compounds. Antioxidant and antibacterial properties of pomegranate peel in in vitro model systems have been reported. The aim of our work is to evaluate the skin effect of grenadine on antioxidant activity. The antioxidant activity of grenadine powder was carried out according to the DPPH method or following the protocol of (Krings and Berger, 2001). Our results of the analyzes carried out on the grenadine powder used are similar to those found in the bibliographical reviews. These results show that the average antioxidant activity of grenadine powder is (32.48 ± 0.39 mg/ml). We will conclude that the results found prove the quality of our grenadine powder and will allow us to continue our scientific and advanced research to make other more efficient techniques.

Keywords : Grenadine powder, Antioxidant activity, DDPH, Diet, Tannins, Flavonoids

Study of the Hepatotoxicity of Fenthion and the Usefulness of the Medicinal Herb Ephedra alata alenda

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ABSTRACT: Using pesticides for domestic or agricultural purposes can have detrimental effects on both human and animal health. These chemical products are known to be the root of numerous cases of intoxication that can result in the emergence of serious illnesses. Currently, research is focused on medicinal plants that have been identified as potential sources of several phytotherapeutic compounds with anti-oxydant properties. This context sets the stage for the current study, which aims to examine the phytochemical screening of the medicinal Saharan plant Ephedra alata alenda as well as its effects on hemato-biochemical changes, oxidative stress, and hepatic disorders caused by Fenthion in male Wistar rats. Our findings demonstrated that chronic exposure to fenthion (1 mg/kg/j) for 30 days had deleterious effects on the body, which were manifested in the general decline in the health of the rats (reduction in body weight, increase in liver weight, and decrease in brain weight), as well as depressive reactions linked to the induction of a liver oxydative stress signaled by a decrease in blood flow to the liver. The various components of this plant, including the flavonoids, alkaloids, and ephedrine, which have a potent ability to neutralize free radicals and protect the brain from oxidative stress, are likely responsible for the preventive effect of E. alata alenda against the toxicity of fenthion.

Keywords: Phytochemical screening, Fenthion, Ephedra alata alenda, Depression, Oxydant stress

Physiological Changes Induced by a Nutritional (hypoprotein diet) in Honeybee

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ABSTRACT: The bee is an indispensable part of agricultural production and food security. This insect faces several threats of anthropogenic and natural origin. Global warming is denounced as one of the major factors in the decline of bees. Estimates have predicted a global warming of 1.5 °C to 4.8 °C by the end of the 21 st century. The consequences of global warming affect not only bees but also the abundance of their honey resources. This can lead to nutritional deficiencies in bees, particularly in protein. To understand how bees react to protein deficiency, we studied the effect of pollen deficiency on the main physiological parameters in two sub-species endemic of Algeria, *Apis mellifera intermissa* and *Apis mellifera sahariensis* under controlled conditions. Emerging workers of both sub-species were reared with two diets: one was pollen-fed, whereas the other pollen-deprived. Several physiological criteria were measured depending on the type of diet and subspecies: the survival of the bees, the amount of total protein in the hemolymph, hypopharyngeal glands development and the ovary development of workers. With the same protein diet, the average life expectancy of *sahariensis* was extended by 5.55 days compared to *intermissa*. Even if deprived of pollen, *sahariensis* lived longer than *intermissa* fed with pollen. In the three age levels, the hypopharyngeal glands were more developed and less affected by pollen deficiency in *sahariensis* than in *intermissa.* The total hemolymph protein was higher in *intermissa* than in sahariensis regardless of the diet, and was also higher in protein-fed than in deprived bees. The ovaries developed more rapidly with a high proportion in *intermissa* than in *sahariensis* regardless of the diet, and were also higher in the bees fed with pollen than those deprived. Pollen deficiency generates physiological alterations and modifications, the amplitude of which varied according to the sub-species of the bee studied.

Keywords: *Apis mellifera intermissa, Apis mellifera sahariensis,* Pollen deficiency, Ovary development, Hypopharyngeal glands, Hemolymph protein, Lifespan

Can Asynchronous Hatching Be Explained by the Brood Reduction Hypothesis in Passerine? Incheon National University

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ABSTRACT: The Brood reduction hypothesis, which explains asynchronous hatching in birds, as an adaptation that enables selective survival of older nestlings when food is for insufficient for the whole brood. This study was conducted in order to determine whether the Brood reduction hypothesis can explain asynchronous hatching in passerines. Infrared cameras were installed inside nest boxes where Great Tits (*Parus major*) were attempting to reproduce in order to determine whether the parents practiced selective feeding of older nestlings. According to the results of the study, no significant difference was observed between the hatching order and the average number of feedings per nestling. In addition, when examining the distribution of food according to hatching order over time, every 30 minutes, beginning at 9 a.m., selective distribution of food to older nestlings was not observed. In conclusion, use of the Brood reduction hypothesis, which supports selective provision of beneficial feeding of older and larger nestlings, to explain the asynchronous hatching of passerines is problematic, thus conduct of future studies focusing on other hypotheses in order to explain the asynchronous hatching of passerines will be necessary.

Keywords: Hatching asynchrony, Brood reduction, Breeding strategies, Food allocation rules, Nestling provisioning

Effect of Different Pretreatments Methods and Convective Drying on Drying Behaviour and Quality Parameters of Strawberry Tree Fruit*

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ABSTRACT: Strawberry tree frui (*arbutus unedo* L.) has shown important medicinal and biological activities. However, few researches have monitored the effect of postharvest processing on quality parameters of arbutus unedo L. The aim of this work was to study the drying kinetics and quality parameters (color and shrinkage) of dried strawberry tree fruit by different pretreatments during convective drying at low temperature. Total color change (ΔE) and shrinkage percentage were assessed after the application of ultrasound and microwave assisted osmotic pretreatments followed by convective drying at 40 °C. Results of drying kinetics and quality attributes were compared to dry samples of strawberry tree fruit with no pretreatment. Convective drying of strawberry fruit samples fruits took from 24 hours to 33 hours, the use of ultrasound decreased the total drying time by 12 % while it was decreased by 27 % after the application of microwave pretreatment. The use of microwave pretreatment resulted in less color change and higher shrinkage percentage after convective drying ($p<0.05$). However, ultrasound assisted osmotic dehydration resulted in a significantly higher color deterioration and lower shrinkage values (59%) as compared to untreated samples.

Keywords: Strawberry tree fruit, Convective drying, Pretreatments, Drying kinetics, Color, Shrinkage

Modeling and Simulation of Low-Temperature Drying Kinetics of Strawberry Tree Fruit*

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ABSTRACT: The aim of this work is to study the thin layer drying behaviour of strawberry tree fruit experimentally, the comparison between the effect of ultrasound and microwave as pretreatments and to perform the mathematical modelling of the kinetics using different thin layer drying models given in the literature. Drying Experiments were performed after the application of ultrasound and microwave assisted osmotic dehydration at inlet drying air temperature of 40ºC and at a fixed drying air velocity of 2 $m.s^{-1}$. Different thin layer mathematical drying models were compared according to their R values and RMSE by non-linear regression analysis. Drying results indicated that the times required for drying of strawberry tree fruit until final moisture content of 15% (w.b.) took from 24 h to 33 h. The use of ultrasound and microwave pretreatment decreased the total drying time by 12 % and 27 %, respectively. The values of the effective moisture diffusivity of strawberry tree fruit drying when applying the Fick's diffusion model were ranged between 6.74×10^{-9} and 9.63×10^{-9} m^2/s, respectively. The Midilli *et al*. model was found to be the best to predict the drying of strawberry tree fruit. The values of drying constant *k* has decreased with the decrease of total drying time under the effect of pretreatments.

Keywords: Modeling, Kinetics, Strawberry Tree Fruit, Convective Drying, Pretreatments, Moisture Diffusivity

Agronomic Evaluation of F1 Hybrid for High-Grain Iron and Zinc Accumulation in Chickpea under Calcareous Soil*

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ABSTRACT: Globally, millions of people have micronutrient deficiency that has long-ranging effects on human health and productivity. To cope with this issue, the biofortification of food crops is an effective way for sustainable production and consumption. Chickpea (*Cicer arietinum* L.) is South Asia's largest-produced food legume and the third largest globally. This study aimed to evaluate the growth and yield performance of the F1 hybrid for high-grain Fe and Zn accumulation in chickpeas under calcareous soil. The nine genotypes of chickpea viz. control (V_o), high Fe (V_1 and V_2), low Fe (V_3 and V_4), high Zn (V_5 and V_6), and low Zn (V_7 and V_8) were investigated, and each treatment replicated thrice. A field experiment was laid out according to the randomized complete block design (RCBD) and performed in Pakistan during 2019-2020. In general, the high Fe F1 hybrids have a positive effect on boosting plant growth (plant shoot length, number of primary and secondary branches per plant) and yield attributes (number of pods per plant, number of seeds per plant, economical and biological yield) as compared to high Zn F1 hybrids and control. Conversely, low Zn F1 hybrids also have a positive effect on pod weight, the number of secondary branches, and biological and economical yield compared to High Zn F1 hybrids. In conclusion, the study provides preliminary information about high Fe hybrids that can be considered appropriate candidates for the biofortification of Fe in chickpeas under calcareous soil.

Keywords: F1 hybrids, Malnutrition, Micronutrient deficiency, Biofortification, Sustainable production

FULL TEXT

ORAL PRESENTATIONS

Tea cultivation in Turkey: A historical perspective

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ABSTRACT: Tea is one of the most important beverage in the world and it is popular throughout the world. Tea is a beverage as old as human history. Türkiye is one of the 30 countries in the world where tea plants are grown and tea is produced economically. The country improves knowledge and and cultivation of tea year by year and as of 2021, it exports tea to more than 30 countries. Tea cultivation in Türkiye is carried out mainly in the Eastern Black Sea Region, from the Georgian border to the Fatsa district of Ordu province. Rize alone meets 80-85% of Türkiye's tea production; Other provinces where tea is grown are Ordu, Giresun, Trabzon and Artvin. Unlike tropical and equatorial regions, where tea cultivation can be done for 12 months, tea is produced only for 6 months of the year in Türkiye and the harvest period, which starts in May every year, is completed in October or November. Since Türkiye is one of the newest countries for tea cultivation, it does not experience many diseases and pests. In addition, it is the one of the country in the world where snows cover tea plantations during winter months, which in this case poses an obstacle for diseases and pests.

Keywords: Tea, cultivation, unique characteristics, harvest period

INTRODUCTION

History

The tea plant was first stumbled upon by accident-as the story goes-in 2737 B.C.E. The emperor at the time was boiling water in his garden when a leaf from the overhanging *Camellia sinensis* tree drifted into his pot. The combination yielded a drink that compelled him to research the tree further, uncovering both medicinal and palatable properties (Chen et al., 2020). *Camellia sinensis* (or tea plant) is used to make most traditional caffeinated teas, including black tea, white tea, oolong tea, and green tea. This plant originated near the southwest region of China as an evergreen forest shrub. The leaves are glossy green with serrated edges and are similar in both shape and size to a bay leaf (Rath, 2020; Jiang et al., 2021).

Two varieties of the tea plant make up some of the most popular types of tea. *Camellia sinensis* var. *sinensis* (Chinese tea) is native to China and thrives in cool temperatures and high elevations. It is commonly grown on mountain slopes, producing a sweeter, gentler taste indicative of both green tea and white tea. *Camellia sinensis* var. *assamica* (Assam tea or Indian tea), on the other hand, thrives in the Assam region of Northern India. This plant is considered more tropical than its Chinese variety, growing larger and producing bigger leaves (due to a climate with plenty of rain and warm temperatures). This variety is used for robust teas like black tea or oolong (Ma et al., 2021; Chen et al., 2022)

World production

The most important producer is shown dark green color on map (Figure 1). Worldwide 5.966.467 tonnes of dry tea is produced per year. China is the largest tea producer in the world with 2.414.802 tonnes dry tea production volume per year. India comes second with 1.252.174 tonnes yearly production. China and India produce together more than 60% of World's total dry tea amount (FAO, 2022; Zha et al., 2022).

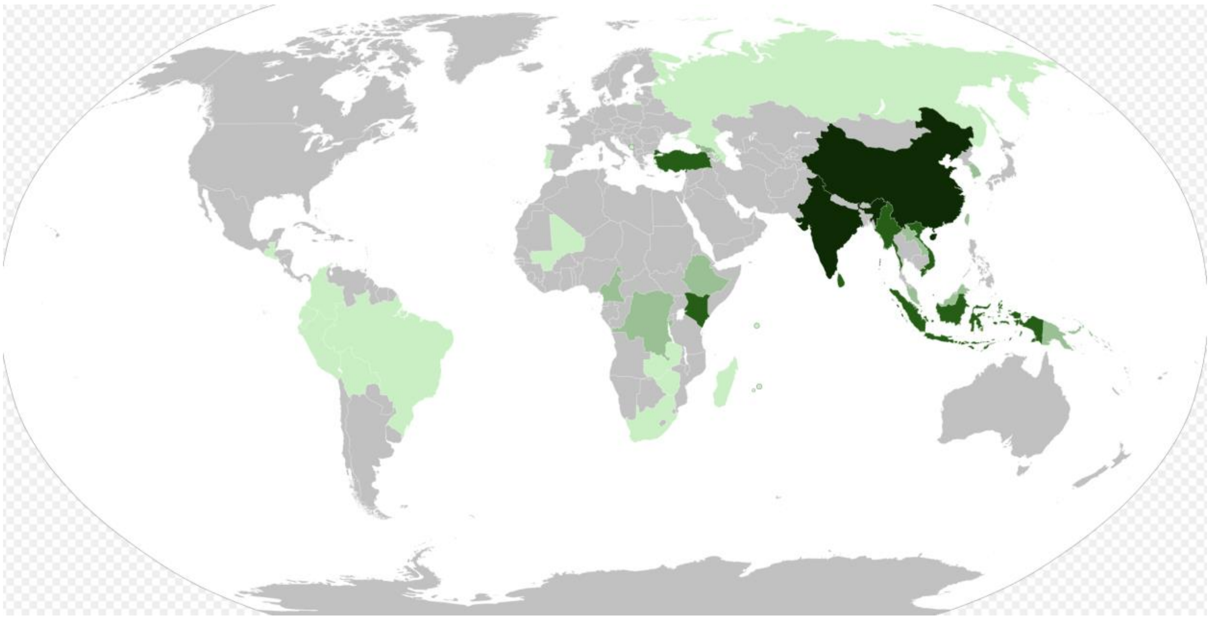

Figure 1. Tea growing areas in the World (green colored areas)

In terms of fresh tea leaves production, China produce 13.757.000 tonnes, and followed by India (5.482.186 tons), Kenya (2.338.000 tons), Türkiye (1.450.000 tons), Sri Lanka (1.302.000 tons), Vietnam (1.073.000 tons), Indonesia (563.000 tons), Bangladesh (393.000 tons), Argentina (339.288 tons) and Uganda (320.737 tons) (Table 1).

The results indicated that tea can be grown in different continentals in the World from Asia to Southern America (Figure 2).

Table 1. Fresh tea leaves production by countries

Ranks	Countires	Production of Tea Leaves in 2021
		(Tonnes)
1	China, mainland	13,757,000
2	India	5,482,186
3	Kenya	2,338,000
4	Türkiye	1,450,000
5	Sri Lanka	1,302,000
6	Viet Nam	1,073,000
7	Indonesia	563,000
8	Bangladesh	393,000
9	Argentina	339,288
10	Uganda	320,737
Source : FAOSTAT, 2022		

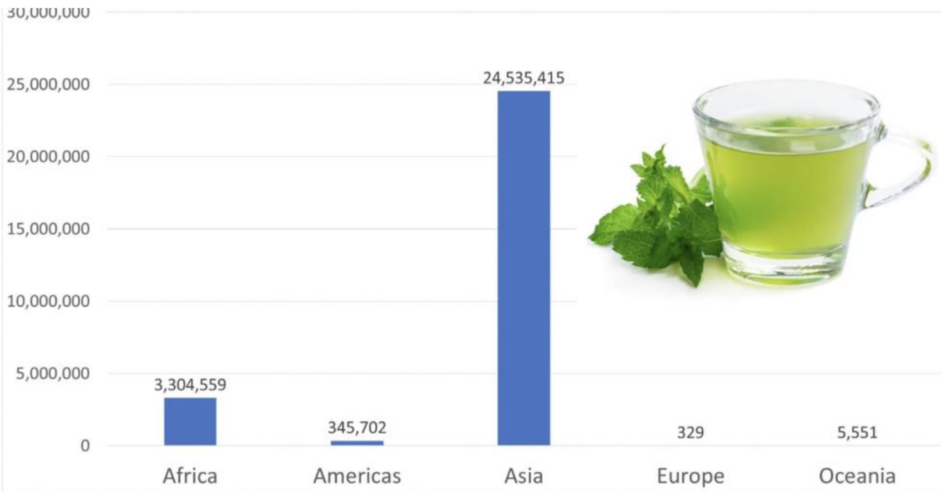

Figure 2. Production of tea leaves based on continent in 2021 (tonnes)

World production and harvested areas of tea are also growing steadily from 2000 year to 2021 year (Figure 3). But the increase is more visible area harvested rather than production amount increase.

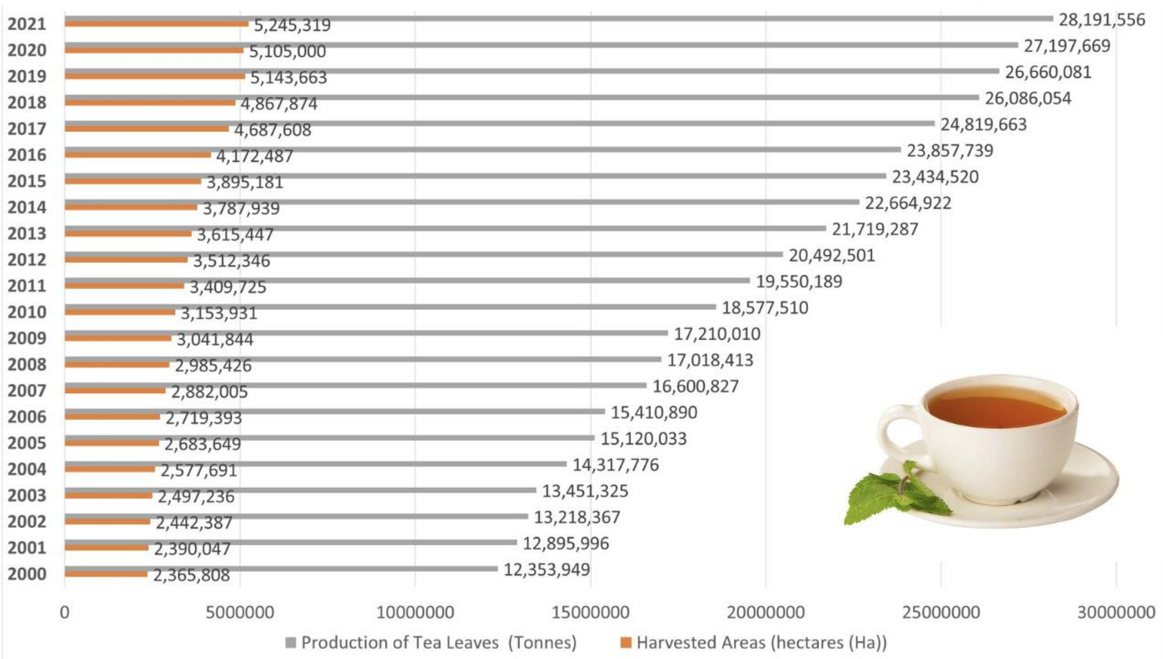

Figure 3. World's production of tea leaves and harvested areas from 2000 to 2021

History of tea in Turkey

Although there have been some attempts to grow tea in Turkey since the beginning of the 20th century, the history of tea production in the country dates back to the 1940s. The first known attempt to grow tea was during the Ottoman Empire, during the reign of Sultan II. It was realized with the planting of tea saplings and seeds brought from the Far East during the reign of Abdulhamid in Bursa; however, this attempt was inconclusive due to the unsuitable ecological conditions. In 1918, botanist Ali Rıza Erten, one of the instructors of Halkalı Higher Agricultural School, and Zihni Derin, General Manager of Agriculture after the First World War, conducted research on growing tea in the Eastern Black Sea Region. As a result of Zihni Derin's work in Rize, the first tea plantation was established in 1923 (Kafkas et al., 2009; Erturk et al., 2010; Ercisli, 2020; Yildirim and Karaca, 2022). Rize, Trabzon, Artvin, Giresun and Ordu are the main tea production area in Türkiye (Figure 4).

The following year, again with Zihni Derin's efforts, "Law No. 407 on Growing Hazelnut, Orange, Tangerine, Lemon and Tea in Rize Province and Borcka District" was accepted. However, due to unfavorable economic conditions, this initiative did not continue. The unfinished enterprise was revived with the work of a delegation from Ankara University Faculty of Agriculture, which was sent to the region in the second half of the 1930s. Raşit Hatipoğlu, who was in the delegation, wrote a book called "Tea Economy in Turkey"; after these studies, the first fresh tea leaf harvest and large-scale dry tea production in Türkiye started in 1938 (Seyis et al., 2018; Yurteri et al., 2019).

The first tea factory in Rize was ordered to England in 1940, but it was opened in 1947 with tea machines that could be sent after World War II. Dry tea production in Türkiye came to meet the domestic consumption in 1965 and tea imports stopped. After this date, tea exports began.

In 2004, Turkey became one of the largest tea markets in the world with a tea production of 205.500 tons (6.4% of the world's total tea production). Türkiye ranked first in the world with 3.16 kg tea consumption per capita, followed by Ireland and UK with 2.19 and 1.94 kg per capita.

Figure 4. Tea producing areas in Turkey

According to the data of the World Food and Agriculture Organization (FAO), 2% of the world's tea farming areas are in Türkiye and the country has a 4% share in world tea production in 2022.

According to the latest information, in the Eastern Black Sea Region approximately 205.118 producers are engaged in tea agriculture on an area of 791.287 decares. While the fresh tea crop yield has changed between 1.250-1.400 thousand tons on average in the last four years, it has exceeded 1.269 thousand tons in 2022 (TUIK, 2023).

There are 207 tea factories engaged in production in the tea sector. Of these, 47 are belongs to Çaykur (Turkish state owned tea producing company) and 144 are privately owned tea factories. 104 of these factories are located in Rize, 26 in Trabzon, 12 in Giresun and 2 in Artvin. The portfolio of Çaykur in general includes ice tea, green tea, organic tea and black tea.

In 2022, 1.269.238 kg of fresh tea was processed. 241.000 tons of dry tea was obtained from this fresh tea. 54.49% of the fresh tea produced was processed by the private sector and 45.50% by ÇAYKUR.

In the report published by the United Nations (UN) Food and Agriculture Organization (FAO); It is predicted that after 10 years, Turkey's annual tea consumption, which is approximately 270 thousand tons, will increase to 400 thousand tons. The Turkish tea industry should take this report into account and prepare the industry in this direction.

Some of the tea gardens (plantations) have already completed their economic life, and some of them have only a short time left to complete their economic life. Aged and inefficient tea gardens should be renewed urgently. This is the one of the most important problem of tea sector of Türkiye (Erturk et al., 2008; Yurteri et al., 2019). For the production of quality fresh leaves, the project of renewing the aged tea gardens with quality tea types within a certain plan should be implemented, and a medium-term agricultural policy of 5-15 and 30 years should be established for this. In addition, it is necessary to prevent new tea gardens made with seeds (generative propagation technique) and unlicensed in an uncontrolled manner, and to renew the gardens by multiplying high quality tea varieties with cuttings and tissue culture by vegetative propagation technique (Beris et al., 2005; Seyis et al., 2018; Yurteri et al., 2019).

Another important problem is the decrease the area in the amount per family by dividing the tea plantations by inheritance. Since the amount of products obtained from tea farming areas, which have become smaller through inheritance, has decreased, income to meet the needs of individuals cannot be obtained. In order to solve the problems arising from the shrinkage of tea agricultural lands, methods such as upper-use model, leasing of gardens or cooperatives can be developed that will make the sector more qualified and more economical (Seyis et al., 2018; Yurteri et al., 2019)

Authors' Contributions

SE designed and analyzed the research, SE studies arranged. GI worked on the preparation of GI 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.

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The Effects of Magneto Priming on Bread Wheat Germination and Seedling Development

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ABSTRACT: The aim of this study was to investigate how magneto priming affects germination and seedling development of bread wheat (*Triticum aestivum* L. cv. Besoztaja). Seeds were surface sterilized with sodium hypochlorite (3 %) and ethanol (80%). Seeds were imbibed in Hoagland's medium for six hours. After that, different static magnetic field forces (SMFF) (Control: No SMFF; 7.5-SMFF: 7.5 mT SMFF; 15-SMFF: 15 mT SMFF and 22.5-SMFF: 22.5 mT SMFF) were applied to imbibed seeds for one hour. 10 seeds were planted into petri dishes with bi-layered filter paper wetted with Hoagland's medium to calculate germination rate and Vigor Index. Magneto primed seeds were grown in hydroponic culture for 12 days to obtain physiological data. 15-SMFF application caused significantly increase in germination rate compared to other SMMF applications. Similarly, Vigor Index of 15-SMFF application was significantly higher than other SMFF applications. SMFF applications caused significant changes in shoot and root fresh and dry weights of bread wheat seedlings. On the other hand, shoot and root length and chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were not affected from SMFF applications. The investigation of the effects of magneto priming on different stress models could be considered as further studies.

Keywords: Magnetic field, Magneto priming, Wheat, Germination

INTRODUCTION

Seed priming is a well-established technique that has been widely adopted in agriculture and horticulture to improve seed quality, seedling establishment, and crop yield. There are various methods of seed priming, including hydropriming, osmopriming, biopriming, and hormone priming, which differ in the solution used, duration of soaking, and other parameters. Overall, seed priming offers a simple and cost-effective way to enhance the performance of seeds and improve the productivity and tolerance of crops in different growing conditions (Rathod and Anand, 2016). A static magnetic field (SMF) is a magnetic field that does not change over time. It is produced by a permanent magnet or a direct current flowing through a wire (Zhang, 2023). Unlike an alternating magnetic field, which fluctuates at a specific frequency and induces electric currents, a static magnetic field creates a magnetic force that can interact with magnetic materials or charged particles (Valberg et al., 1997).

Static magnetic fields are widely used in various applications such as medicine, industry, and research (Kabeel et al., 2015). Recent studies have also explored the potential of static magnetic fields in agriculture, particularly as a tool to enhance plant growth and productivity (Radhakrishnan, 2019). This technique, known as magneto-priming or magnetic field priming. Magneto-priming is a technique used in agriculture to enhance the growth and yield of crops. It involves exposing seeds or plants to a magnetic field prior to planting, which is believed to increase germination rates, accelerate plant growth, and improve overall plant health (De Souza et al., 2016).

This technique is based on the principle that magnetic fields can stimulate the movement of charged particles within plants, which can in turn trigger various physiological responses (Galland and Pazur, 2005). While the scientific evidence supporting magneto-priming is still limited, it has gained attention as a potential tool for sustainable agriculture that could help increase crop productivity and reduce the need for synthetic fertilizers and pesticides. In this context, magneto-priming has emerged as an area of active research and development, with ongoing efforts to optimize the technology and explore its potential applications. The aim of this study was to investigate how magneto priming affects germination and seedling development of bread wheat (*Triticum aestivum* L. cv. Besoztaja).

MATERIAL AND METHOD

Materials

Bread wheat seeds (*Triticum aestivum* L. cv Bezostaja) were used as plant material in this study. All chemicals were purchased from Sigma-Aldrich, Carlo Erba, Isolab, Tekkim and Duchefa.

Sterilization

Wheat seeds were surface sterilized with sodium hypochloride (3 %) for 10 minutes. Seeds were washed with sterile distilled water for four or five times. Additionally, seeds were treated with ethanol (80 %) for 30 or 40 seconds. Surface sterilized seeds were rinsed with sterile distilled water for several times. Seeds were used for further studies. Hoagland's Medium (hydroponic culture) was used to grow plants.

Magneto priming

Surface sterilized wheat seeds were imbibed in Hoagland's medium for six hours. After that, different static magnetic field forces (SMFF): Control: No SMFF; 7.5-SMFF: 7.5 mT SMFF; 15-SMFF: 15 mT SMFF and 22.5-SMFF: 22.5 mT SMFF) were applied to imbibed seeds for one hour.

Germination Test

10 seeds were planted into petri dishes with bi-layered filter paper wetted with Hoagland's medium to calculate germination rate and Vigor Index. Germination rate (1) and Vigor Index were calculated at the end of the fifth day as following formulas

Germination Rate (%) = (Germinated seeds/Sowed seeds) X 100

Vigor Index = (Seedling Length) X (Germination Rate)

Physiological parameters

Magneto primed seeds were grown in hydroponic culture for 12 days to obtain physiological data. Length, fresh and dry weights both of shoot and root tissues were measured and Relative Water Content (RWC) was calculated (Smart and Bingham, 1974).

RWC (%) = (Turgid weight - Fresh weight) / (Turgid weight - Dry weight) X 100

Determination of the pigment content

Pigment content was determined according to Lichtenthaler (1987). Chlorophyll a, Chlorophyll b, total chlorophyll and carotenoid contents were determined.

Statistical analysis

Data was presented as mean ± SEM (standard error of mean). GraphPad Prism 8 program was used as statistical program. One Way Anova Fisher LSD test was applied to compare groups. $P<0.05$ was accepted as statistically significant.

RESULTS AND DISCUSSION

Germination rate of 15mT application was statistically higher than 7.5 mT and 22.5 mT applications (Figure 1). The highest vigor index value was calculated for 15 mT application. This application caused significantly increase in vigor index value according to other SMF applications (Figure 1).

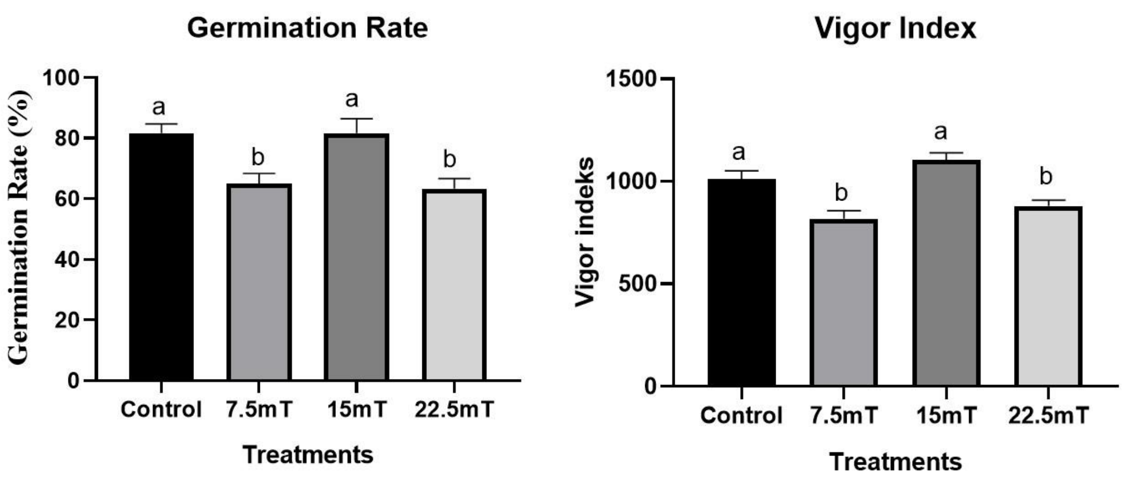

Figure 1. Germination rate and Vigor Index.

There was no significantly difference between treatments for shoot length and RWC. Magneto priming with 15 mT treatment caused significantly increase in shoot fresh weight according to Control seedlings.

The highest shoot dry weight was measured in the 22.5 mT treated seedlings. Additionally, 22.5 and 15 mT treatments caused increase in shoot dry weight compared to Control (Figure 2 and 3).

There was no significantly difference between treatments for root length and RWC. Magneto priming caused significantly increase in root fresh weight according to Control seedlings (Figure 4). The highest root dry weight was measured in the 22.5 mT treated seedlings. Additionally, 22.5 and 15 mT treatments caused increase in root dry weight compared to Control (Figure 4 and 5).

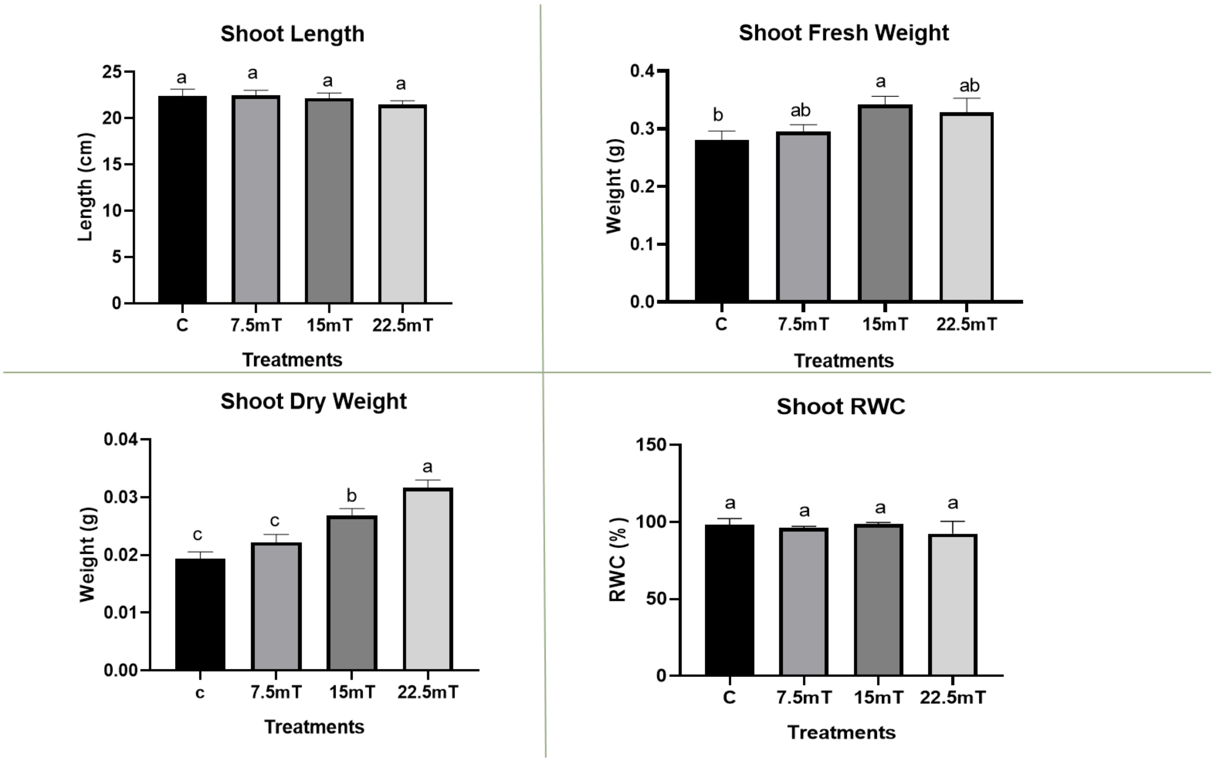

Figure 2. Physiological effects of magneto priming to wheat shoot tissue. Different letters on the column indicate significantly difference between groups ($p<0.05$).

Figure 3. Shoot length. Lines indicate 5 cm.

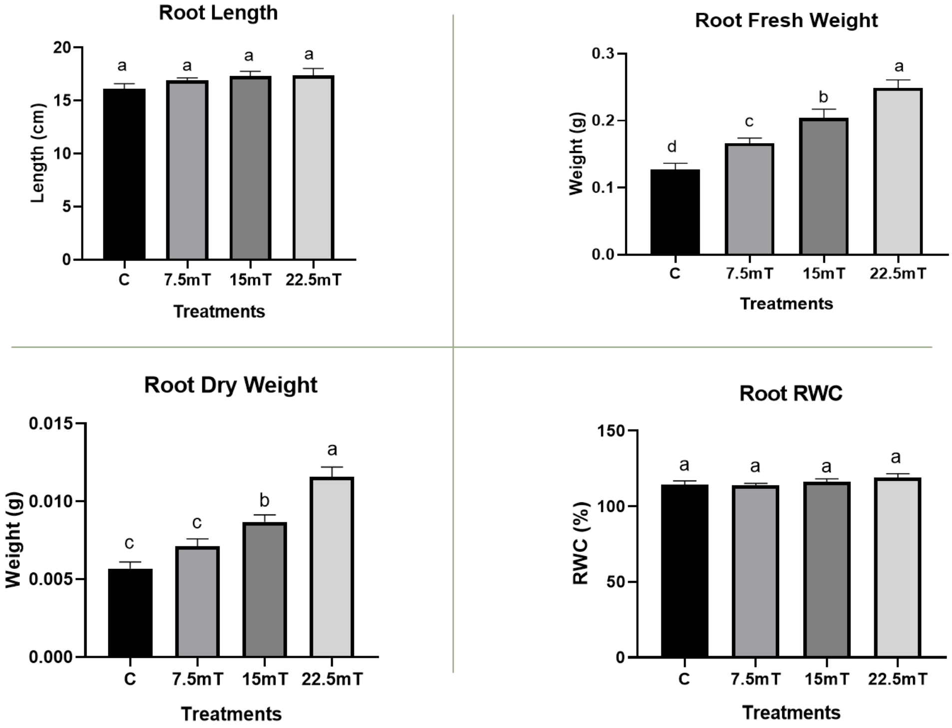

Figure 4. Physiological effects of magneto priming to wheat shoot tissue. Different letters on the column indicate significantly difference between groups (p<0.05).

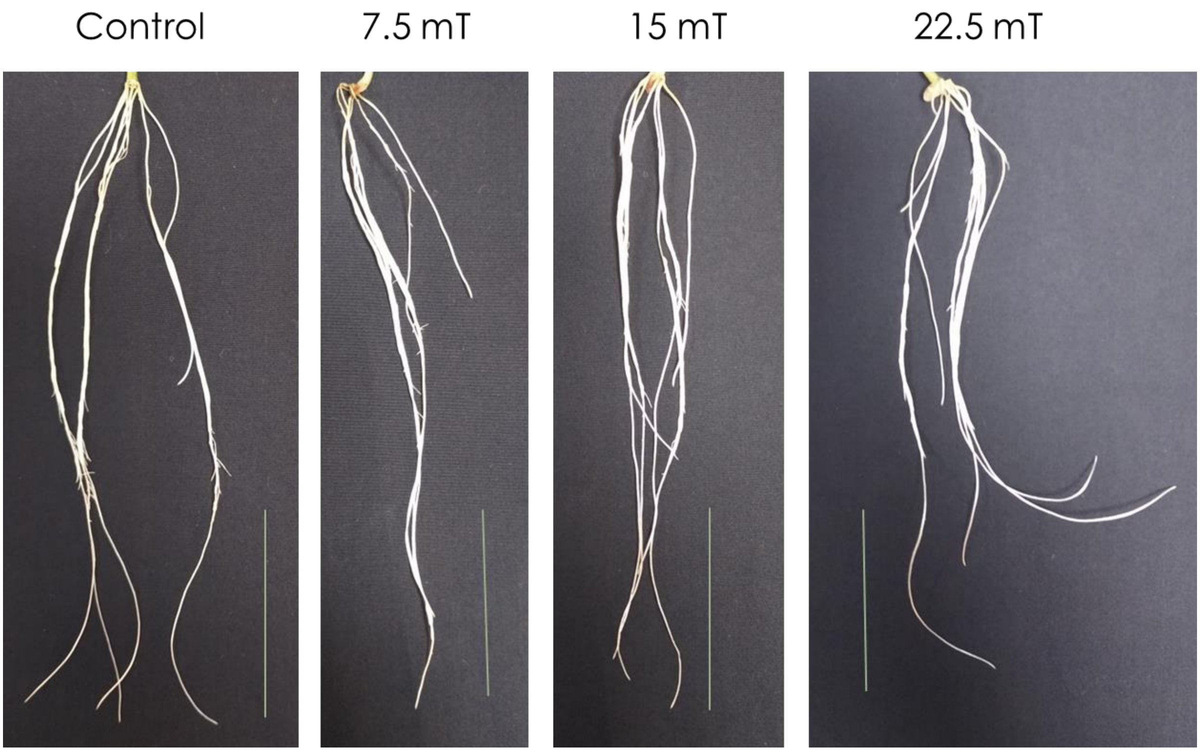

Figure 5. Root length. Lines indicate 5 cm.

Magneto priming did not affect pigment content of bread wheat seedlings (Figure 6).

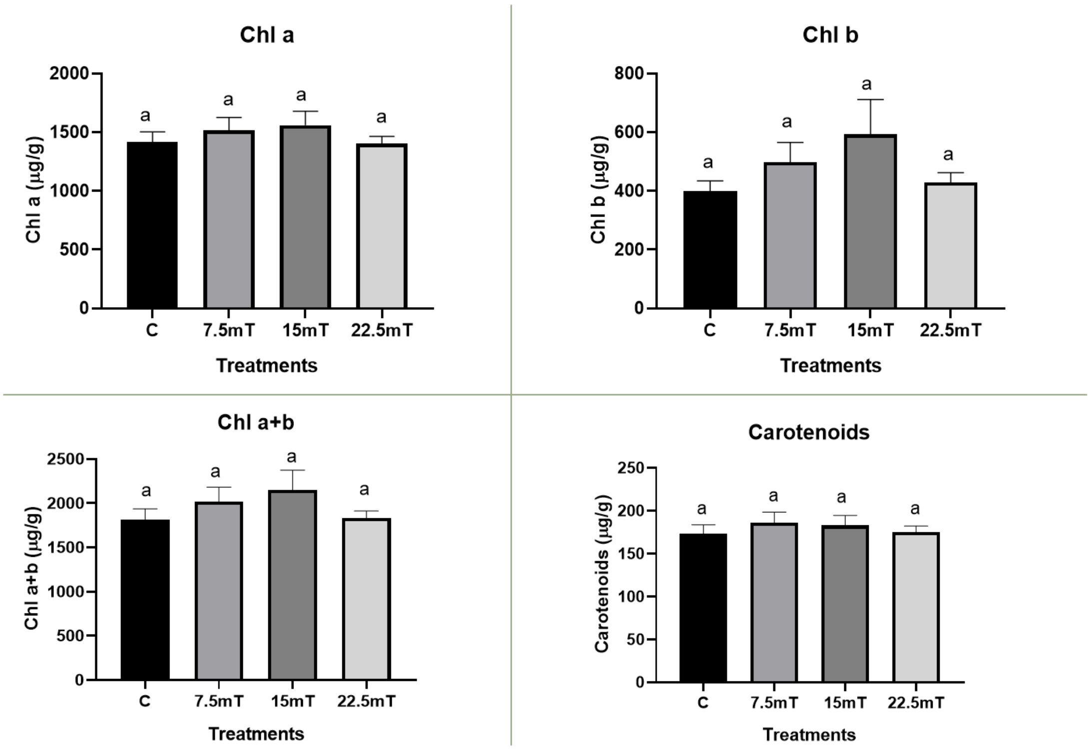

Figure 6. Physiological effects of magneto priming to pigments. Different letters on the column indicate significantly difference between groups ($p<0.05$).

Magneto priming, also known as magnetic field priming or magnetic stimulation, refers to the use of a magnetic field to enhance the germination, growth, and overall performance of plants.

This technique involves exposing seeds or plants to a specific magnetic field prior to sowing or transplanting, which is believed to stimulate cellular processes and promote stronger root development, faster growth, and improved stress tolerance (Kataria et al., 2019). Magneto priming has gained increasing interest in recent years as a promising and eco-friendly approach to enhance crop productivity and mitigate the effects of various environmental stressors such as drought, salinity, and heavy metal toxicity. In this context, numerous studies have investigated the potential benefits of magneto priming in different crops and under various growth conditions, highlighting its potential as a sustainable and cost-effective solution to improve agricultural productivity and ensure global food security (Baghel et al., 2019; Kataria et al., 2019).

CONCLUSION

SMFF applications caused significant changes in shoot and root fresh and dry weights of bread wheat seedlings. On the other hand, shoot and root length and chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were not affected from SMFF applications. The investigation of the effects of magneto priming on different stress models could be considered as further studies.

Statement of Conflict of Interest

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

Authors' Contributions

AB, ÖB and MEE designed and analyzed the research, AB, ÖB and MEE studies arranged. AB and ÖB 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.

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Effect of Essential Oil of *Ferula* sp. against *Pseudomonas viridiflava*, 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 *Ferula communis* and *Ferula orientalis* around the world and grows naturally at Erzurum province of Turkey against *Pseudomonas viridiflava* isolates, which are the agent of Soft Rot for some fruits and vegetables. For this purpose, 8 isolates of *P. viridiflava* which have been determined as the agent of Soft Rot in previous studies performed in plants such as potatos, onions, pepper, tomato, pumpkin, parsley and watermelon. As the positive control, Streptomycin antibiotics sold as ready produce were used. According to the obtained results, the essential oil has the antibactericidal effect of 0,9-25 mm against 8 isolates of *P. viridiflava*. It has been observed that the antibiotics used as the positive control has the antibacterial effect of 17-23 mm. In conclusion, the essential oil has the lethal effect against 8 *P. viridiflava* isolates which are agents of Soft Rot. It is assesed that these essential oil extracted from *Ferula communis* and *Ferula orientalis* can be used against these Soft Rot pathogens.

Keywords: Antibacterial activity, *Satureja hortensis*, Soft rot

INTRODUCTION

Belonging to Apiaceae family, genus *Ferula* consist of 180-185 species, which makes it the third largest genus in the family. These include disulfide compounds coumarin derivatives, sesquiterpene compounds, aromatic lactones, daucane esters (1). It contains more than 150 species, mainly in the Mediterranean and Central Asia. For hundreds of years, products from *Ferula* sp. have been used in traditional medicine for skin infections, diarrhea, killing intestinal parasites, malaria, and microbial diseases (2,3,4,5). Ferula sp. in Turkey are called çakşir, çakşir grass or çaşir. *Ferula* L., the third largest genus of the Apiaceae family, is 18 in the flora of Turkey, only 9 of which are endemic (6,7).

Herbal extractions and essential oils have been used for pharmacological purposes such as antibacterial, anti-fungal, antiviral, antiparasitic, insecticidal, and antispasmodic throughout history. Currently, they are bing used in the pharmaceutical, sanitary, cosmetic, agricultural, and food industries [8]. Essential oils are a type of liquid that is subtle, highly concentrated, aromatic, and volatile. This natural oils are mixtures of complex and volatile compounds produced as secondary metabolites by aroma plants [9]. Essential oils are valuable not only for their natural protective function for host plants but also because they possess properties that are several times more potent than dried herbs. Antibacterial, antimicrobial, antiviral, and anti-fungal properties, as well as certain specific therapeutic effects, render essential oils a precious factor [10-11].

The aim of the present study was to evaluate the antibacterial effect of *Ferula communis* and *Ferula orientalis* essential oil against *P. viridiflava strains* isolated from different plants.

MATERIALS AND METHODS

Bacterial Strains and Plant Materials

In this study, eigth bacterial strain was used, which cause soft rot on some plants. Whole strains were tested formerly and they were highly virulance strains. The aerial parts of *Ferula communis* and *Ferula orientalis* were collected in Erzurum (Turkey) during flowering between July- September 2022, and were air dried in the shade.

Isolation of The Essential Oils

The dried plant samples (500 g) were subjected to hydro distillation using a Clevenger-type apparatus for 4 h. The oil was extracted with $CHCl_3$ and then were dried over anhydrous Na_2SO_4 and stored under N_2 atmosphere at 20 ^{0}C in a sealed vial until use. The essential oil yield, which were based on dry materials of the plant samples.

Determination of Antibacterial Activities

Antibacterial activity assays were carried out according to [9] with a minor modification. The essential oil, by filtration by 0.45 µm Millipore filters. Bacterial suspension (100 µl) containing $1x10^8$ CFU/ml of bacteria spread by a sterile swab on Triptic Soy Agar (TSA) medium. The discs (6 mm in diameter) were impregnated with 12,5 µl of the essential oil (1g/ml dimethyl sulfoxide-DMSO) solutions, and put in the middle of the inoculated plates.

The bacterial cultures were incubated at 27±2 °C for 48 h, and then inhibition zones were measured in diameter (mm) around of the discs. DMSO used as negative control. Streptomycin used as positive control. The assays were performed with three replicates.

Determination of Minimal Inhibition Concentration (MIC)

The minimal inhibition concentration (MIC) values were determined by using the modified agar well diffusion method [10]. In the agar-well diffusion technique, a two-fold serial dilutions of the essential oil was prepared by diluting 10% DMSO to achieve a decreasing concentration range from 800 µm/ml to 3,125 mµ/ml. Using 100 µl of suspension containing $1x10^8$ CFU/ml of bacteria spread on TSA plates. The discs were impregnated with 12.5 µl of essential oil solutions. Then, they were put in the middle of inoculated TSA agar plates. The bacterial cultures were incubated at 27±2 °C for 48 h. The least concentration of the essential oil showing a clear zone of inhibition were taken as the MIC. DMSO was used as negative control. Streptomycin was used as positive control. The assays were performed with three replicates.

RESULTS AND DISCUSSION

The antibacterial activity of essential oils *Ferula communis* and *Ferula orientalis* were tested and the results that obtained are given in Table 1. It was determined that essential oil produced inhibition zones at varying ranges to the total of 8 bacterial isolates tested. The highest inhibition zone was observed against F-139 isolate with 25 mmin both applications . The MIC values for bacterials were determined to be 15.62-31.25 µL ml^{-1}. It was observed that the highest inhibition zone of the streptomycin antibiotic used as a positive control was against F-139 and F-176 isolates with 25 mm and the lowest inhibition zone was against F-786 isolates with 17 mm.

Table 1. Antibacterial activities of essential oil of *Ferula communis* and *Ferula orientalis*, 10% DMSO andStreptomycin

		Ferula communis		***Ferula orientalis***		**10% DMSO**		**Streptomycin**
Strain No	**Bacteria**	**DD**	**MIC**	**DD**	**MIC**	**DD**	**M**	**DD**

							IC	
F-108	*P. viridiflava*	10	31,25	11	31,25	-	-	23
F-128	*P. viridiflava*	13	31,25	12	31,25	-	-	22
F-139	*P. viridiflava*	**25**	**15,62**	**25**	**15,62**	-	-	25
F-173	*P. viridiflava*	13	31,25	10	31,25	-	-	21
F-176	*P. viridiflava*	13	31,25	12	31,25	-	-	25
F-421	*P. viridiflava*	10	31,25	18	31,25	-	-	19
F-764	*P. viridiflava*	11	31,25	10	31,25	-	-	22
F-786	*P. viridiflava*	14	31,25	10	31,25	-	-	17

DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 1.25 mg of the fractions; MIC, minimal inhibitory concentration in mg/ml; –, not active.

Therefore, the essential oil of the *Ferula communis* and *Ferula orientalis* plants have a lethal effect against all of the *P. viridiflava* pathogens which especially used in this study and causing soft rot in different hosts, suggests that the possibility of using this vegetable oil is high in combating these diseases.Copper compounds and antibiotics are used in the struggle against plant pathogenic bacteria and these applications have many disadvantages. Many studies have reported that phytopathogenic bacteria are resistant to many antibiotics over time. Therefore, the use of antibiotics is prohibited in many countries. For example; there are a huge number of studies indicating that there exist resistant strains of *Xanthomonas campestris* pathogens to kanamycin, ampicillin, penicillin and streptomycin [12,13,14]. In this study, it was observed that there was a medium or high resistance in pathogenic bacteria against the antibiotic tested.

As a result, the most important motivation of this study is being the first application of essential oil obtained from naturally grown *Ferula communis* and *Ferula orientalis* plants in Turkey against soft bruising of *P. viridiflava*. Successful results obtained from the essetial oil of the plant used in the study are also the first results obtained for this group of pathogens. The fact which has increased the importance of the study is the antibiotic used in the study has less effect compared to the essential oil. This is evidence that pathogens have become more resistant to antibiotics over time. For this reason, the results obtained from this study have included the conclusions and recommendations to organic agriculture and sustainable agriculture systems that have increased importance in recent years. Concentrations effective from non-working results will be tested in practical applications, and if the expected results are achieved, targeting and marketing of products intended to fight against these pathogens is targeted.

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Root Residues and Productivity Of Plants In Different Types Of Rotation And Continuous Crops

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ABSTRACT: The article mainly talks about the determination of root mass, amount of tiller residue and productivity of plants with different root structure in studies using short-rotational and continuous cropping. It is known that in the intensive farming system, soil fertility is increased by providing the necessary amount of organic and mineral fertilizers, its proper cultivation and taking necessary meliorative measures, and most importantly, by creating effective biological diversity in agricultural lands. So, it is important to give more space to the main plants, especially cereals, in the rotation crops. It should be noted that agricultural plants cause a large amount of organic matter to enter the soil in the form of root mass and stump residue, which in turn improves soil fertility with the nutrients it contains. During the conducted research, it was determined that the root mass of plants with different root structures and the amount of root residues are higher in rotational crops. According to the obtained results, the amount of root and shoot residues collected in the 0-40 cm layer of the soil per hectare is 3.8 s in winter wheat, 2.6 s in barley, 3.5 s in peas, 3.0 s in soybean, 6.1 in corn. etc. an increase of 2.1 s was observed for fodder peas and 2.0 s for rape. The results obtained from the studies show that the root mass and root residues, siderates, which enrich the soil with organic substances and nutrients, are of great economic importance. It is possible to increase the productivity by using cereal, cereal-legume and siderate plants in short-rotational crops in peasant farms.

Keywords: Root mass, Root residue, Plant rotation, Continuous cropping, Biological diversity, Productivity

INTRODUCTION

In our republic, research scientists are studying the system of measures that show the positive effect of soil fertility on its biological activity and the productivity of cultivated plants. Only an efficient farming system ensures the proper use of land and maximum yield with less labor and resources. Among the agrotechnical measures carried out in the direction of increasing soil fertility and increasing the productivity of cultivated agricultural plants by effectively using the arable land, the application of rotational crops is of particular importance.In the intensive farming system, soil fertility is increased by providing the necessary amount of organic and mineral fertilizers, its proper cultivation and taking necessary melioration measures, and most importantly, by creating effective biological diversity in agricultural lands (Boutin C. and et al., 2008).

So, it is important to give more space to the main plants, especially grain, in the rotation crops (I. Jaskulska and et al., 2012). There are wide opportunities for this in the Republic. In rapidly developing regions, intensive placement of fodder crops in rotational crops without harming the specific weight of various types of agricultural crops (cereals, cotton, tobacco, vegetables) is a must. In this case, it means protecting and restoring soil fertility by creating species diversity, as well as creating a fodder base in accordance with the current demand of animal husbandry, taking into account the specific characteristics of natural and economic zones. Scientifically based rotation of plants in the fields it also causes the increase of nutrients in the soil and their efficient use, the protection of soils from erosion by creating favorable water-physical properties, and the minimization of weeds, diseases and pests.

As a result of numerous studies, it has been proven that legumes play an important role in the restoration of soil fertility as the predecessors of the main crops. Because leguminous plants absorb nitrogen from the air and enrich the soil with nitrogen (C. Boutin et al., 2008, D. Jorgenson Ho et al., 2014, Ogorodnikov L.P., et al.,2015). These plants play an important role in the formation of forage and food base, as well as in changing the chemical composition of the soil. Compared to cereals, more humus, total nitrogen and other nutrients are accumulated in legumes (H.Shimono et al.,2002.

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 (T.H.Tamrazov, 2022).

MATERIAL AND METHODS

Numerous experiments were conducted on the territory of the Absheron Auxiliary Experimental Farm Research Institute of Crop Husbandry of in two schemes (rotational and continuous cropping schemes), three replicates under irrigation conditions. The soils of the Absheron AEF area have low fertility and are poorly supplied with basic nutrients and atmospheric sediments. The varieties of winter wheat "Gobustan", barley "Jalilabad-19", pea "Sultan", soybean "Biyson", corn "Zagatala-420", rapeseed "Radikal" and fodder pea "Azerbaijan 1508" were used as the object of the research. The root mass of plants with different root structures, the amount of tiller residues and productivity were determined in the studies using short-rotational and continuous crops.

RESULTS AND DISCUSSION

Agricultural plants cause a large amount of organic matter to enter the soil in the form of root mass and stump residue, which in turn improves soil fertility with the nutrients it contains (T.H.Tamrazov et al., 2022). In the conducted studies, the root mass of plants with different root structures and the amount of root residues were higher in rotational crops. According to the obtained results, the amount of root and shoot residues collected in the 0-40 cm layer of the soil per hectare is 3.8 s in winter wheat, 2.6 s in barley, 3.5 s in peas, 3.0 s in soybean, 6.1 in corn. etc. an increase of 2.1 s was observed for fodder peas and 2.0 s for rape (Figure 1).

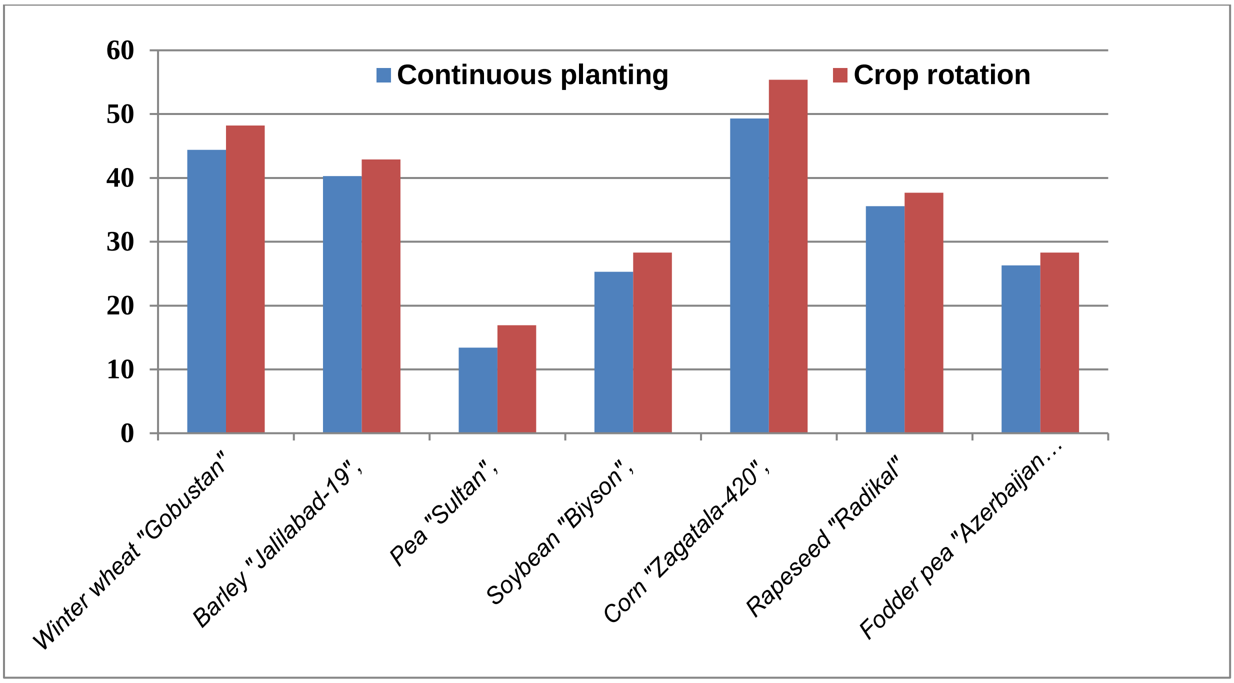

Figure 1. Depending on the planting schemes, the root-root residues of plants in the 0-40 cm layer of the soil, s/ha

It should be noted that the amount of root mass per hectare in a layer of 20-40 cm is 6.4 s in wheat, 6.0 s in barley, 2.1 s in peas, 3.5 s in soybean, it was 11.7 s in corn, 3.0 s in rapeseed and 4.5 s in fodder peas. In order to regulate the protein balance of the nutrition of the population and strengthen the food base of farm animals, the cultivation of cereals and legumes has become widespread in many countries of the world.In order to regulate the protein balance of the nutrition of the population and strengthen the food base of farm animals, the cultivation of cereals and legumes has become widespread in many countries of the world (Table 1).

Table 1. Productivity of plants at alternation and continuous crops, centner/ha

Crops	Plants									
	Wheat Gobustan Buğda	Barley Jalilabad-19	Pea Sultan	Soybean "Biyson		Corn "Zagatala-420		Fodder pea "Azerbaijan 1508		Rapeseed Radikal
	Grain			Grain	Green mass	Grain	Green mass	Grain	Green mass	Green mass + siderate
Continuous planting	36,7	34,8	8,4	22,1	248,9	63,8	585,9	34,3	576,2	369,0
Crop rotation	39,7	37,3	10,9	23,9	273,5	69,1	632,3	35,6	593,0	411,3

As can be seen from the table, the grain yield of winter wheat is 36.7-39.7 centners per hectare, depending on the planting scheme; 34.8-37.3 centners of barley; 8.4-10.9 centners of peas; 22.1-23.9 centners of soybeans; the yield of corn was 63.8-69.1 centners and fodder peas was 34.3-35.6 centners. In contrast to continuous cropping, the yield of green mass per hectare increased from 248.9 centners to 273.5 centners in the soybean bean formation phase, from 585.9 centners to 632.0 centners in the milk-wax maturity phase of corn. increased from 576.2 centners to 593.0 centners in the phase of fodder pea formation.

CONCLUSION

In the studies, the productivity of rapeseed variety "Radical" was studied both as green mass and siderate. Rapeseed as green mass was harvested in the third decade of April, the average yield was 292.0-269.3 s according to planting schemes. Then the field was watered again, after a month the plants were crushed and mixed with the soil. The yield of rapeseed as a siderate is 19.6 t/ha more than under rotational cultivation.

The results obtained from the studies show that the root mass and root residues, siderates, which enrich the soil with organic substances and nutrients, are of great economic importance. It is possible to increase the productivity by using cereal, cereal-legume and siderate plants in short-rotational crops in peasant farms. From the results of the given forecasts and numerous studies, it is known that in order to maintain the soil fertility parameters in the intensive farming system, serious consideration should be given to the creation of biological diversity in the rotational cropping system in order for the mentioned plants to have high ecological stability.

ACKNOWLEDGEMENT

First of all, we are very sorry for the disaster in our brotherly country! I ask God to have mercy on the dead! We express our gratitude to you for creating conditions for us to participate in the conference. I hope that the submitted article will make the conference more relevant.

Funding

The study was conducted by ourselves at the Institute

Statement of Conflict of Interest

The purpose of publishing this article is to publish our research and participate in the conference

Authors' Contributions

The results of the conducted research were extensively analyzed using 7 different plant samples on a table and a picture. I would like to mention that the role of continuous and rotational cropping schemes in increasing soil fertility in the country and direct productivity has been determined.

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Hawthorn Fruits: Medicinal Use for Centuries

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ABSTRACT: Hawthorn (*Crataegus* spp.) is one of the most important wild edible fruits found in Anatolia naturally and consumed by local people for centuries. Its resistance to diseases and pests under natural conditions, the fact that it contains many species with different fruit colors and appeals to different areas of use has made it possible to use it in Anatolia for hundreds of years. All hawthorn trees or shrubs propagated by seeds natural way in Turkey. In general it is a thorny tree or shrub with pink and white flowers that can reach almost 10 meters in height. It has red-brown, red or yellow fruits with 1 or 5 seeds. The slightly sour fruits of this tree, namely the hawthorn fruit, are eaten. It is rich in substances with abundant antioxidant properties. Hawthorn, which has a round appearance as small tree or shrub, is sometimes used as an ornamental plant. In Turkey, it grows in general as shrub in rocky and stony places, on slopes facing streams, in mountainous environments or in forests. In Turkey usually in October, hawthorn fruit is offered for sale on the stalls, in many markets with high price compared to the other pome fruits. The plant is well known for its medicinal properties and centuries it has been used in Anatolia in traditional medicine to treat different ailments.

Keywords: Hawthorn, *Crataegus*, Medicinal, Anatolia

INTRODUCTION

Medicinal Properties

For millennia, hawthorn (*Crataegus* spp.) has aided cultivars and people around the world by extending itself as an outstanding medicine because it bears copious pharmacological actions. Moreover, it is also equipped with ample nutrients that are extremely vital for marvelous sound health and well-being. It is high in calcium, phosphate, magnesium (Shahat et al., 1998; Leskovac et al., 2007; Tadic et al., 2008; Kumar et al., 2012). The medicinal properties of hawthorn (*Crataegus* spp., a genus comprising approximately 300 species) have been utilized by many cultures for a variety of therapeutic purposes for many centuries (Tassell et al., 2010; Barros et al., 2011; Yang and Liu, 2012; Chen et al., 2013; Zhao et al., 2019).

In the Western world cardiovascular disease (CVD) has become one of the single most significant causes of premature death. For many years the studies are shown that hawthorn is especially important for the heart health. Due to the normalization of cardiovascular system functions, it is consumed as a heart support (Figure 1). It helps increase blood flow and oxygen in the heart. It strengthens the walls of blood vessels. It is very beneficial for nervous heart palpitations, heart rhythm disorders, severe infectious diseases, heart failure, muscle weakness, high blood pressure and arteriosclerosis after a heart attack. It is very rich in vitamin C. It strengthens memory (Bahorun et al., 2003; Bahri-Sahloul et al., 2009; Kostic et al., 2012; Alirezalu et al., 2018; Copra-Janicijevic et al., 2018; Moldovan et al., 2021)

The identification of constituent groups such as bioflavonoids and proanthocyanidins has shed light on some of the beneficial effects of *Crataegus* on the cardiovascular system. The bioflavonoids now being well established as possessing significant antioxidant activity (Alirezalu et al., 2018; Copra-Janicijevic et al., 2018; Moldovan et al., 2021) (Table 1). Berries, leaves, and flowers of hawthorn are phytochemically similar in composition, and differing primarily in the ratio of specific flavonoids and procyanidins present (Figure 2).

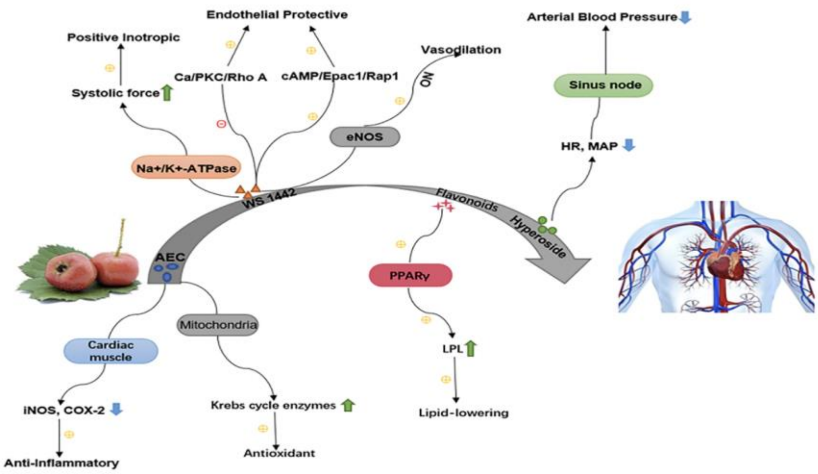

Figure 1. Mechanism of action of hawthorn in the protection against cardiovascular disease. (1). Alcoholic extract of *Crataegus oxyacantha* (AEC) pretreatment maintained mitochondrial antioxidant status and prevented mitochondrial lipid per-oxidative damage and decrease in Krebs cycle enzymes induced by isoproterenol in rat heart.; AEC can act on myocardial tissue to reduce iNOS expression and downregulate COX-2 to exert anti-inflammatory effects. (2). Hawthorn extract WS1442 increases contractility and increases positive muscle strength by inhibiting the Na+/K+ -ATPase pump; WS1442 acts at the serine 1177 site to phosphorylate eNOS and increase NO-mediated vasodilatation; WS1442 effectively protects the vascular endothelium by inhibiting the Ca/PKC/Rho A pathway and activating the cAMP/Epaci/Rap1 pathway. (3). Hawthorn flavonoids exert hypolipidemic effects by acting on the PPARγ pathway to increase LDL expression in blood vessels. (4). High doses of hawthorn ginsenoside can cause significant reductions in heart rate (HR) and mean arterial pressure (MAP), and induce sinus node (Zhang et al., 2022).

Hawthorn berries are rich in hyperoside, while leaves contain higher levels of vitexin-2-rhamnoside. Significant levels of vitexin-2-rhamnoside found in flowers. Flowers contain higher levels of flavonoids, the leaves containing the highest levels of oligomeric procyanidins (OPCs). Higher levels of procyanidins present in leaves (Vanhaelen and Vanhaelen-Fastre, 1989; Kingston, 2007; Zick et al., 2008).

Centuries have witnessed the usage of hawthorn as a helping hand in digestive problems and abdominal pain. It is loaded with prebiotics that enhances the growth of your normal flora which are of great importance in the maintenance of gastrointestinal health. The fiber-loaded berries are also always there to save you from constipation by increasing the bulk of stool (Corzo et al., 2015; Nazhand et al., 2020).

A clinical trial discloses the anti-diabetic and anti-hyperlipidemic activity of *Crataegus* species. Researchers found that the mean fasting blood glucose levels jumped down from 223.6 mg/dl to 183.34 mg/dl, HbA1C reduced 15% (from 8.5 mg/dl to 7.2mg/dl), triglycerides reduced 32% (from 235.5 mg/dl to 160 mg/dl), cholesterol reduced 40% (from 310 mg/dl to 187.6 mg/dl), HDL increased 25% (from 52.4 mg/dl to 69.2 mg/dl) and LDL reduced 26% (from 155.2 mg/dl to 115.5 mg/dl) (Abdulrahim and Al-Shawabkeh, 2017).

Table 1. Depiction of areas of influence of cardiovascular system and role of hawthorn to encounter those disorders.

Antioxidant	Antiatherosclerotic	**Hypotensive**	**Action on cardiac cells**
Direct scavenging of ROS (Reactive oxygen species)	Antioxidant activity	Antioxidant activity	Antioxidant activity
Enhanced super oxide dismutase and catalase activity	Down regulation of capsase-3	Vasodilation through NO synthesis	Down regulation of capsase-3
Protective function for α-tocopherol	Regulation of lipoprotein lipase expression ·	Weak ACE activity	Inhibit cAMP (cyclic adenosine monophosphate) which increases coronary blood flow, positive ionotropy and slight increase in heart rate
Protection of gap junction	Inhibits dietary cholesterol absorption	Relaxes smooth muscles	Chronotrophic and antiarrythmic effects
Intracellular communication	Raises excretion of bile acids		
Inhibition of lipoxygenase activity			

It could be suggested that it prevents oxidation of LDL that prohibits the formation of free radicals which are attributed to distort the integrity of cardiovascular system i.e. blood vessels (Leskovac et al., 2007; Tadic et al., 2008). Rajendran et al. (1996), demonstrated that *Crataegus* exerts controlling of hepatic LDL-receptors, which enhances influx of plasma LDL-cholesterol into the liver and cholesterol degradation to bile acids, released within bile so overwhelming biosynthesis of cholesterol. On the other hand, hawthorn have showed anti hyperglycemic effect appeared in the reduction of fast blood glucose and Hb A1C in patients (Abdulrahim and Al-Shawabkeh, 2017). They proposed that the presence of flavonoids has a vital role in regulating the level of glucose in plasma as deduced in lowering enzymes activities that convert starch into free glucose (Shahat et al., 2002; Al-Hallaq et al., 2013).

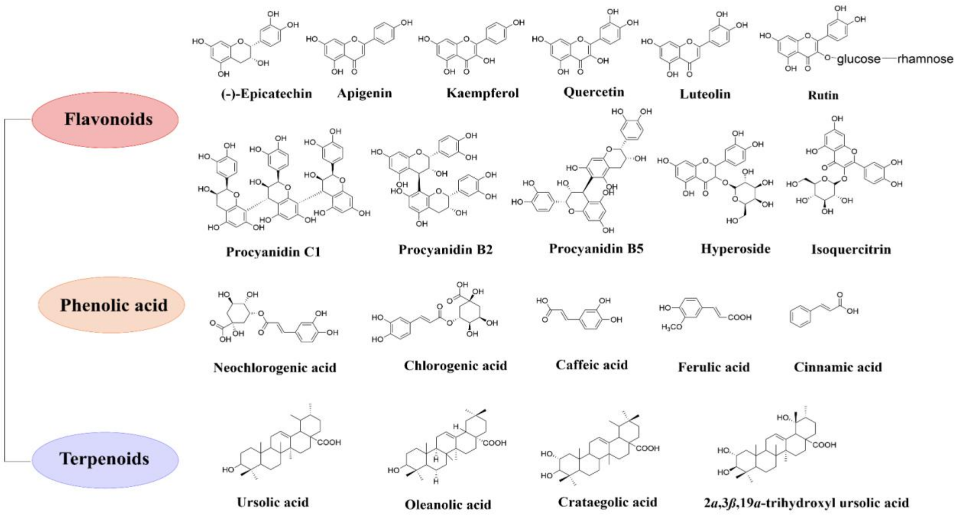

Figure 2. The main bioactive compounds of hawthorn

BODY

Traditional Use of Hawthorn in Anatolia and Its Benefits

Hawthorn species widely distributed throughout Anatolia, and has been using frequently in traditional medicine (Ercisli, 2004; Yilmaz et al., 2010; Coklar et al., 2018).

● It accelerates the healing process of heart disease considerably. It is also good for rhythm disorder and is also used as an alternative treatment method.

● It is good for the elimination of damage to the muscles of the heart after a heavy infection and for heart failure problems.

● It is effective in the recovery of arteriosclerosis that occurs after high blood pressure. It minimizes the risk of heart attack.

● It helps in expelling the accumulated fluids, that is, in removing edema.

● It is also used as an antidepressant because it helps to reduce nervous problems.

● It is useful in eliminating diarrhea problem. However, if consumed in excess, it can cause constipation.

● Especially if people who have had a heart attack consume this fruit, it strengthens the heart.

● It relieves vomiting. It regulates the stomach.

● It is known that it is good for chest pain, migraine and headaches.

● It is an effective fruit that prevents vascular occlusion.

The leaves of the hawthorn tree are boiled and consumed in the form of tea in Anatolia. Consuming hawthorn leaf is very beneficial for people with vascular and heart diseases. It is a treatment method used to open clogged veins (Alinterim, 2012; Ercisli et al., 2015).

The leaves and seeds of the hawthorn tree are also very useful. The seeds of hawthorn fruit are used in the treatment of heart failure, nervous heart palpitations, heart rhythm disorders, heart muscle disorders, high blood pressure and arteriosclerosis. It is known that hawthorn plant or vinegar is good for veins and heart. It is effective in strengthening the heart muscles and increasing blood circulation in the heart vessels. At the same time, hawthorn vinegar has a property that expands the veins (Kadas et al., 2014). Hawthorn fruit also has many known benefits. Some of these are those (Arslan et al., 2011; Batu, 2012):

● Fights cholesterol.

● It has an immune-boosting effect.

● It enables people to take precautions against signs of aging.

● It helps to protect cardiovascular health.

● It provides the prevention of cancer as it is used in the prevention of diseases that are usually deadly and very dangerous.

● One of the most important features of the hawthorn plant is that it has a protective effect against all cancers.

CONCLUSION

The fruit, leaves and seeds of the hawthorn plant all have their own benefits. But the common good thing is for the heart. For this reason, every person should consume hawthorn fruit regularly.

Statement of Conflict of Interest

The authors declare that they are no conflict of interest.

Authors' Contributions

Halil İbrahim Sağbaş and Sezai Ercişli designed and analyzed the research. They arranged studies, and 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.

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Role of Exogenous Amino Acid in Salt Stressed Broccoli Seedlings

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ABSTRACT: In this study, the effect of exogenous applied amino acid in broccoli seedlings under salt stress was investigated. In the study, two amino acids (Gluten amin 6.5 and Protein 7.5) were applied to the root zone of the plant 3 times with one-week intervals. The salt solutions prepared with 100 mm NaCl were given to the plant as irrigation water. In the study, the effects of the applications were evaluated in terms of plant fresh and dry weight, plant height, stem diameter, tissue electrical conductivity (TEC) and mineral content. It was determined that the plant fresh and dry weight, plant height and stem diameter of the broccoli seedlings decreased, while the TEC value increased with salt application. However, the negative effects of salt on these parameters were alleviated by amino acid applications, and both applications had a significant effect. In addition, it was determined that the mineral content (except Cl and Na), which decreased with salinity, showed a lower decrease with applications. In this study, it was concluded that the negative effect of salt stress on broccoli seedlings can be alleviated by exogenous amino acid application.

Keywords: Broccoli, Salinity, Amino acid, Stress

INTRODUCTION

Salinity is one of the most devastating abiotic stresses in the world that drastically reduces the production and quality of agricultural products. Salt stress shows the effect of more than 20% of arable land in the world (Hasanuzzaman and Fujita, 2022). The first stage of reduction in plant growth by salinity is due to the osmotic effect, and the second stage, which has a slower effect, is the result of salt toxicity in the leaves (Parihar et al., 2015). The osmotic effect of salinity leads to decreases in growth rate with the effect of water deficiency, which occurs as a result of the salt in the soil solution reducing the plant's ability to take up water. The second stage, which is the ion effect of salinity, is that excessive salt enters the plant with transpiration, causing damage to the cells in the transpiration leaves and further reduction in growth (Greenway and Munns, 1980; Parihar et al., 2015). Osmotic stress is caused by the excess of $Na+$ and $Cl-$ ions, which reduce the osmotic potential in the soil and prevent water uptake and nutrients. Oxidative stress of salinity leads to the formation of reactive oxygen species (ROS) (hydrogen peroxide, superoxide ions, singlet oxygen, peroxides, etc.) that are toxic to biomolecules such as proteins, lipids, nucleic acids, etc. This situation causes the normal functioning of the cell to be disrupted and the growth and development of the plant to decrease (Rasool et al., 2013). Damage to the plant by salt stress starts from the germination stage and then affects all the main processes such as growth, photosynthesis, plant-water relationship, nutrient order and yield.

Numerous studies have been conducted to reduce the effect of salt stress on plants. In these studies, some applications made exogenous to the plants are included. One of them is the exogenous application of amino acids to plants, which are also involved in the endogen metabolism of plants and have an effect on the formation of different responses to stress. Proline and other amino acids, which are involved in plant endogenous metabolism, accumulate when the plant is exposed to stress. Amino acids accumulated in plants act as osmolytes, regulating ion transport, modulating stomatal opening, affecting the synthesis and activity of some enzymes, gene expression and redox homeostasis (Rai, 2002). In previous studies, it has been determined that amino acid application has important effects against various stress factors such as salinity (Peña Calzada et al., 2022), drought (Haghighi et al., 2020), temperature (Matysiak et al., 2020) and heavy metal stress (Wang et al., 2017) in plants.

Broccoli (*Brassica oleracea* var. italica), belonging to the Brassicaceae family, is a very popular and widely consumed vegetable. Broccoli, which has an important place in human nutrition in terms of vitamins and minerals it contains, is very beneficial for health as it contains various polyphenols and antioxidants (Nagraj et al., 2020). Broccoli is generally considered moderately tolerant to salinity (Di Gioia et al., 2018; Ali et al., 2022). As a matter of fact, it was determined that salt stress controls the leaf photosynthetic mechanism, stimulates redox homeostasis and increases the activities of antioxidant enzymes, thus enabling broccoli plants to tolerate moderate NaCl stress (Ali et al., 2022).

There are studies on the effects of salt stress on broccoli, but studies on how amino acid application affects broccoli seedlings under salt stress are insufficient. In this study, the effects of amino acid treatments on broccoli seedlings under salt stress were investigated in terms of some morphological features and mineral content.

MATERIAL AND METHOD

Broccoli (*Brassica oleracea* var. italica) was used as a plant material in the pot study carried out in the controlled greenhouse conditions. To obtain seedlings, the seeds were sown in viols containing peat:perlite (2:1). Seedlings with 2-3 leaves were planted in 1.3 liter pots in a mixture of soil, peat, sand and manure (2:1:1:1). After planting the seedlings, 30 ml of amino acid solution (Gluten amin 6.5 and Protein 7.5) was applied to the root zone of the plant. The contents of these products are given in Table 1. The treatments were repeated three times at one-week intervals. Salinity applications (0 and 100 mM NaCl) as irrigation water were started with irrigations after the first amino acid application. According to the results of the measurements made with the soil moisture meter, the irrigations were made at intervals of 3 days according to the soil field capacity. The study was terminated 40 days after the seedling planting and various measurements were made. Plant fresh and dry weight, plant height, stem diameter, tissue electrical conductivity (TEC) and mineral content were examined. The data obtained from the study arranged according to completely randomized design was analyzed using the SPSS program. Data were subjected to variance analysis and differences of means were determined by Duncan multiple comparison test.

Table 1. Some ingredients of the products used in the study

Total amino acid (on natural sample (%))	**Gluten amin 6.5**	**Protein 7.5**
Aspartic acid	2,99	1,26
Gamma-aminobutiric acid	<0,05	<0,05
Glutamic acid	5,79	15,39
Alanine	3,63	1,03
Arginine	3,17	1,37
Phenylalanine	1,12	2,31
Glycine	7,82	1,40
Hydroxyproline	4,32	<0,05
Histidine	0,55	0,78
Isoleucine	0,83	1,24
Leucine	2,01	2,71
Lysine	2,28	0,63
Methionine	0,50	0,69
Proline	4,63	5,34
Serine	1,59	2,19
Tyrosine	0,72	1,37
Threonine	1,10	1,06
Tryptophan	0,12	0,41
Valine	1,31	1,50
Total	44,50	40,70

RESULTS AND DISCUSSION

In the study, the effects of the treatments on plant height, stem diameter, plant fresh weight, plant dry weight and TEC in broccoli seedlings under salt stress are given in Figure 1, 2 and 3.

Plant height, stem diameter, plant fresh weight and plant dry weight decreased with salinity, however, this decrease was lower with amino acid applications compared to the control. Salinity caused decrease 43% in plant height, 14% in stem diameter, 68% in plant fresh weight and 57% in plant dry weight. However, the decrease in amino acid applications (Gluten amine 6.5 and Protein 7.5) in salinity conditions is 30-36%, 2-3%, 48-57% and 43-45% for these parameters, respectively. In addition, the applications significantly increased plant growth in salt-free conditions. Increases in plant height, stem diameter, plant fresh weight and plant dry weight with amino acid applications (Gluten amine 6.5 and Protein 7.5) are 10-6%, 15-11%, 31-28% and 15-24%, respectively. TEC increased with salt stress. However, the increase in TEC with amino acid applications was at a lower level.

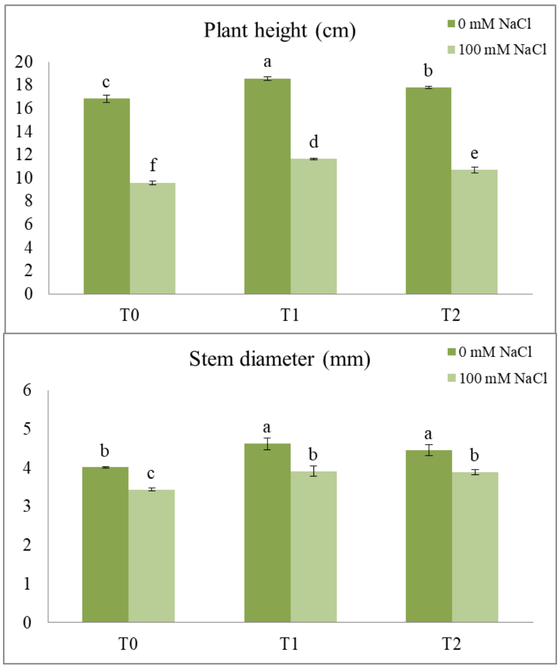

Figure 1. The effects treatments and salinity on plant height and stem diameter of broccoli seedling. There is no statistical difference between same letters given in each bar ($p<0.001$). T0: Control (no treatment), T1: Gluten amine 6.5, T2: Protein 7.5.

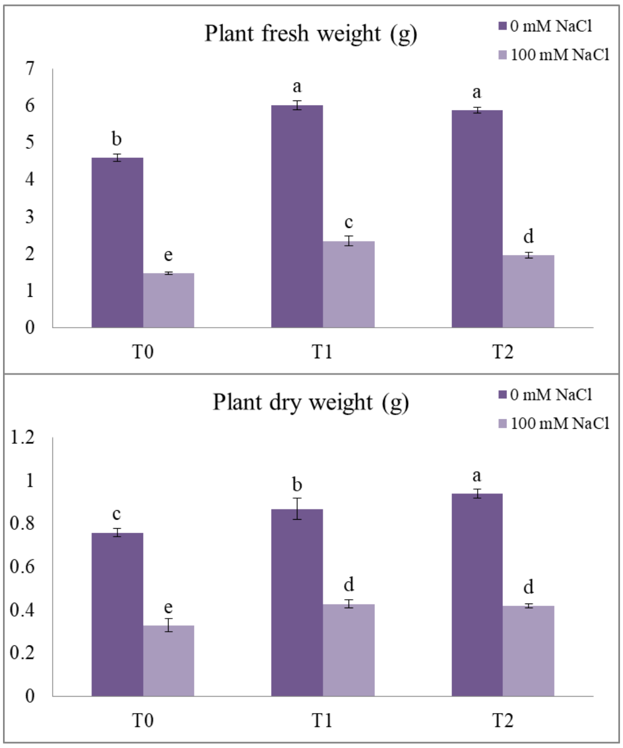

Figure 2. The effects treatments and salinity on plant fresh and dry weight of broccoli seedling. There is no statistical difference between same letters given in each bar ($p<0.001$). T0: Control (no treatment), T1: Gluten amine 6.5, T2: Protein 7.5.

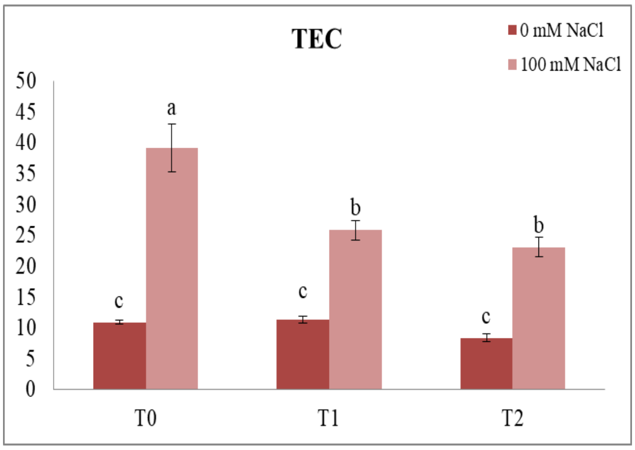

Figure 3. The effects treatments and salinity on TEC value of broccoli seedling. There is no statistical difference between same letters given in each bar ($p<0.001$). T0: Control (no treatment), T1: Gluten amine 6.5, T2: Protein 7.5.

In our study, significant reductions in growth and development of broccoli seedlings with salinity were also observed in terms of the parameters examined. Similarly, Ali et al. (2022) reported that with increasing NaCl, plant leaf area, shoot length, root length, shoot and root dry weights decreased and antioxidant enzyme activities (SOD, CAT and APX) increased in broccoli. In addition, Di Gioia et al. (2018) determined that salinity stress reduced plant growth mainly in the first growth stage and then reduced broccoli head average weight.

In our study, it was determined that the negative effect of salt stress on broccoli seedlings can be alleviated by amino acid application, so that plants can grow without much damage to plant development in a salty environment. Similarly, it was determined that the application of L-cysteine, L-methionine and amino acid mixtures in broccoli increased root and shoot weight and plant growth (Shekari and Javanmardi, 2017).

The effects of the treatments on mineral content of broccoli seedlings under salt stress are given in Figure 4 and 5. Except for Na and Cl, there was a decrease in the mineral content of broccoli seedlings with salinity. Compared to the control, the N, P, K, Ca, Mg, S, Mn, Fe, Zn and B contents of the plants decreased by 20%, 21%, 13%, 29%, 31%, 28%, 20%, 22%, 30% and 19%, respectively in 100 mM salt conditions. However, amino acid applications provided significant increases in these mineral substances both in salt-free and salty conditions. The increase occurred with amino acid applications (Gluten amine 6.5 and Protein 7.5) in salt-free environment was 52-39%, 45-51%, 57-56%, 41-35%, 28-19%, 43-14%, 53-12%, 33-41%, 57-22% and 10-1%, respectively. Similarly, significant increases occurred in saline conditions. Plant Na and Cl content increased with salinity, and the increase in these mineral substances was lower with amino acid applications.

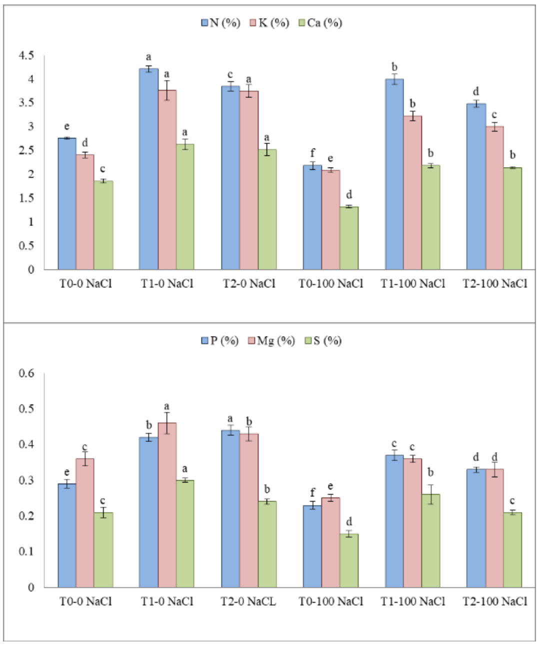

Figure 4. The effects treatments and salinity on N, K, Ca, P, Mg and S content of broccoli seedling. There is no statistical difference between same letters given in each bar (p<0.001). T0: Control (no treatment), T1: Gluten amine 6.5, T2: Protein 7.5.

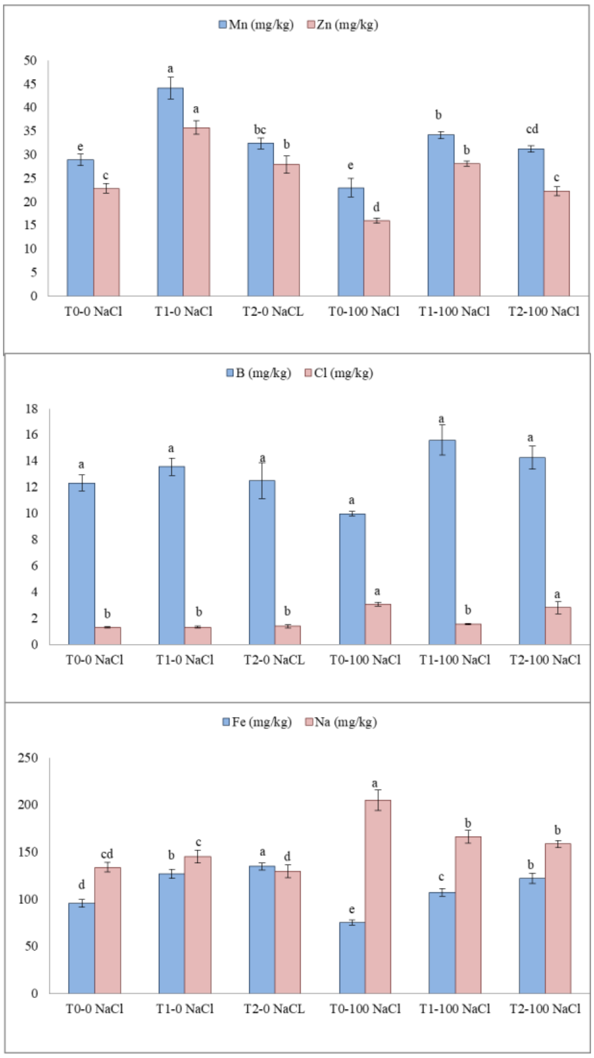

Figure 5. The effects treatments and salinity on Mn, Zn, B, Cl, Fe and Na content of broccoli seedling. There is no statistical difference between same letters given in each bar ($p<0.001$). T0: Control (no treatment), T1: Gluten amine 6.5, T2: Protein 7.5.

It is observed that salinity causes decreases in plant mineral concentrations, except for Na and Cl. In another study, salinity increased the concentrations of Na^+ and Cl^- ions in two broccoli cultivars and caused deterioration of endogenous mineral levels in shoots and roots, depending on the variety and salt concentration (Zaghdoud et al., 2012). In another study, it was determined that Na and Cl accumulation increased with a decrease in Ca, K, Mg, NO_3, total P and PO_4 contents in broccoli leaves and roots with increasing soil salinity (Ali et al., 2022).

As a result of this study, it was determined that amino acid application changed the effect of salt stress on plant mineral substance content. In previous study, it was determined that both shoot and root Na content increased and K, Ca, Mg and P accumulation decreased in maize and bean plants with salinity. However, it was determined that amino acid applications changed the selectivity of Na, K, Ca and P in both species, restricted Na uptake and increased K uptake, K/Na ratio, C and P selectivity (Abd El-Samad et al., 2011).

CONCLUSION

As a result, the mitigating effects of the Gluten amine 6.5 and Protein 7.5 used in the study are thought due to the various amino acids they contain. Exogenous application of these products can be important for growth and development of plants grown in problematic soils.

Statement of Conflict of Interest

The authors declare that they are no conflict of interest.

Authors' Contributions

ME, EY and MT designed and analyzed the research, ME, EY and UT studies arranged. All authors contributed to the writing of the manuscript and took part in the process of publication of the manuscript and read and approved it.

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Drought Acclimation on Drought Stress Resistance in Eggplant (*Solanum melongena* L.) during Seedling Growth

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ABSTRACT: In recent years, drought has been emphasized as one of the important abiotic stress factors negatively affecting agricultural production. The fact that the existing water is mostly used in agricultural production requires more effective use of the decreasing water resources. The effects of drought acclimation in eggplant (*Solanum melongena* L.) during seedling growth in greenhouse were examined as pot experiment. Treatments were non-acclimated and non-stressed (NA), drought acclimated and non-stressed (DA), non-acclimated and drought stressed (NAS), drought acclimated and drought stressed (DAS). In the study, the effects of applications on plant dry weight, root dry weight, plant height, stem diameter, leaf area, tissue relative water content (RWC) and tissue electrical conductivity (TEC) in eggplant seedlings were determined. Plant dry weight, root dry weight, plant height, stem diameter, leaf area, tissue relative water content decreased while TEC increased with drought stress. However, it was observed that DAS ameliorated effects of severe water constraints on seedling growth of eggplant. As a result, it has been determined that drought acclimation has positive effects on seedling development and can be considered as an application that can be done under water limited conditions.

Keywords: Eggplant, Water deficit, Acclimation

INTRODUCTION

Today, drought is more emphasized as one of the most important abiotic stress factors limiting agricultural production. Researches have focused on how to continue plant production in drought stress or what applications will be made against drought stress. Drought reduces leaf size, stem elongation and root growth, and causes deterioration in plant growth and plant-water relationship, and a decrease in water use efficiency (Farooq et al., 2009; Farooq et al., 2012). Plants develop a set of responses to overcome drought stress. Some plants increase stress resistance after being exposed to low levels of stress. This condition, expressed as acclimation, depends on plant genotype and environment (Banik et al., 2016). In nature, it is known that plants experience cycles of water-deficiency stress and are exposed to multiple sequential or simultaneous environmental stresses. Mild water stress in the early stage of seedling growth can provide resistance to severe water stress encountered in the later growth and development period (Selote and Khanna-Chopra, 2010). As a matter of fact, it was determined that the application of mild drought adaptation in wheat seedlings caused higher water content in the roots and acclimatization and increased resistance in wheat roots (Selote and Khanna-Chopra, 2010).

Eggplant is one of the vegetable crops that is widely produced and consumed in Turkey and in the World. In the world, 59 million tons of eggplant has been produced on an area of approximately 2 million hectares in 2021 (FAO, 2023). It is estimated that the current situation in eggplant growing areas will change in the future due to climate change (Plazas et al., 2019). Eggplant has a low tolerance to abiotic stresses such as drought. With drought, the growth and yield of the eggplant plant decreases (Zayova et al., 2017). However, it is also stated that the resistance is higher than several vegetable crops. It was determined that photosynthetic pigments decreased and proline, malondialdehyde, phenolics and flavonoids increased in eggplant with water stress (Plazas et al., 2019).

Although there are many studies on the mechanism of drought stress tolerance in eggplant, research examining drought adaptation and recovery in terms of drought stress resistance is lacking. In this study, the effect of drought acclimation on eggplant seedlings was investigated in terms of increasing tolerance to drought stress.

MATERIAL AND METHOD

This study was conducted under controlled greenhouse conditions. Eggplant (*Solanum melongena* cv. Pala) was used as a plant material. To obtain seedlings, the seeds were sown in viols containing peat:perlite (2:1). Seedlings with about 2-3 leaves were planted in 2,5-liter pots with mixture of soil, peat and sand (3:1:1). After planting the seedlings, the plants were firstly irrigated according to the field capacity. The seedlings were grouped into four treatments, namely, non-acclimated and non-stressed (NA-100% of field capacity), drought acclimated and non-stressed (DA-80% of field capacity and then 100% of field capacity), non-acclimated and drought stressed (NAS-100% of field capacity and then 40% of field capacity), drought acclimated and drought stressed (DAS-80% of field capacity and then 40% of field capacity). The irrigation processes are shown schematically in Figure 1.

Irrigations were made according to soil moisture meter and made at intervals of about 3 days with respect to the field capacity. The study was terminated 50 days after the seedling planting and various measurements were made. Plant and root dry weight, plant height, stem diameter, leaf area, tissue electrical conductivity (TEC) and relative water content (RWC) were determined.

The data obtained from the study arranged according to completely randomized design was analyzed using the SPSS program. Data were subjected to variance analysis and differences of means were determined by Duncan multiple comparison test.

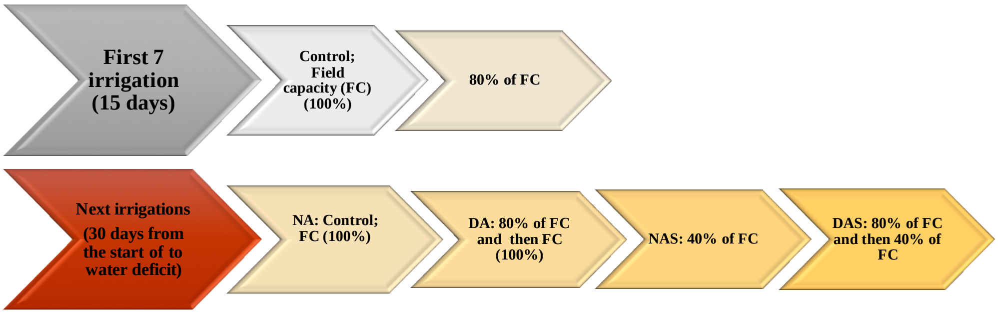

Figure 1. The irrigation processes

RESULTS AND DISCUSSION

In the study, the effects of irrigation on plant growth are given in Figure 2-8. In the NAS application, where severe water restriction was applied, there was a significant decrease in plant growth compared to 100% irrigation (NA). With NAS, plant dry weight, root dry weight, RWC, plant height, stem diameter and leaf area decreased by 68%, 77%, 19%, 56%, 37% and 50%, respectively. On the other hand, with the application of DAS, in which acclimation was performed, these parameters increased by 31%, 26%, 7%, 12%, 3% and 9%, respectively. The decrease in plant dry weight, root dry weight, plant height, stem diameter and leaf area were lower in plants to which drought was applied after the acclimation (DAS) process. TEC value increased significantly with drought, 90% increase occurred with NAS, 5% increase was observed with DAS, respectively.

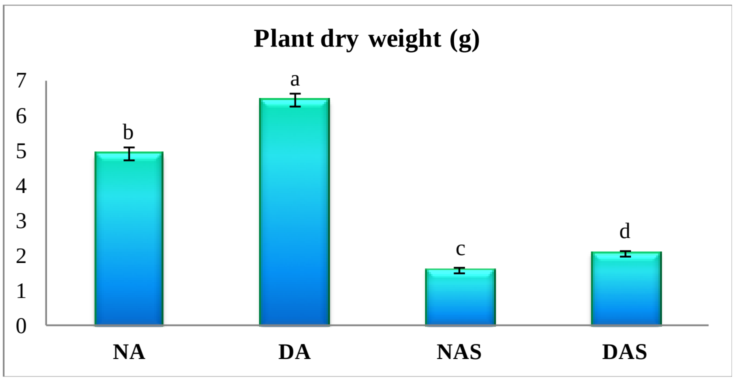

Figure 2. The effects of irrigation on plant dry weight of eggplant.

There is no statistical difference between same letters given on each bar (p<0.001). Non-acclimated and non-stressed (NA-100% of field capacity), drought acclimated and non-stressed (DA-80% of field capacity and then 100% of field capacity), non-acclimated and drought stressed (NAS-100% of field capacity and then 40% of field capacity), drought acclimated and drought stressed (DAS-80% of field capacity and then 40% of field capacity).

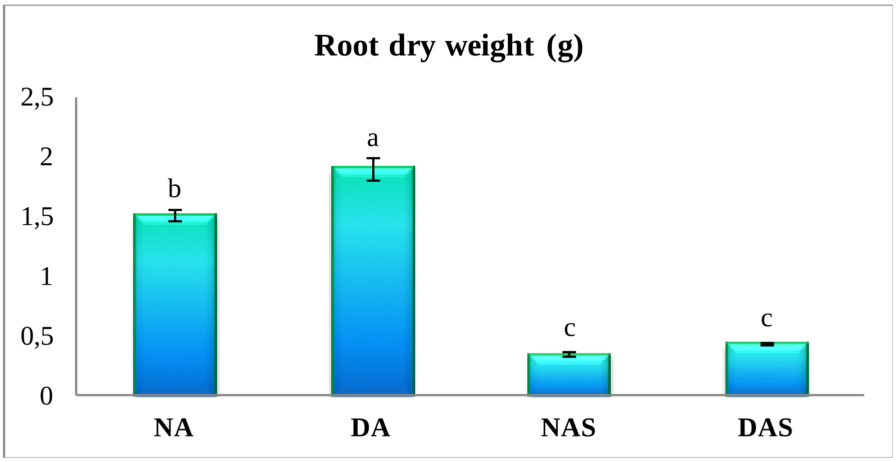

Figure 3. The effects of irrigation on root dry weight of eggplant.

There is no statistical difference between same letters given in each bar ($p<0.001$). Non-acclimated and non-stressed (NA-100% of field capacity), drought acclimated and non-stressed (DA-80% of field capacity and then 100% of field capacity), non-acclimated and drought stressed (NAS-100% of field capacity and then 40% of field capacity), drought acclimated and drought stressed (DAS-80% of field capacity and then 40% of field capacity).

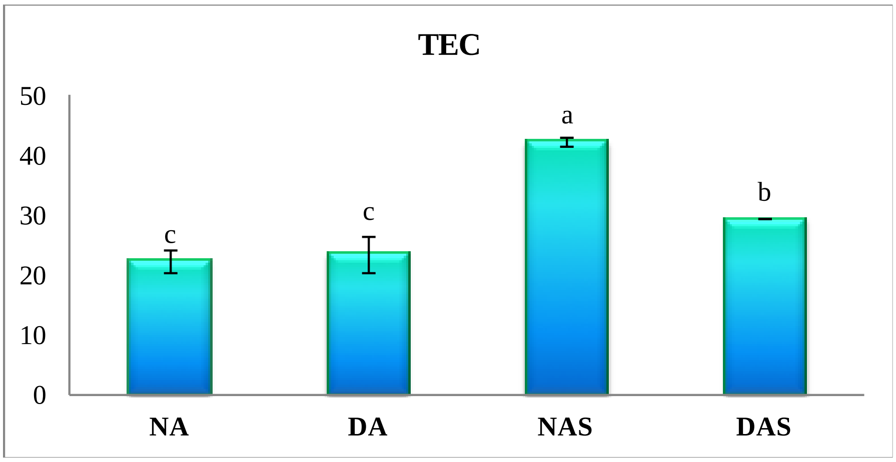

Figure 4. The effects of irrigation on TEC of eggplant.

There is no statistical difference between same letters given in each bar ($p<0.001$). Non-acclimated and non-stressed (NA-100% of field capacity), drought acclimated and non-stressed (DA-80% of field capacity and then 100% of field capacity), non-acclimated and drought stressed (NAS-100% of field capacity and then 40% of field capacity), drought acclimated and drought stressed (DAS-80% of field capacity and then 40% of field capacity).

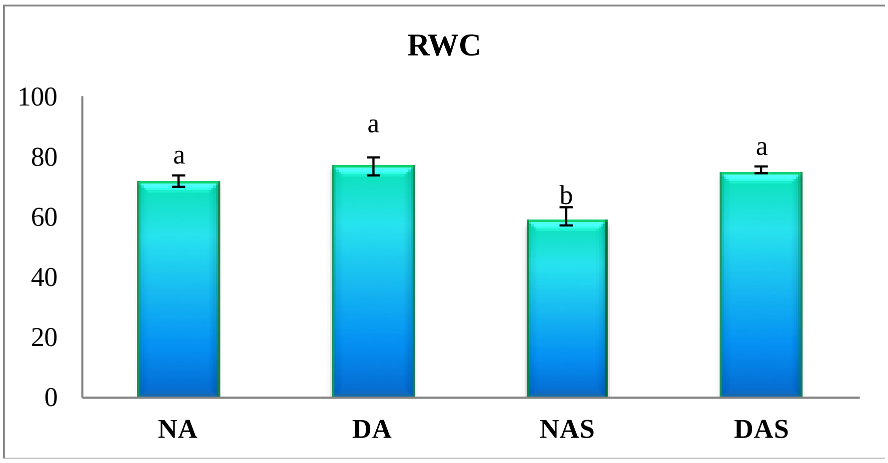

Figure 5. The effects of irrigation on RWC of eggplant.

There is no statistical difference between same letters given in each bar ($p<0.001$). Non-acclimated and non-stressed (NA-100% of field capacity), drought acclimated and non-stressed (DA-80% of field capacity and then 100% of field capacity), non-acclimated and drought stressed (NAS-100% of field capacity and then 40% of field capacity), drought acclimated and drought stressed (DAS-80% of field capacity and then 40% of field capacity).

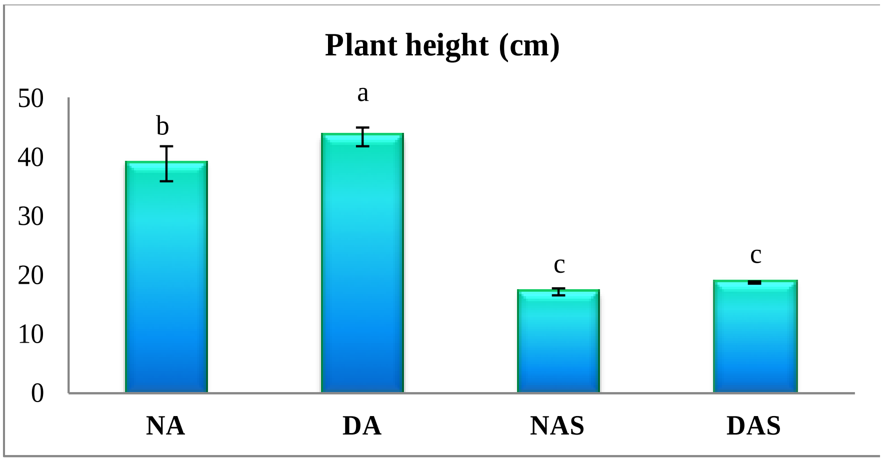

Figure 6. The effects of irrigation on plant height of eggplant.

There is no statistical difference between same letters given in each bar ($p<0.001$). Non-acclimated and non-stressed (NA-100% of field capacity), drought acclimated and non-stressed (DA-80% of field capacity and then 100% of field capacity), non-acclimated and drought stressed (NAS-100% of field capacity and then 40% of field capacity), drought acclimated and drought stressed (DAS-80% of field capacity and then 40% of field capacity).

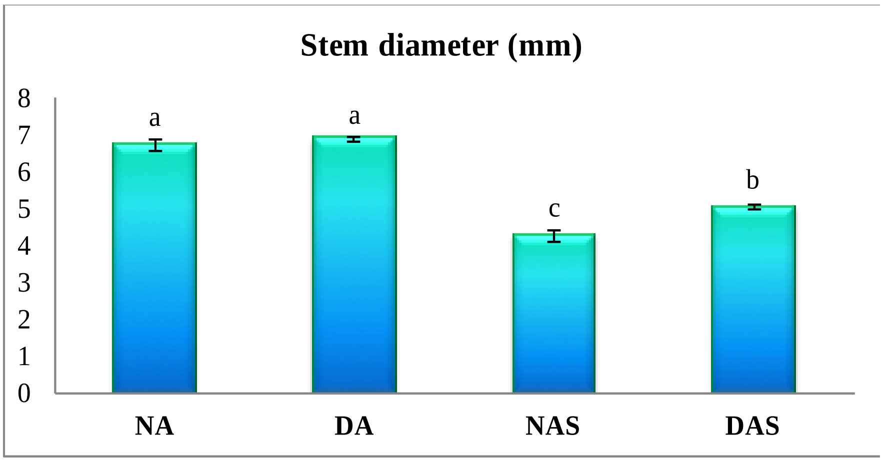

Figure 7. The effects of irrigation on stem diameter of eggplant.

There is no statistical difference between same letters given in each bar ($p<0.001$). (DA-80% of field capacity and then 100% of field capacity), non-acclimated and drought stressed (NAS-100% of field capacity and then 40% of field capacity), drought acclimated and drought stressed (DAS-80% of field capacity and then 40% of field capacity)

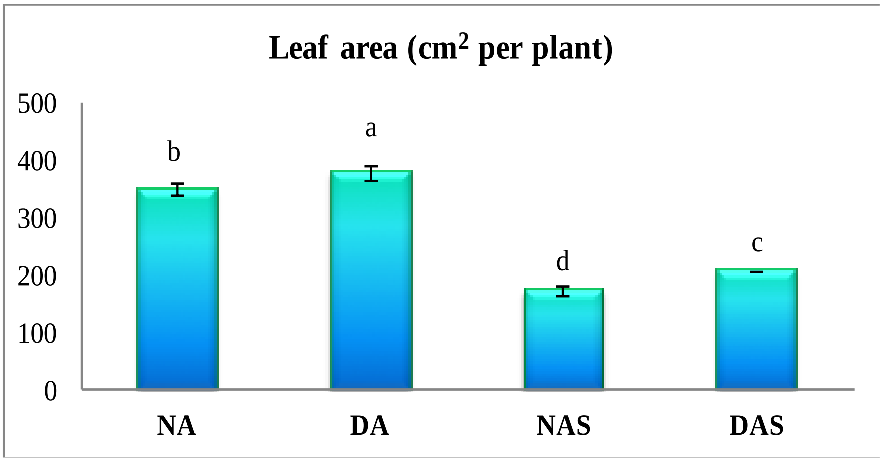

Figure 8. The effects of irrigation on leaf area of eggplant.

There is no statistical difference between same letters given in each bar ($p<0.001$ Non-acclimated and non-stressed (NA-100% of field capacity), drought acclimated and non-stressed (DA-80% of field capacity and then 100% of field capacity), non-acclimated and drought stressed (NAS-100% of field capacity and then 40% of field capacity), drought acclimated and drought stressed (DAS-80% of field capacity and then 40% of field capacity).

Drought stress causes detrimental effects on growth and development by causing changes in plant metabolism. According to the results, plant dry weight, root dry weight, RWC, plant height, stem diameter and leaf area under drought stress conditions were significantly reduced compared to well-watered plants. Similarly, the relative water content (RWC) and photosynthetic efficiency decreased with drought stress, thus inhibiting eggplant growth and yield (Semida et al., 2021).

Plants adapt to drought stress using a variety of strategies, including drought escape, avoidance, and tolerance. Drought tolerance is the ability of the plant to show growth, development and economic efficiency in sub-optimal irrigation. Also, the ability to conserve leaf area, maintain vegetative growth, and increase cell membrane stability is considered stress tolerance (Abobatta, 2019). However, adaptation to drought is important in terms of reducing the negative effects of stress on the plant. Therefore, acclimation will be important in terms of increasing tolerance to drought.

In our study, acclimatization of eggplant seedlings had a mitigating effect on parameters measured. The decrease in plant growth with drought was lower in acclimated plants. Similarly, acclimated wheat plants were found to maintain higher root RWC under severe water stress conditions than the non-acclimatized plant (Amoah et al., 2019; Selote and Khanna-Chopra, 2010). It has been noted that acclimation is important in the adaptive response to stress, enabling it to retain higher water content in the root tissue. This has provided an important role in gaining resistance, especially the higher water holding ability during dehydration (Selote and Khanna-Chopra, 2010). In a study, it was found that programmed irrigation is the most appropriate method after 55-60% or 70-75% of the existing soil water is depleted, and irrigation after 85-90% of the existing soil water is consumed causes significant decreases in the parameters examined in eggplant (El-Miniawy, 2015). Pre-treatment of drought acclimation followed by drought stress reduced leaf wilting, induced thicker cuticular layer and more open stomata of potato plant compared to drought stress directly under stress (Banik et al., 2016). Also, acclimation improves the ability of wheat plants to survive water stress that will occur in the later stages of growth and development by using physiological, biochemical and molecular mechanisms (Amoah et al., 2019).

CONCLUSION

As a result of this study, it is thought that acclimatization with a moderate water restriction in the initial stage of plant development may increase the tolerance level of eggplant seedlings against severe water stress that will occur in the later stages of development. It will be possible to obtain clearer results by examining the effects of acclimation application against drought during the flowering and fruit formation periods of the plant.

Statement of Conflict of Interest

The authors declare that they are no conflict of interest.

Authors' Contributions

ME, EY and MT designed and analyzed the research, ME, EY and UT studies arranged. All authors contributed to the writing of the manuscript and took part in the process of publication of the manuscript and read and approved it.

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Variation of Physiological Parameters of the Effect of Pesticide Residues: Analysis Using Artificial Neural Networks

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ABSTRACT: The residual pesticides on vegetables and fruits have a detrimental effect on the living organism. In this study, changes in biochemical parameters were recorded on rats. Serum studies performed on rats treated with chlorpyrifos showed a significant decrease in the following parameters: urea, transaminases, blood glucose compared to control groups. However, the results of the treated rats show a significant increase in levels of the parameters of ALB2, cholesterol, creatinine and triglyceride. A significant increase in alkaline phosphatase activity and rate the total protein was observed. 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 process these data by the artificial intelligence techniques including artificial neural networks. Such as artificial neural networks 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 variation of biochemical parameters, it is extensible and allows the introduction of other variables that are not considered in this study.

Keywords: Artificial neural networks, Pesticides residues, Physiological parameters, Mouse

INTRODUCTION

The broad spectrum organophosphate insecticide chlorpyrifos is the most widely used insecticide in Algeria. Chlorpyrifos is a broad spectrum insecticide [1] and has many uses. Chlorpyrifos is an organophosphate insecticide. Is acutely toxic to bees, birds, mammals, aquatic life, and certain species of algae, the rat LD50 is 135 mg/kg. Like all organophosphates, chlorpyrifos and chlorpyrifos-oxon kill insects and other animals, including humans, because of this toxicity to the nervous system. Acute Toxicity Symptoms of acute chlorpyrifos poisoning in humans include headache, nausea, dizziness, muscle twitching, weakness, increased sweating and salivation, and fluidfilled lungs. These symptoms are common to all organophosphate insecticides.

Unconsciousness, convulsions, and death can result with sufficient exposure [2]. A variety of different kinds of exposure to chlorpyrifos can cause acute toxicity. Direct skin contact with the insecticide, either as a solid or in water can be toxic. Ingestion, breathing of vapors, or contact with chlorpyrifos-treated soil is also toxic [3]. Pesticides are also considered a biologically active chemical of great value to agriculture [4]. Kidney plays a vital role in the maintenance of an organism's internal environment, being the key to the regulation of extracellular fluid volume and composition as well as acid–base balance. It is also a target of toxic chemicals, which can disrupt its functions, and cause temporary or permanent derangement of homeostasis. The purpose of the present study is to determine the effect of organophosphate insecticide chlorpyrifos in order to evaluate the effect of its sub lethal toxicity on some organs in mouse wistar albino strain and evaluate through an experimental study its effects on some biochemical parameters and this from a level of sub acute and acute exposure.

MATERIAL AND METHODS

A. Chemical Material

Pesticide: Chlorpyrifos tested is selected from those commonly used for phytosanitary treatments of fruits and vegetables in the region of Setif.

B. Biological Material

We used in this study male mouse and female albino Wistar weighing between 150 and 350 g and from the Pasteur Institute in Algiers.

C. Evaluation of Acute Toxicity in Rats in vivo

The acute toxicity test to estimate irritating or sensitizing effects that appear between 1 and 14 days after administration of a single dose CPF.

D. The Median Lethal Dose LD_{50}

For chlorpyrifos, the LD 50 is between 82 and 270 mg/kg body weight of mouse.

E. Acute Toxicity

The CPF is diluted and administered orally to animals in a single dose by stomach tube.

Five lots have received a dose of 1/5 of the LD50. The control group received saline.

F. Sub Acute Toxicity

Rodents treated orally with chlorpyrifos dissolved in distilled water. A dose of 1/20 of LD 50, which corresponds to 22 mg/kg/week they are taken at a mouse of one day a week for 6 weeks.

G. Observation of Animal Behavior

Male rats appear to be more sensitive than female rats. This sensitivity may be due to physiological differences. It can also be justified by the fact that cytochromes P450 are more degradable in females than males due to their activity.

RESULTS

Consequences of CPF Intoxication

An increase in relative liver weight in females than in males.

A significant decrease in the relative mass of the brain.

A significant increase in the relative weight of the kidneys.

A slight increase in the relative weight of the spleen in females.

A significant increase in the relative weight of the lungs.

A significant decrease in the relative weight of the heart.

A significant decrease in the relative weight of ovaries.

A significant increase in the relative weight of the testes.

Effects of CPF on Biochemical Parameters

The effect of CPF showed a significant decrease in the concentration of uric acid, the increase in LDL concentration, increasing the concentration of alkaline phosphatase and the significant decrease in the concentration of aspartate aminotransferase (Figure 1-5).

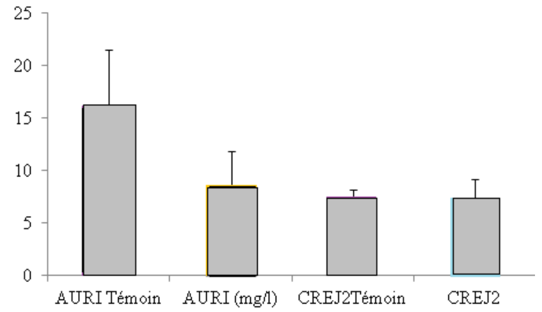

Figure 1. Effect on phosphatase alcaline

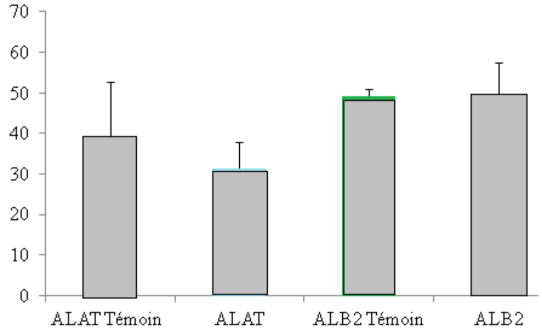

Figure 2. Effect on LDL concentration

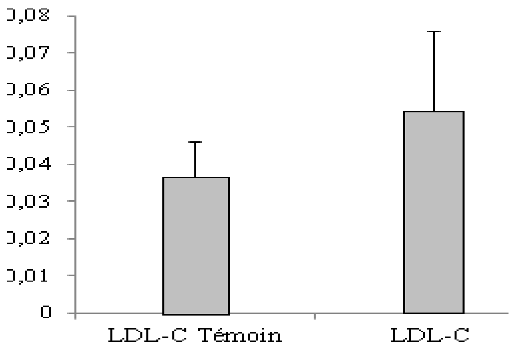

Figure 3 Effect on phosphatase alkaline

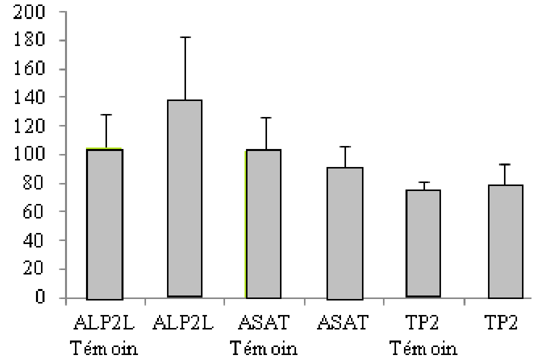

Figure 4 Effect on total proteins

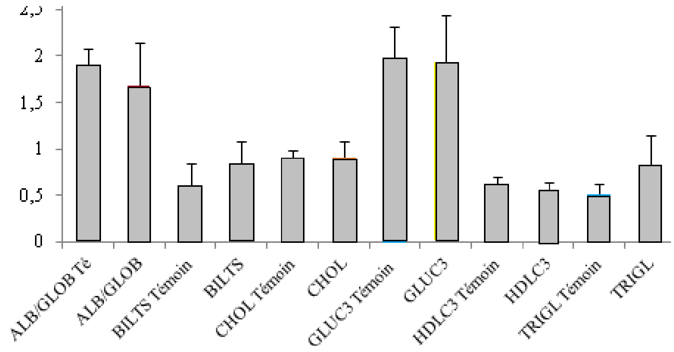

Figure 5. Effect on aspartate aminotransferase concentration

Application of Artificial Neural Networks

Since the parameters that characterize the effects on the body are characterized by imprecision (significantly increase, decrease substantially), we found it useful to analyze these variations by the principles of artificial neural networks. As input parameters of the system, we consider the weights of the organs studied. As an output parameter of the system we consider the concentration of chlorpyrifos administered. The system established, can make the match between the two spaces of the inputs and output.

ANN Principles

Neural networks are designed to mimic the performance of the human brain. There is inputs level, output level, and a variable number of internal (or hidden) layers. The inputs are connected to hidden layer and they are in turn connected to output. As the neural network learns from a data set, the connection weights are adjusted. Data are fed into the input nodes, processed through the hidden layer(s), and the connection weights to the output nodes are adjusted. Neural nets are categorized based on their learning paradigm. Neural networks can reveal unexpected and otherwise undetectable patterns in large data sets. The major weakness in neural network solutions is the fact that the methods by which a relationship is discovered are hidden and therefore not readily understood or explained [5]. In the simplest way, a cooperative model [6];

[7], can be considered as a preprocessor wherein artificial neural network (ANN) learning mechanism determines the training data [8].

B. Expression of the Problem

Mapping of the space of parameters involved in the toxicity effect of *CPF* on organs, we consider inputs [weight of the brain, kidneys, spleen, lungs, heart, ovaries, testes]; The CPF diluted and administered orally to animals in a single dose by stomach tube is considerate as output of system.

Fig. 1 describes the topology with eight inputs extensible, two hidden layers, and an output (3-2-1) in the terminology of models of artificial neural networks. Wij et Wjk are weights, which represents the connection between the inputs and the output of the system. Weights contain all the information about the network. The objective is the training of the network to reach the minimum value of the reading error at the output observed [9] (Fig. 6).

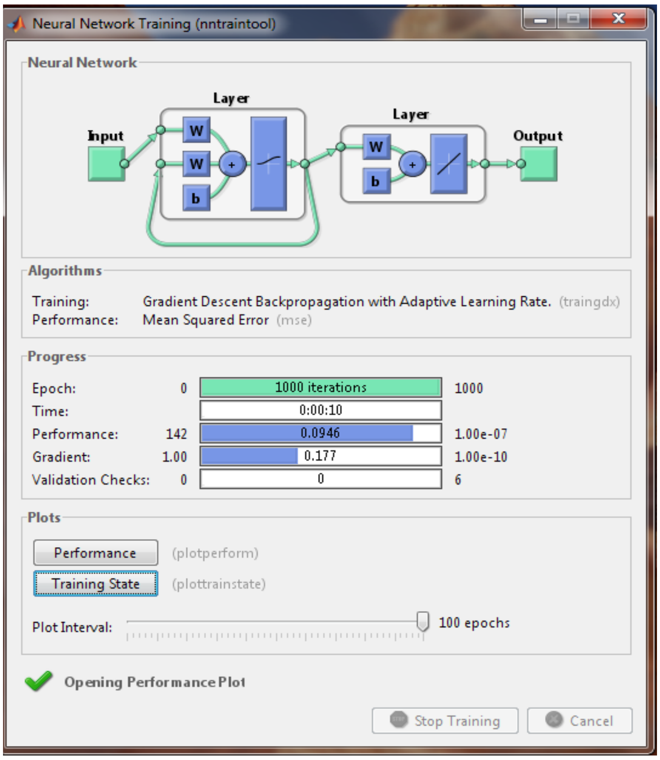

Figure 6. Structure and block diagram of the system

C. Model

Using examples consists of 100 tests of 50 different combinations of factors involved in the process toxicity effect with all possible combinations. We choose to keep 10 tests (50%)

while 10 tests (50%) are used for learning. A priori, the relationship between these two spaces is complex (in particular non-linear) which justifies the use of a multilayer network.

D. Learning of the Neural Network

It is in this case to introduce different data to the input in correspondence with the degree of weight variations. To achieve this, the method is a kind of imitation of the brain: if the answer is correct, it is, but if there is an error, we must modify the network so as not to repeat the mistake. Is repeated several hundred times the operation, until the system has the smallest error value as possible

Note: To change the system, just work on the weights [W] which are in the form of real numbers linking neurons. As these weights involved in the sum made by each neuron (the sum is weighted), it is possible to modify the network by changing their values without changing the network itself. That said, it is not clear how much weight we need to modify these. The goal is to achieve convergence towards a minimum error. In our case, after 142 iterations, the error is 0.0946 (Fig. 1) with a gradient of 0.17 to 1000 iteration (Fig. 7).

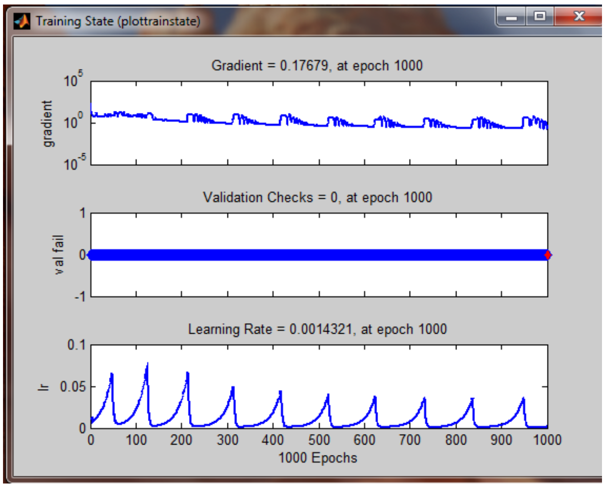

Figure 7. Error correction functions

The result after training is shown in Fig. 8. The proposed program predicts the degree of weight organs variation based on the input parameters according CPF concentration. Test values are fully consistent with the recorded values (Fig. 9).

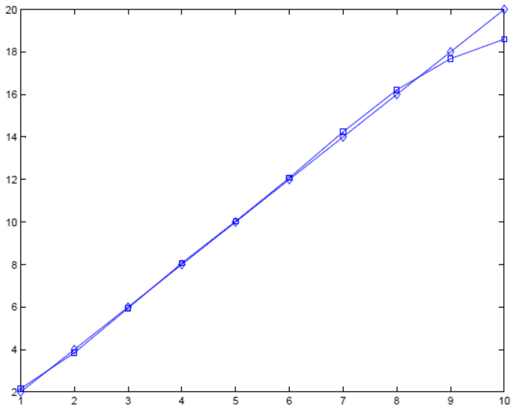

Figure 9. Example of application between the measured values and test values

RESULTS AND DISCUSSION

In practice farmers argue their investment by integrating irrigation potential soil quality and the environment used and implicitly establishes a link between the quality and risk of disease. The proposed system can predict the degree of damage depending on the nature of the environment used.

By introducing random values to the input of the system allows us to instantly read the result to predict the output. The lack of precision that can occur is subject to many variables to the system input.

The present study showed that repeated exposure to low-level PCF led to a deterioration of biochemical parameters of mousses showing pathological changes in tissues such as kidney, liver, brain, and genitals organs. Pesticides can contaminate the ecosystem.

This study represents a contribution to the correct evaluation of the extent of the health risk due to exposure to pesticide residues, especially chlorpyrifos. It would also be necessary to test bioaccumulation and bioconcentration in animals to better study their misdeeds on health.

Statement of Conflict of Interest

The authors have no conflict of interest.

Authors' Contributions

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

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Effects of Microfluidization and Ultrasonication Pre-Treated Whey Addition on Some Quality Parameters of Bread*

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ABSTRACT: Bread is insufficient in terms of nutritional values such as amino acids, minerals and fatty acids, since it is generally made from refined flour today. Therefore, many additives have been used to enrich the bread. Although there are studies on the use of whey to enrich bread, disadvantages such as a reduction in bread volume and water absorption limit its use. Therefore, pre-treatment that can lead to modifications in the molecular structure of whey and improve its technological properties is being investigated. This study aimed to investigate the effects of microfluidization and ultrasonication pre-treated whey addition on some quality properties of bread. While microfluidization and ultrasonication strengthened the effects of whey on ash, unsalted ash, volume, crust/core ratio, hardness, and cohesiveness, they reversed its effects on moisture content, elasticity, and color of bread. Microfluidization supported the effects of whey on acidity, weight and chewiness properties of bread, while ultrasonication reversed these effects. In the sensory analysis, the highest scores were obtained in the ultrasonication treated whey added bread (USWB) and later in the microfludization treated whey added bread (MFWB). Results suggest that microfluidization and ultrasonication pre-treatment may have a potential to improve the use of whey to enrich bread.

Keywords: Microfluidization, Ultrasonication, Whey, Bread

INTRODUCTION

Bread is included in the daily meals of most people from all over the world because it is cheap, hearty, and accessible (Basaran, 2022). Bread is produced by fermenting and baking the dough obtained by mixing wheat flour, yeast, salt and water in appropriate proportions (Gębski et al., 2019). However, the nutritional value of bread is not sufficient in terms of amino acids such as lysine, dietary fiber, essential fatty acids and minerals due to the separation of germ and bran during the milling stage of wheat (Pasqualone et al., 2017; Meral and Karaoğlu, 2019). For this reason, malnutrition is becoming increasingly common in low-income societies where bread is used as a staple food. Therefore, researchers are focusing on the studies of the functionality of breads that are fortified, especially protein, amino acids, fibre, and minerals (Sajdakowska et al., 2019; Brouns et al., 2022; Zain et al., 2022).

Whey, which is a by-product of cheese-making facilities, is a rich source of protein (14%), lactose (70%), lipids (4%), minerals (9%) and vitamins (Blažić et al., 2018). However, whey is a serious burden to the environment due to its high biological and chemical oxygen demand (BOD:40-60 g/L and COD: 50-80 g/L) and high organic and inorganic substance content. In addition, this burden is increasing given the global growth rate of whey production (2%) and the difficulties in treatment plants and costly advanced tec hnology requirements (Zotta et al., 2020; Buchanan et al., 2023). Therefore, the management of whey and increasing of its usage fields are very crucial for environmental concerns. The fact that whey is especially rich in branched-chain essential amino acids, lactose, lipids, vitamins and minerals and the proven health effects of whey proteins contribute to increasing its usage in the food industry (Khan and Selamoglu, 2019; Zandona et al., 2021).

One of the fields where whey is evaluated is its use in bread dough (Cansız et al., 2020; Guiné et al., 2020; Ferreyra et al., 2021; Tsanasidou et al., 2021; Ingrassia et al., 2022; Dopazo et al., 2023). It has been reported that the direct use of whey in bread dough increases the nutritional value of bread and provides benefits in terms of Maillard reaction due to its lactose content. However, its disadvantages are stated as it reduces water absorption capacity, reduces the volume of bread by limiting the work of yeast due to high osmotic pressure, and causes undesirable changes in the properties of the crumb (Van Riemsdijk et al., 2011; Wronkowska et al., 2015). Different technical approaches that can be used as a pretreatment are being investigated in order to obtain high benefits from whey and to reduce the disadvantages that may limit its use in bread dough.

There has been increasing interest in microfluidization and ultrasonication process that can enhance techno-functional properties including the protein functionality, stability, foaming, water holding capacity, textural, and the health benefits of food products by producing smaller particles sizes (Oboroceanu et al., 2014; Yildiz et al., 2016; Koo et al., 2018; Khatkar et al., 2018a; Liu et al., 2019; Alliod et al., 2019; He et al., 2022). Several studies demonstrated that treatment of whey with microfluidizer (MF) and ultrasound (US) can increase shelf life, surface hydrophobicity, enhance solubility, foaming, and nutritional properties of whey (Shen et al., 2017; Monteiro et al., 2020; Vidotto et al., 2022). However, no study has been found on the contribution of whey treated with these processes to bread properties. Therefore, the effects of whey treated with MF, and US, on some quality parameters of bread were investigated in this study.

MATERIAL AND METHOD

Materials

Wheat flour, refined salt, and baker's yeast (Saccharomyces cerevisiae) were obtained from Ankara Halk Ekmek Un Fabrikası A.Ş. (Ankara, Turkey). Some properties of wheat flour are 87.1% of dry matter, 31.97% of wet gluten, 11.19% of dry gluten, 12.9% of moisture, 0.56% of ash, 26 cm3 of sedimentation, 869 s of falling number. Whey was obtained from Atatürk Orman Çiftliği Süt Fabrikası (Ankara, Turkey). It included 5.73% of dry matter, 0.38% of ash, 0.165% of acidity, 0.3% of fat.

Microfluidization Process

Microfluidization process was carried out at Middle East Technical University (Ankara, Turkey). The process conditions set as 11.000 psi at 750 bar using a Microfluidizer equipment (M-110Y, Microfluidics, USA) with two consecutively connected interaction chambers having micro-channel diameters of 200 µm and 100 µm, respectively (Bigikocin et al., 2011; Yildiz et al., 2016). It has 100-500 mL/min flow rate, 24x41x76 cm^3 volume, 25 kg weight. The process was first tested on water. Then, the process was started by putting whey into the sample cup. Whey exposed to pressure in the closed system was transferred directly from the product outlet point of the device to the sterile plastic bottle and thus preserved under refrigerator conditions.

Ultrasonication Process

Ultrasonication process was carried out using an ultrasonic processor (UP400S, Dr. Hielscher GmbH, Stuttgart, German) with H14 probe (100 mm length and 14 mm diameter) at a constant frequency (24 kHz) at Middle East Technical University (Ankara, Turkey) (Rather et al., 2015). 400 W of power, 100% amplitude, and 105 W/cm2 of the acoustic power density were used as study parameters. The prope was immersed into 2 cm depth in glass baker. All experiments were performed in 250 mL of glass beakers. Whey samples were treated for 5 min. Temperature was kept constant with a peristaltic pump (working at 7.5 L/min) connected to a water bath (Memmert, Germany). Whey samples treated with ultrasound were kept in sealed glass bottles stored in at 4 °C until used in bread making.

Bread Baking Procedure

Bread baking was carried out according to AACC 10-10B with minor modification (AACC, 2000) at Ankara Halk Ekmek Un Fabrikası A.Ş. (Ankara, Turkey). Treated and untreated whey samples were added to the bread dough consisting of refined salt (45 g), yeast (203 g), flour (3 kg) and water (1.8 L) at a rate of 25% of the water absorption capacity of the flour. Following the kneading process (4 min slow and 18 min fast in the spiral kneader), the dough was weighed and divided into 3 equal parts and rested for 10 min. After cutting and shaping, the bread samples were left to fermentation process for 75 min at 25±1 °C. Then, the bread samples were baked in an oven (190 °C inlet temperature and 242 °C outlet temperature) for 28 min. Breads taken out of the oven were cooled at room temperature. Taking into account the analyzes to be made, nine bread samples were obtained for each treatment group.

Bread Quality

Microscopic Imaging of Bread Samples

Bread samples enriched with whey treated and untreated were cut in 3 mm thick. Then, bread slices were photographed using a stereomicroscope (Nikon SMZ 1500, Japan, 40X) after 1, 2, and 5 days.

Experimental Analyzes

Experimental analyzes of bread samples enriched with whey treated and untreated were performed according to Nwosu et al. (2014) with minor modification. In experimental analysis, moisture, ash salt, acidity, bread weight, dough yield, volume, specific volume, and crust core ratio were examined.

Texture Analysis

The texture properties of the bread samples were determined using a texture analyzer (Lloyd TA Plus, UK) in the Instrumental Analysis Laboratory, Department of Food Engineering, Hacettepe University (Ankara, Turkey). Bread samples enriched with whey treated and untreated were cut into 1.25 cm thick slices with the same saw knife on the 1st, 2nd and 3rd days after cooking. The cut two slices of bread were placed on top of each other and texture measurements were made in this way (Çetiner et al., 2017). Hardness values of bread samples were obtained by compressing the bread in the rate of 25% with stroke 1 mm/s. The cylinder prop diameter was 3.5 cm.

Color Analysis

Values of brightness from 0=black to 100=white (L*), redness to greenness (a*), yellowness to blueness (b*) of bread samples (crumb and crust) were analyzed using a Minolta Spectrophotometer (CM-3600d, Japan) according to CIE L*, a*, b* system. The colour of bread samples was expressed as the average of six L∗, a∗, and b∗ measures on three bread slices. A white calibration plate was used to standardize the equipment prior to colour measurements (García -Segovia et al., 2020).

Sensory Analysis

Panelists (10 people) were composed of equal numbers of men and women aged 23-35. Bread samples were coded with letters and randomly presented to the panelists. Bread samples were evaluated in terms of bread internal structure, crust, shape symmetry, color, taste and smell. Panelists used numeric expressions from 1 (dislike extremely) to 5 (like extremely) for evaluation (Bilgiçli and İbanoğlu, 2015).

Statistical Analysis

All statistical evaluations were performed using a statistical software (SPSS 15.0 for Windows, SPSS Inc., Chicago, IL). Significant differences between means were tested using Duncan's multiple range test. Significant differences were accepted at $p<0.05$ levels. All the experiments were performed in triplicate and three independent experiments. All data are given as the mean±SE (standard error) (Karaoğlu and Kotancılar, 2006).

RESULTS AND DISCUSSION

Microscopic Imaging of Bread Samples

The microscopic images of the inner structure of the bread samples of Normal bread (NB), Normal whey added bread (NWB), Microfludization treated whey added bread (MFWB), Ultrasonication treated whey added bread (USWB) after 1, 2 and 3 days in room temperature are given in Figure 1. The holes seen in the inner structure of the bread are gas bubbles caused by the work of the yeast. It is understood that there are small and very big gas holes in the normal bread (NB) samples, and these holes show a heterogeneous distribution. When the images taken on the 1st and 2nd days are examined, it can be stated that the addition of whey causes the gases produced by the yeast to be partially retained and the formation of more homogeneous and smaller gas holes in the NWB samples compared to the NB samples. While fewer small and few medium-sized gas holes were seen in the inner structure of the MFWB samples, there are a small number of small gas holes and a larger number of medium-sized gas holes with a homogeneous distribution in the inner structure of the USWB samples.

Bread Samples	**Time (days)**		
	1.day	**2.day**	**5.day**
NB			
NWB			
MFWB			

USWB

Figure 1. The microscopic images of the inner structure of the bread samples: NB: Normal bread, NWB: Normal whey added bread, MFWB: Microfludization treated whey added bread, USWB: Ultrasonication treated whey added bread

Experimental Analyzes

The variance analysis and the Duncan multiple comparison test results of the experimental analysis measurements of the bread samples in this study are given in Table 1 and Table 2, respectively.

Table 1. The variance analysis results of the experimental analysis measurements of the bread samples

Variation source	SD	Moisture		Ash		Unsalted Ash		Acidity		Weight		Volume		Crust core ratio	
		KO	F	KO	F	KO	F	KO	F	KO	F	KO	F	KO	F
Treatment	3	3.275	959.8*	0.007	181.7*	0.007	181.7*	0.44	116.8*	5.85	10.69*	137.5	7.333*	0.017	39.2*
Error	4														

Table 2. The Duncan multiple comparison test results of the experimental analysis measurements of the bread samples

Bread Samples	Moisture (%)	Ash (%)	Unsalted Ash (%)	Acidity	Weight (g)	Volume (cm^3)	Specific volume (cm^3/g)	Crust core ratio
NB	37.17±0.03^{a}	2.31±0.00^{a}	0.56±0.01^{a}	4.25±0.00^{a}	259.50±^{a} b	490±0.00^{a}	1.888±0.01^{a}	0.867±0.01^{a}
NWB	35.54±0.42^{b}	2.40±0.01^{b}	0.655±0.01^{b}	4.80±0.07^{b}	258.37±^{b} c	482±3.54^{a}	1.867±0.01^{a}	0.745±0.03^{b}
MFWB	37.91±0.07^{c}	2.41±0.01^{b}	0.665±0.01^{b}	4.95±0.07^{b}	257.25±^{c}	470±7.07^{b}	1.827±0.03^{a}	0.682±0.02^{c}
USWB	38.49±0.08^{d}	2.44±0.01^{c}	0.695±0.01^{b}	3.95±0.07^{c}	261.26±^{a}	482±3.54^{a}	1.847±0.01^{a}	0.665±0.00^{c}

NB: Normal bread, NWB: Normal whey added bread, MFWB: Microfludization treated whey added bread, USWB: Ultrasonication treated whey added bread. Means with different lowercase letters in the same column are statistically different at $P<0.05$

The addition of treated and untreated whey significantly changed the moisture, ash, unsalted ash, acidity, weight, crust/core ratio measurements of the bread (Table 2). While untreated whey caused a decrease in the moisture, volume, specific volume and crust/core ratio values of bread, it led to an increase in the ash, unsalted ash and acidity values of breads compared to normal bread samples. Kittisuban et al. (2014) reported that whey caused a decrease in volume and specific volume values of breads in the absence of hydrocolloids. In addition, the low water holding capacity of whey proteins may cause a decrease in specific volume. In this study, untreated whey decreased the moisture content of bread. This may be associated with the low water-binding capacity of whey proteins. Because the ability of gluten proteins to form hydrogen bonds with water molecules is higher than whey proteins (Ferreyra et al., 2021). Sahagún and Gómez (2018) reported that gluten-free bread samples containing whey protein exhibited the lowest specific volume. The changes in the specific volume of bread can also be related to the amount of whey used. Zhou et al. (2018) reported that 5-15% whey protein supplementation non-significantly decreased the specific volume of bread, while 20-30% whey protein supplementation insignificantly increased it compared to the control group.

The bread samples with the highest moisture, ash, unsalted ash and weight values and the lowest acidity and crust/core ratio were the USWB. Chandrapala et al. (2011) reported that ultrasonication process led to minor changes in secondary structures (α-helix or β-sheets) and hydrophobicity of whey protein. In addition, they suggested that ultrasonication lasting less than 5 min increases the surface hydrophobicity of the whey protein concentrate and ultrasonication lasting more than 5 min may result in more extensive bonding by reducing the surface hydrophobicity as a result of partial denaturation of the proteins causing the surface hydrophobicity. The fact that the USWB has the lowest acidity value can be explained by the denaturation of proteins with the effect of acoustic cavitation on the ultrasonication process. In this way, a decrease in the number of amino acids and a decrease in acidity can occur. It has been showed that ultrasonication significantly reduced in sizes of whey molecule and the band intensity of low molecular weight molecules (β-lactoglobulin and α-lactalbumin) and increased solubility of whey (Khatkar et al., 2018b).

The highest acidity and lowest weight, volume, and specific volume values were obtained in the MSWB samples. The increase in acidity value of NWB and MFWB samples compared to NB can be explained by the increase in amino acid presence from whey proteins. Previous studies have reported that microfludization can change the structure of proteins β-lactoglobulin (β-lg), α-lactalbumin (α-la), and bovine serum albumin (BSA) found in whey, and improve their binding affinity and interaction with hydrophobic molecules (Zhong et al., 2019; Vidotto et al., 2022). A significant increase in moisture, weight values and a significant decrease in volume and crust core ratio were observed in MFWB samples compared to NWB samples (Table 2). Chen et al. (2023) reported that microfluidization decreased free sulfhydryl and amino group content of whey protein concentration and increased surface hydrophobicity, emulsifying stability index, and interface protein adsorption. The decrease in amino acid content in whey may lead to a decrease in the presence of amino acids added to the bread formulation, which may explain the low acidity value.

Texture Analysis

The variance analysis and the Duncan multiple comparison test results of the texture measurements of the bread samples in this study are given in Table 3 and Table 4, respectively.

Table 3. The variance analysis results of the texture measurements of the bread samples

Variation sources	SD	Hardness		Cohesiveness		Elasticity		Chewiness	
		KO	F	KO	F	KO	F	KO	F
Treatment (A)	3	13.707	11.12*	0.001	2.009	0.214	1.349	113.59	6.55*
Time (hour) (B)	2	598.24	485.5*	0.042	115.53*	0.030	0.191	2303.5	132.89*
AxB	6	62.373	50.62*	0.002	4.370*	0.090	0.57	440.88	25.435*
Error	24								

Table 4. The Duncan multiple comparison test results of the texture measurements of the bread samples

Bread Samples	Hardness (N)	Cohesiveness	Elasticity (mm)	Chewiness (Nmm)
NB	18.898±7.19a	0.476±0.07a	5.569±0.41a	49.098±17.99a
NWB	17.362±9.12b	0.481±0.04a	5.230±0.47a	41.624±19.29b
MFWB	15.909±3.21c	0.497±0.01a	5.345±0.28a	41.604±5.49b
USWB	17.019±7.40b	0.484±0.02a	5.507±0.43a	43.273±15.25b

NB: Normal bread, NWB: Normal whey added bread, MFWB: Microfludization treated whey added bread, USWB: Ultrasonication treated whey added bread. Means with different lowercase letters in the same column are statistically different at P<0.05

According to Table 4, hardness, elasticity, and chewiness values were decreased in the treated and untreated whey added bread samples compared to the NB samples. In contrast, Guiné et al. (2020) reported that whey added bread had the highest hardness and chewiness values compared to the normal breads. Ozturk and Mert (2018) stated that the dissolved proteins in whey can provide softer products by contributing to the formation of homogeneous structure. In this study, the lowest hardness and chewiness values and the highest cohesiveness value were obtained in the MFWB samples. the microfluidization pre-treatment caused a decrease in the hardness values and an increase in the springiness and cohesiveness of breads (Ozturk and Mert, 2018). Microfluidization may have contributed to the softness of the product by breaking down the whey molecules, resulting in a higher surface area, decreasing the stability of hydrophobic structures, and improving the hydrophilic property (Dissanayake and Vasiljevic, 2009).

Color Analysis

The variance analysis and the Duncan multiple comparison test results of the color measurements of the bread samples in this study are given in Table 5 and Table 6, respectively.

Table 5. The variance analysis results of the color measurements of the bread samples

Variation sources	SD	L*		a*		b*		C*		h	
		KO	F	KO	F	KO	F	KO	F	KO	F
Treatment (A)	3	11.317	3.265*	4.24	8.42*	1.448	1.337	3.234	2.739	9.884	8.44*
Part (B)	2	495.93	143.1*	756.4	1503*	1752.1	1618*	2209.3	1871*	1736.8	1484*
AxB	6	7.962	2.297	2.99	5.94*	1.030	0.95	1.979	1.676	5.939	5.07*
Error	36										

Table 6. The Duncan multiple comparison test results of the color measurements of the bread samples

Bread Samples	L*	a*	b*
NB	64.867±4.32^{a}	7.016±5.52^{a}	26.29±1.08^{a}
NWB	62.532±5.85^{b}	8.300±6.27^{b}	27.07±1.03^{a}
MFWB	64.040±5.24^{ab}	7.125±5.69^{a}	26.40±0.87^{a}
USWB	63.626±5.06^{ab}	7.233±6.21^{a}	26.61±0.46^{a}

NB: Normal bread, NWB: Normal whey added bread, MFWB: Microfludization treated whey added bread, USWB: Ultrasonication treated whey added bread. Means with different lowercase letters in the same column are statistically different at P<0.05 according to Duncan multiple comparison test

According to color measurements, there was a decrease in L* values in treated and untreated whey added bread samples compared to the NB samples. Similarly, Zhou et al. (2018) reported that L* value in the crumb of the concentrated whey protein (CWP) added bread samples was lower than the normal bread samples. In addition, they stated that the increased CWP ratio (from 5% to 10%) in the bread samples further decreased the L* value. The same results (a decrease in L* values of bread) with 20% CWP were demonstrated by Ferreyra et al. (2021). In this study, treated and untreated whey caused an increase in a* values of breads (Table 6). Sahagún and Gómez (2018) reported that the addition of whey caused a decrease in L* brightness values and a darker color by increasing a* values of gluten-free bread samples. The decrease in L* brightness values can be associated with the effect of the increase in the presence of amino acids on the Maillard reaction. The lysine amino acid, which is high in whey proteins, is the primary reactive amino group that reacts with reducing sugars in the Maillard reaction (Chen et al., 2015). In addition, considering the contribution of lactose in whey to Maillard reaction, the increase in a* values can be explained (Abd El-Salam and El-Shibiny, 2018). In this study, although there was no significant change, the addition of whey caused an increase in the b* color values of the bread samples. Chudy et al. (2020) reported that the riboflavin content in whey may cause slight changes in the b* color values of breads.

The changes in L* and a* values in the NWB samples were statistically significant compared to the NB samples. However, color changes in MFWB and USWB samples were not significant compared to the NB samples. According to this, it was understood that the addition of whey affected the color measurements of the bread, but microfludization and ultrasonication processes limited this effect on the color of bread samples due to modifications in whey. The color values in the MFWB samples were closer to the control group values compared to those of the USWB samples. Microfludization and ultrasonication processes may have caused an increase in riboflavin release by reducing the size of micelles in whey. In addition, it has been reported that microfludization and ultrasonication processes can cause modification in the secondary structure of whey proteins and change their affinity and reaction efficiency (Chen et al., 2016; Abd El-Salam and El-Shibiny, 2018; Dev et al., 2021; Gantumur et al., 2023).

Sensory Analysis

The variance analysis and the Duncan multiple comparison test results of the sensory analysis of the bread samples in this study are given in Table 7 and Table 8, respectively.

Table 7. The variance analysis results of the sensory analysis of the bread samples

Bread Samples	L*	a*	b*
NB	64.867±4.32^{a}	7.016±5.52^{a}	26.29±1.08^{a}
NWB	62.532±5.85^{b}	8.300±6.27^{b}	27.07±1.03^{a}
MFWB	64.040±5.24^{ab}	7.125±5.69^{a}	26.40±0.87^{a}
USWB	63.626±5.06^{ab}	7.233±6.21^{a}	26.61±0.46^{a}

Table 8. The Duncan multiple comparison test results of the sensory analysis of the bread samples

Bread Samples	Bread Crumb	Bread Crust	Symmetry	Taste	Odour	Color
NB	4.0±0.82^{a}	3.3±0.95^{a}	4.0±0.47^{a}	3.1±0.88^{a}	3.7±0.82^{a}	3,8±0,63^{a}
NWB	4.1±0.57^{ab}	3.5±0.85^{a}	4.1±0.57^{a}	3.2±1.03^{a}	3.4±0.84^{a}	3,8±0,63^{a}
MFWB	4.6±0.52^{bc}	3.9±0.74^{a}	4.2±0.42^{a}	3.6±0.84^{ab}	3.4±0.97^{a}	4,2±0,63^{a}
USWB	4.8±0.42^{c}	4.0±0.67^{a}	4.2±0.42^{a}	4.1±0.57^{b}	3.4±0.97^{a}	4,4±0,70^{a}

NB: Normal bread, NWB: Normal whey added bread, MFWB: Microfludization treated whey added bread, USWB: Ultrasonication treated whey added bread. Means with different lowercase letters in the same column are statistically different at $P<0.05$

According to the sensory analysis, the addition of untreated whey improved the crumb, crust, symmetry, and taste properties of bread, but only resulted in a significant change for bread crumb. However, microfludization and ultrasound treatments strengthened these effects of whey. In particular, there was a significant difference in bread crumb and taste properties compared to NB and NWB samples. In the sensory analysis of bread crumb, bread crust, symmetry, taste, odour and color of bread samples, the highest scores were obtained in the USWB samples and later in the MFWB samples. There are no reports on the effect of ultrasound and microfluidization on sensory characteristics of whey added breads. However, Barukčić et al. (2015) reported that ultrasonication treatment (480 W, 55 °C, and 10 min) improved the sensory properties of whey. Similarly, Herrera-Ponce et al. (2022) stated that the panelists' scores caused a significant increase in taste (sweetness) of ultrasound treated whey beverage compared to the control beverage. In addition, the role of volatile compounds in taste change is known, and it has been reported that the ultrasound process provides the release of volatile compounds by the acoustic cavitation and the mass transfer of gases (Monteriro et al., 2018; Guimarães et al., 2019). Maillard reaction is very important, especially in color and crust acceptance of breads, and it has been reported that the ultrasound process improves the glycosylation of whey proteins (Perusko et al., 2015; Jiang et al., 2018). These views support that the highest scores were given by the panelists on the USWB samples.

CONCLUSION

In this study, effects of microfludization and ultrasonication of whey on some physicochemical, texture, color, and sensory properties of bread are investigated. Microfludization and ultrasonication processes caused a decrease in the sizes of gas holes and provided a homogenous structure in bread samples. While the highest acidity and cohesiveness and lowest weight, volume, specific volume, hardness and chewiness values were obtained in the MSWB samples, the highest moisture, ash, unsalted ash and weight values and the lowest acidity and crust/core ratio were obtained in the USWB samples. Microfludization and ultrasonication processes reversed effects of whey on the color of bread samples due to modifications in whey. In the sensory analysis of bread crumb, bread crust, symmetry, taste, odour and color of bread samples, the highest scores were obtained in the USWB samples and later in the MFWB samples. Based on these findings, it is suggested to investigate the use of microfluidization and ultrasonication pre-treated whey both in other bakery products and potential foods.

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Statement of Conflict of Interest

The authors declare that they are no conflict of interest.

Authors' Contributions

S.Ç, F.A and T.F.B designed and analyzed the research. 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.

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A Study of Soil Physico-chemical Properties in the North West Region of Lake Fetzara, Annaba, Algeria

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ABSTRACT: The Characterization of soils in the north-West of Fetzara Lake in Annaba region reveals that the roles played by soil formation factors appear to be influenced by the nature of the vegetation cover dominated by annual herbaceous plants with a significant and consistent supply of fresh organic matter. The objective of this work is to examine the physico-chemical characterizations of the soil and their functioning in the region of Lake Fetzara to define and enhance the quality and pedological richness of this region and to assess the state of soil organic matter. this soil is used as pastoral land without any anthropique intervention so it's favourable for organic matter evolution that serves an important function in the environment. We have adopted the following approach: fifteen profiles have been collected we sampled the soil from 0 to 30cm, The soil samples were dried, crushed and sieved at 2mm to obtain fine fraction which will be used for the physico-chemical analyses.On these samples we carried out a characterization of the physico-chemical properties ,The characterization focused on the estimating of organic Matter by (LOI) method and also analyzed the Granulometric fractions, PH , moisture%, The results show that we are in the presence of soil slightly calcareous , with a sandy clay to clay-sandy loam texture, very rich in organic matter. The richness of these soils in organic matter is linked to the permanent contributions of the plant cover and in the absence of disturbance or other agent modifying the natural evolution of this contribution. This base has provided the means to explain the significant role played by the organic carbon content of these soils. in the conservation of environmental characteristics and the increase of soil quality,improved sustainable methods are needed to enhance OM stocks in soils , especially that We are in an extremely critical period of climate change and it is taken into consideration as a method of reducing the greenhouse effect,so, it is crucial to put all precautions in place to safe this particularly fragile balance.

Keyword: Soil characterization, Organic Matter , Soil preservation , Soil biodiversity ,lake Fetzara, Annaba

INTRODUCTION

When averaged across biomes,Land-use change is the factor that have the largest worldwide impact on biodiversity by the year 2100, due to its catastrophic effects on habitat availability and consequent extinctions of species (Sala, O. E., 2000), The interaction between human activity, the biosphere's carbon (C) cycle, and projected climate change is currently the subject of considerable political and socioeconomic attention (Ostle et al. 2009). The Soils appear to be a solution for mitigating climate change, as they represent the largest terrestrial reservoir of organic carbon and are in high interaction with the atmosphere (Jacobson et al., 2000; Scharlemann et al., 2014).

The balance between the soil and vegetation is promoted by the various states of organic matter, which provides specific property on the soil. The organic materials are known for being generally dark in color, which provides for a better absorption of heat and sunlight, which would increase both biological activity and physicochemical reactions (Bernoux, 2004).

So The Soil organic carbon (SOC) plays a key role in determining soil properties, plant nutrients and land-atmosphere carbon exchange, but is affected by various natural and anthropogenic disturbances such as fires. loss, climate change, land use change or soil pollution Attributed to the importance of soil carbon in the global carbon cycle and potential feedbacks to climate change, how SOC responds to disturbances is of great interest-(Falahatkar et al., 2014; Yang et al., 2015).

Our work is part of this process knowledge of soils, their characteristics and their functions also the status of organic matter in the pastoral land of Lake Fetzara region.

MATERIAL AND METHOD

We sampled the soil from 0 to 30cm from the deph of the profile, in numbered and hermetically sealed bag.

Preparation of soil samples:

Figure1. preparation of fine soil

Soil samples were air dried, crushed and sieved at 2 mm to obtain fine earth which will be used for physico-chemical analyses.

Physico-chemical analyzes:

Organic Matter (LoL method):

Produced by incineration of the soil after passage in a muffle furnace at 480°C for 4 hours, it is expressed as a percentage of the dry weight of the soil (Benslama-Zanache, 1998).

Calculation Formula: 100*(p2-p0)-(p3-p0)/(p2-p0).

Particle size:

The particle size analysis of a soil consists in determining the proportion of the various classes of particle size. The soil analyzes separate the particles into three distinct classes: either sand (from 2 to 0.05 mm), silt (from 0.05 to 0.02 mm) and clay (less than 0.02 mm). (Damay and Julien, 1995). This allows us to know certain characteristics of the soil, such as the ability of roots to penetrate it, the ability of the soil to retain water, or its vulnerability to compaction. And so the granulometry is carried out according to the international method, (by the use of the Robinson pipette).

Calculation formula:

A%=(p1-p0)*400.

A+L%=(p1-p0)*400

L%=(A+L)-A=L%

SF%=(p1-p0)*10

SG%=(p1-p0)*10

The water pH:

It is the measure of the acidity of a suspension of earth in water, with an earth/water ratio normalized (1/5). It also indicates the concentration of “H+” ions present in the water. (Morel, 1986). There pH reading is done on the pH meter when the needle is stabilized and after resting for at least one time of suspension (Ben Amara, 2007).

Hygroscopic humidity (H):

Hygroscopic moisture represents the quantity of water that a soil can retain under the conditions natural drying. It is the quantity of water retained on the external surface of the soil particles and in equilibrium with the pressure and atmospheric humidity.The evaluation of hygroscopic humidity involves drying the soil in the open air and then drying it in the oven for 24 hours at 105°C (Benslama, 2005).

RESULTS AND DISCUSSION

The measurement results of the various parameters are presented below.

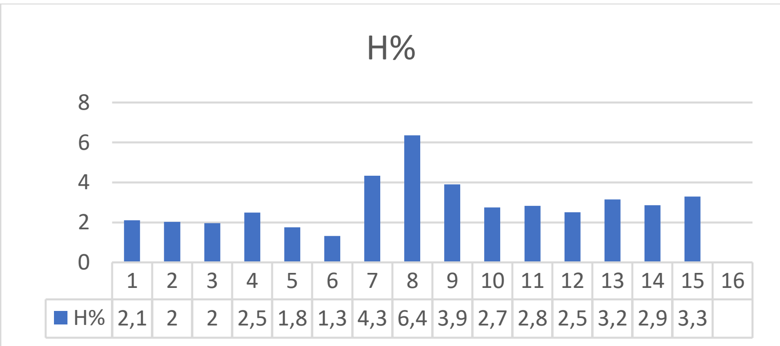

Figure 1. Variation in the Hygroscopic Moisture Content of the Soils of Lake Fetzara.

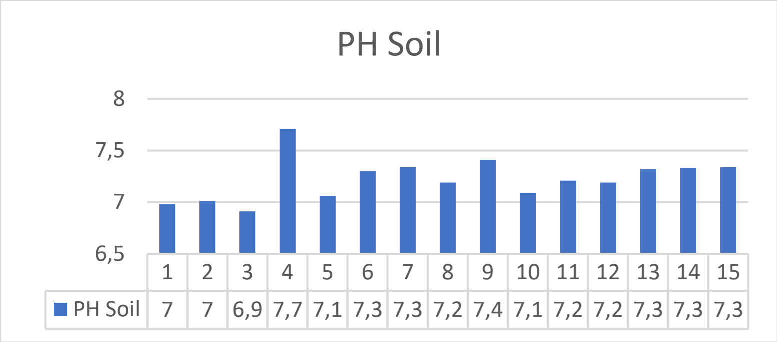

Figure 2. pH Soil measurement

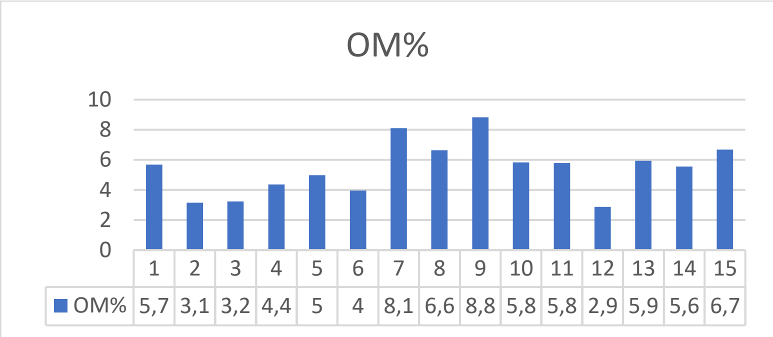

Figure 3. volution of organic carbon in the soil

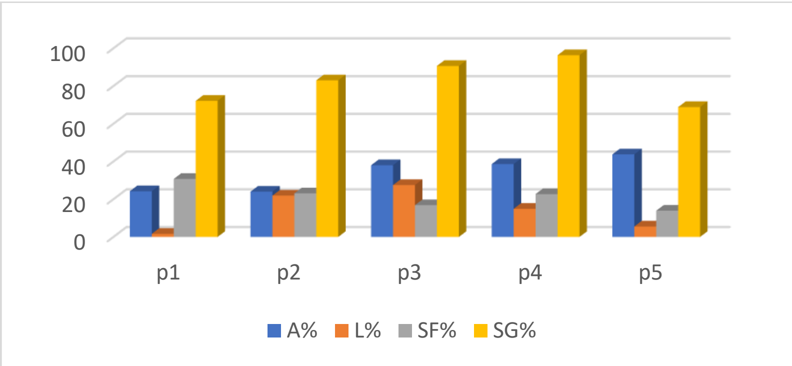

Figure 4. Distribution of texture components

DISCUSSION

The results show that the soils are in a state of very high desiccation. We find that this moisture remains below 7%, and it's higher in stations seven and eight than in the other profiles with a content of less than 4%. This situation may be related to the nature of the soil particles and the organic matter content which seems to play a fundamental role in the evolution of soil moisture. The results show that we are in the presence of soil, moderately rich in organic matter we note that the majority of our soils have a carbon content higher than 2%.

Examination of figure N°4 Shows that profile N°5 is more clayey than the others, which explains its strong ability to retain and protect organic matter while profile N° 1 has a higher fine sand content probably linked to its position close to the dunes, the results of the PH soil show that the soil is in a good acid-base state.

CONCLUSION

The combined action of climatic factors and the diversity of plant cover on a rock tender promotes the alteration and release of mineral elements which in turn ensure a good maintenance of a dense and diversified plant cover. in the northwest part of Lake Fetzara and the Particle size analysis revealed a sandy clay to loamy sandy clay texture expressing the result of the weathering of the parent rock in situ, The richness of these soils in organic matter is linked to the permanent contribution of the plant cover.and in the absence of disturbance or other agent modifying the natural evolution of this contribution. therefore, very clear to note that there is a difference in the content of organic matter in the soil linked to the quality of the contribution, and to the configuration of the land.

So, Knowledge the dynamics of organic matter in connection with the plant cover and the nature of the rock is considered as a means of combating the greenhouse effect, and plays an important role in the conservation of physico-chemical and biological properties and considered as a decision-making tool.

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Statement of Conflict of Interest

The authors declare no conflict of interest.

Authors' Contributions

Chaima Dahmani and Mohamed Benslama designed and analyzed the research, chaima dahmani studies arranged 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.

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Determination of some Plant Growth Characteristics of Potential Biofertilizer Bacterial Strains

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ABSTRACT: In this study; It is aimed to obtain bacterial isolates that may have biofertilizer potential from the soil samples taken from the root parts of different plants grown in Erzurum province and to determine some plant growth promoting properties of these isolates. As a result of the isolations made for this purpose; A total of 78 bacterial isolates were obtained. Nitrogen fixation and phosphate solubility properties of the obtained isolates were determined, and stock cultures of isolates that could be potential biofertilizers were made. According to the results obtained; UG-24, UG-62, UG-64, UG-65, UG-71 and UG-72 isolates gave strong positive results and the effects of these isolates on the growth of different plants, individually or in combination, will be investigated.

Keywords: Biofertilizer, PGPR, Phosphate Solubility

INTRODUCTION

Bacteria that are free-living, promoting plant growth, used in biological control or as biological fertilizers (BG) are called plant growth promoting rhizobacteria (PGPR). PGPRs are generally grouped as biofertilizers that increase the nutrient ratio in the plant, phytostimulators that promote plant growth with plant hormone production, rhizoremediators that break down organic pollutants, and biopesticides that control diseases with antibiotic and antifungal metabolite production (Antoun and Prevost 2006). It is important that PGPRs secrete plant hormones such as auxins, cytokinins and giberalins, as well as their mechanism of stimulating effect on root development and crop yield, and their ability to fix nitrogen (Anonymous 2006). Not only do plants produce plant hormones, but also many bacteria in contact with plants, both beneficial and harmful, can produce one or more of these substances (Fuentes-Ramirez and Caballero-Mellado 2006). Auxins are the plant hormones most secreted by PGPRs in terms of quantity. It is generally accepted that auxin production rather than nitrogen fixation is the main factor in stimulating rooting and increasing plant growth (Bloemberg and Lugtenberg 2001). It is stated that soil microorganisms have the ability to synthesize cytokinins in relation to plants (Anonymous 2006). Another bacterial synthesized hormone that plays a role in promoting growth is giberalin. The future of biofertilizers based on hormone-producing bacteria looks promising. Many trials have shown that bacterial addition increases the phytohormone level in plants (Fuentes-Ramirez and Caballero-Mellado 2006). PGPRs mostly belong to the genera Acetobacter, Azospirillum, Azotobacter, Bacillus, Paenibacillus, Pseudomonas, Rhizobium, Rhodobacter, Rhodosprilum, and Serratia (Burdman et al, 2000; Çakmakçı, 2005a, b). Within these genera, many soil bacteria that grow in the plant rhizosphere, root zone, on or inside are recognized as plant growth promoters by many different mechanisms mentioned above, and vegetative and generative growth in plants (vegetables, fruits, ornamental plants, some trees, cereals, etc.) at varying rates. they show an enhancing effect (Vessey 2003; Niranjiyan et al. 2006).

As a result, in order to ensure sustainability in existing agricultural systems, obtaining and accurately and reliably identifying bacteria with PGPR properties, which are of great agricultural importance, should be the primary goals of researchers working on this subject. In addition, the properties of these identified bacteria that promote plant growth should be determined and intensive studies should be carried out on their practical use. For this reason, this study was planned and carried out to determine which properties of bacteria promote plant growth.

MATERIAL AND METHODS

Preparation of used solutions and media

The preparation methods of the solutions and media used during the research are as follows:

1. 70% ethyl alcohol: It was prepared by adding 26 ml of sterile distilled water (sdH_2O) to 70 ml of ethyl alcohol (96%) and stored at -20°C (Klement et al., 1990).

2. Nutrient agar: 28 g of nutrient agar mixture (Oxoid) was added into 1 L of distilled water (dH2O). The medium was sterilized in an autoclave at 121°C for 15 minutes and cooled to 45°C, then poured into sterile petri dishes and left to solidify (Lelliot and Stead 1987).

3. Nutrient broth: 13 g of nutrient broth content (Oxoid) was added to 1 L of dH_2O, and the mixture was sterilized in an autoclave at 121°C for 15 minutes (Lelliot and Stead 1987).

4. Lauryl broth (LB): The broth prepared by adding 10 g peptone, 10 g NaCl and 5 g yeast extract to 1 L dH_2O was sterilized in an autoclave at 121°C for 15 minutes (Klement et al., 1990).

5. 30% glycerol: It was prepared by adding 30 ml of glycerol into 70 ml of sdH_2O and sterilizing it in an autoclave at 121°C for 15 minutes (Klement et al., 1990).

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.

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.

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 (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.

RESULTS AND DISCUSSION

Bacteria used as biological control agents or biofertilizers in agriculture are called plant growth promoting bacteria= PGPB. A total of 78 bacterial isolates were obtained as a result of the isolations made from soil samples taken from the rhizosphere of plants grown in different areas in Erzurum. In order to determine whether the isolates obtained have PGPB potential, their phosphate dissolving and nitrogen fixation properties were determined and the results were shown in Table. 1. According to these results; UG-24, UG-62, UG-64, UG-65, UG-71 and UG-72 strains gave strong positive results for both traits. As a result, in this study, bacteria with PGPB properties were isolated from soil samples taken from the rhizosphere of leguminous forage plants widely grown in our region, and their plant growth promoting properties (nitrogen binding, phosphate sources, etc.) were determined. Similarly, phosphate solubility of 16 isolates was found to be strongly positive, and when evaluated in terms of nitrogen fixing properties, 25 isolates were found to be strongly positive. In addition, when the results were examined, it was determined that there were many isolates with positive effects of both nitrogen fixer and phosphate solvent. When the results are evaluated in general, it is thought that the obtained isolates may have a high potential to become PGPB. Generally, the dominant phosphate forms are Fe and Al compounds in acid soils and Ca phosphates in calcareous soils. Microorganisms are the main factor in the natural P cycle. Bacteria solubilize insoluble inorganic phosphate in the form of tricalcium, dicalcium, rock phosphate and hydroxy apatite (Goldstein 1986, 1995), and organic acids produced by microorganisms are considered to be the main mechanism in the solubility of mineral phosphates (Leyval and Berthelin 1989, Salih et al. 1989, Hadler et al. et al. 1990). Similarly, some bacterial organisms (Rhizobium spp.) in the soil live in symbiotic relationship with some plants (Legumes). They form nodules in the root regions of these plants and fix the free nitrogen (in the form of N2) in the atmosphere and convert it into a forum (ammonia=NH3 ; nitrate=NO3 -) that plants can use. Thus, such plants do not need to be supplemented with nitrogen-containing fertilizers in the environment where they are grown, and they leave a nitrogen-rich soil in the plants that will be grown in the same soil after them. In addition, some bacterial organisms are found freely in soils in low concentrations and have the ability to fix nitrogen without being in a symbiotic relationship with legume plants (Elkoca et al., 2008; Şahin et al., 2004). It is possible to eliminate the constant dependence of the plants to be grown on nitrogen fertilizers by increasing the population of such bacteria in the soil or infecting the non-existent soils. However, since these bacteria live freely in the soil, their populations decrease completely or partially in winter seasons. Therefore, the populations in the soil should be supported continuously with annual or seasonal applications.

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.1 Some plant growth promoting properties of potential biofertilizer bacteria, host and location data

Strain no	Host Plants	Location	N-Free Medium	Phosphate solubility (P)
UG1	*Matricaria chamomilla*	Karaçoban	K^+	-
UG2	"	"	+	+
UG3	"	"	+	-
UG4	"	"	+	-
UG5	"	"	-	-
UG6	"	"	-	-
UG7	"	"	+	K^+
UG8	"	"	+	+
UG9	"	"	+	K^+
UG10	"	"	+	-
UG11	"	"	+	-
UG12	"	"	+	+
UG13	*Mentha*	Karaçoban	-	-
UG14	"	"	+	-
UG15	"	"	-	-
UG16	"	"	+	+
UG17	"	"	+	K^+
UG18	"	"	+	+
UG19	"	"	K^+	-
UG20	"	"	+	-
UG21	"	"	+	+
UG22	*Urtica* spp.	Karaçoban	K^+	+
UG23	"	"	+	+
UG24	"	"	K^+	K^+
UG25	"	"	+	+
UG26	"	"	K^+	+
UG27	"	"	+	-
UG28	"	"	+	K^+
UG29	"	"	K^+	+
UG30	"	"	Z^+	K^+
UG31	"	"	Z^+	+
UG32	"	"	Z^+	+
UG33	"	"	K^+	+
UG34	"	"	K^+	+
UG35	*Centaurea depressa*	Karaçoban	Z^+	K^+
UG36	"	"	Z^+	K^+
UG37	"	"	-	Z^+
UG38	"	"	Z^+	Z^+
UG39	"	"	Z^+	K^+
UG40	"	"	Z^+	-
UG41	"	"	Z^+	+

UG42	"	"	+	-
UG43	"	"	Z$^+$	-
UG44	"	"	Z$^+$	+
UG45	"	"	+	Z$^+$
UG46	"	"	Z$^+$	+
UG47	"	"	Z$^+$	-
UG48	*Ferula* spp.	Sercan	+	+
UG49	"	Göz mountain	K$^+$	+
UG50	"	Sercan stream	K$^+$	+
UG51	"	Sercan stream	K$^+$	-
UG52	"	"	K$^+$	+
UG53	"	Aktaş	K$^+$	+
UG54	"	"	K$^+$	-
UG55	"	Sercan stream	K$^+$	+
UG56	"	"	+	+
UG57	"	Aktaş	+	+
UG58	"	"	K$^+$	+
UG59	"	Saz mountain	K$^+$	+
UG60	Mentha	Karaçoban	K$^+$	+
UG61	"	"	K$^+$	+
UG62	"	"	K$^+$	K$^+$
UG63	"	"	+	K$^+$
UG64	*Conium maculatum*	Karaçoban	K$^+$	K^{++}
UG65	Mentha	Karaçoban	K$^+$	K$^+$
UG66			+	+
UG67	*Echium vulgare*	Karaçoban	+	-
UG68	"	"	-	+
UG69	"	"	K$^+$	+
UG70	"	"	Z$^+$	K$^+$
UG71	"	"	K$^+$	K$^+$
UG72	"	"	K$^+$	K$^+$
UG73		Oltu	+	-
UG74	"	"	+	-
UG75	"	"	+	-
UG76	"	"	+	-
UG77	"	"	+	-
UG78	"	"	+	-

*: K$^+$: strong possitive, +: possitive, -: negative

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Determination of Yield and Quality Characteristics of Some Faba Bean Varieties in Bingöl Conditions*

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ABSTRACT: This study was carried out to determine the herbage yield and quality of some faba bean varieties in Bingol ecological conditions. As the material in the study, a total of 10 different faba bean varieties were used: Filiz 99, Kıtık 2003, Salkım, Emiralem, Sevil, Sorgun, Hıstal, Luz De Otono, Reina Mora and Sakız. In the study, plant height, first pod height, green herbage, dry herbage and crude protein yields, crude protein, crude ash, ADF, NDF, digestible dry matter and dry matter intake rates and relative feed value were investigated. In the study, the highest plant height, first pod height, green herbage yield, dry herbage yield, crude protein ratio and crude protein yield were obtained from Hıstal variety. The lowest ADF and NDF ratios and the highest digestible dry matter, dry matter intake and relative feed value were obtained from Luz De Otono variety. As a result, Hıstal cultivars in terms of yield and Luz De Otono cultivars in terms of quality came to the fore by showing superior characteristics in the ecological conditions of Bingöl province.

Keywords: Faba bean, Protein, ADF, NDF, Herbage yield

INTRODUCTION

It has been understood that legumes are the main source of protein in human and animal nutrition, and when crop rotation is applied in many legume cultivation areas in the world, it can increase the productivity level of the soil under certain ecological conditions and reduce the incidence of weeds, diseases and pests (Mwanamwenge et al., 1998). Faba bean, one of the leguminous forage crops, is an annual plant in the legume family (Fabaceae), which gives its name to this family and is cultivated both as human food and as a forage plant for animals. The word "faba bean", which originates from Arabic, means "grass, greenery" in this language. The Ottomans used faba beans in the same sense (Nişanyan, 2014). Faba beans, which originated in the Middle East in prehistoric times, are among the cool climate legumes cultivated and used culturally as a main protein source for human food and animal nutrition (Multari et al., 2015).

Faba bean is a cultural plant with very low costs in terms of cultivation. Since it yields crops in early spring, it is a good pre-plant used in crop rotation. Due to the high amount of nitrogen it fixes in the soil, faba bean is also of great importance in increasing soil fertility as a green fertilizer plant (Özdemir, 2002). Faba bean has an important agronomic value in order to keep the soil structure healthy. In areas where intensive grain cultivation is practiced, crop alternation with faba bean plant prevents soil erosion (Köpke and Nemecek, 2010). Faba bean, which grows very well in temperate climate regions, is more resistant to cold than other edible grain legumes such as beans, peas and cowpeas (Vural et al., 2000; Pekşen et al., 2006).

Faba bean varieties cultivated in field agriculture are systematically categorized under three groups. These are *Vicia faba* var. *minor*, *Vicia faba* var. *equina* and *Vicia faba* var. *major*. There are considerable differences between the groups in terms of morphological and seed characteristics. Morphologically, plant height ranges between 40 and 200 cm, number of leaves between 20 and 70 per plant, dry grass yields between 200 and 480 kg per decare and crude protein ratios up to 25%. In climates with abundant rainfall and mild climates, plant height increases considerably, green yield increases while dry matter ratio decreases (Gençkan, 1983; Manga et al., 1995; Alan and Geren, 2006).

The aim of this study was to determine the faba bean varieties that can adapt well to Bingöl ecological conditions in terms of yield and quality, to learn the cultivation conditions and to introduce this plant to the producers engaged in animal production in the region.

MATERIAL AND METHOD

The experiment was established on May 8, 2022 at Bingöl University Agricultural Research and Application Center located 15 km away on the road to Genç district of Bingöl province. In the study, 10 faba bean varieties were used as plant material. The varieties given in Table 1 were preferred because of their earliness and high productivity.

Table 1. Varieties used in the study and the institutions where the varieties were obtained

No	Varieties	Institutions Where Varieties are Sourced
1	Emiralem	Agrogen Ziraat A.Ş.-Tekirdağ
2	Filiz 99	Ege Tarımsal Araştırma Enstitüsü-İzmir
3	Hıstal	Fito Tohumculuk-Antalya
4	Kıtık 2003	Ege Tarımsal Araştırma Enstitüsü-İzmir
5	Luz De Otono	Fito Tohumculuk-Antalya
6	Reina Mora	Fito Tohumculuk-Antalya
7	Salkım	Ege Tarımsal Araştırma Enstitüsü-İzmir
8	Sevil	Agrogen Ziraat A.Ş.-Tekirdağ
9	Sorgun	Agrogen Ziraat A.Ş.-Tekirdağ
10	Sakız	İntfa Tarımsal Araştırma Merkezi-Konya

According to the data obtained from Bingöl General Directorate of Meteorology, the average temperature of Bingöl province during the growing period of 2022 was 23.2 ^{0}C, the total annual precipitation was 30.2 mm and the relative humidity was 38.1%. It was determined that the 2022 growing period in which the experiment was carried out was warmer, the total annual rainfall was higher and the relative humidity value was lower compared to the average of many years (1975-2022). It was determined that the soil structure in the area where the study was carried out was clayey-loamy, slightly acidic (pH: 6.26), salt-free (0.014%), organic matter (1.09%), lime (0.41%) and potassium (18.27 kg da^{-1}) content was low, and phosphorus (7.60 kg da^{-1}) content was moderate.

The research was conducted in a randomized block design with three replications. The sowing of the experiment was carried out in 6 rows of 5 m in length, with rows spaced 40 cm apart with a hand marker, and with a row spacing of 15 cm. In sowing, 20 kg of seed was used per decare. The experiment was fertilized with 9 kg da^{-1} nitrogen (N) and 9 kg da^{-1} phosphorus (P_2O_5) fertilizers. Weeds were controlled by hand hoeing during the growing period in the plots. Harvesting was done when the lower pods of the plants started to appear. Plant height and first pod height were measured on 5 plants randomly selected from each plot. Green herbage yields were obtained from the mowing of each plot after removing the edge effects and dry matter ratios were determined by drying 500 g plant samples from each plot at 70 ^{0}C in a drying cabinet. Dry matter ratios were multiplied by green herbage yields to obtain dry herbage yields. The samples of faba bean varieties were ground in a mill with a sieve diameter of 1 mm and prepared for chemical analyses. Crude protein analyses were performed by the methods specified in AOAC (1990). The crude protein ratios determined in the dry herbage samples were multiplied by the dry herbage yields per decare and crude protein yields were calculated. Cell wall constituent ADF and NDF were analyzed by the methods described in Van Soest (1963) and Van Soest and Wine (1967), respectively. Digestible dry matter (DDM) and dry matter intake (DMI) ratios and relative feed values (RFV) were calculated. The following formulas were used in the calculations (Morrison, 2003).

DDM= 88.9 - (0.779 x ADF)

DMI= 120 / NDF

RFV= (DDM x DMI) / 1.29

The experiment was conducted in a randomized block design with 3 replications. Tukey test was applied to the values determined as a result of the experiment with JMP statistical package program and the differences and similarities were compared.

RESULTS AND DISCUSSION

Plant height, first pod height, green herbage, dry herbage and crude protein yields and crude protein ratios of some faba bean cultivars were found statistically significant at 1% level (Table 2). The highest plant height was obtained from Hıstal variety with 65.0 cm and this variety was followed by Kıtık 2003 variety (60.9 cm) which was in the same group statistically. The lowest plant height was obtained from Sevil variety with 45.8 cm. The average plant height of faba bean cultivars was 54.7 cm (Table 2). Considering the previous studies; Geren and Alan (2005) found the plant height of faba bean as 79.4 cm under the ecological conditions of Ödemiş district of İzmir province, Soysal et al. (2020) found it as 53.3 cm under the ecological conditions of Siirt province, Başdemir et al. (2020) found it as 59.7 cm in Dicle University Faculty of Agriculture trial area.

The highest first pod height was obtained from Hıstal variety with 35.6 cm and the lowest first pod height was determined in Luz De Otono variety with 26.0 cm. The average value of the first pod height of the faba bean varieties was 29.9 cm. Pekşen et al. (2006) determined the first pod height as 20.18 cm in a 3-year study with 15 faba bean genotypes under Samsun ecological conditions. Kadıoğlu (2019) determined the first pod height as 26.5 cm in the study conducted in the Pasinler trial area of Eastern Anatolia Agricultural Research Institute, Soysal et al. (2020) determined that the first pod height varied between 14.0-14.6 cm in the ecological conditions of Siirt province. It can be said that the reasons for the differences between the values obtained as a result of the research and the values determined by the researchers are the different varieties in the trials and the different soil and climate characteristics of the places where the trials were carried out.

Table 2. Mean values of plant height, first pod height, green herbage yield, dry herbage yield, crude protein yield and crude protein ratios of faba bean varieties

Varieties	PH (cm)	FPH (cm)	GHY (kg da^{-1})	DHY (kg da^{-1})	CPY (kg da^{-1})	CPR (%)
Emiralem	56.33 d	27.93 cde	2676 c	498 b	93.5 c	18.77 ab
Filiz 99	51.90 e	28.60 bcde	1499 f	322 d	46.7 e	14.49 ef
Hıstal	65.00 a	35.60 a	3419 a	716 a	130.9 a	18.28 b
Kıtık 2003	60.93 b	32.00 abc	2882 b	509 b	103.9 b	20.41 a
Luz De Otono	46.87 f	26.00 e	1233 g	252 e	41.8 e	16.53 cd
Reina Mora	50.33 e	28.20 bcde	1471 f	323 d	42.7 e	13.22 f
Sakız	52.40 e	30.87 bcd	1859 e	330 d	59.4 d	17.99 bc
Salkım	57.80 cd	31.20 bcd	1197 g	249 e	40.8 e	16.28 d
Sevil	45.80 f	27.07 de	1099 g	251 e	45.8 e	18.23 b
Sorgun	59.67 bc	32.33 ab	2168 d	428 c	64.4 d	15.03 de
Average	**54.70**	**29.98**	**1950**	**388**	**67.0**	**16.93**
CV (%)	**1.72**	**8.3**	**2.88**	**5.06**	**4.67**	**3.34**

Statistically significant differences at 1% level were found between the varieties in terms of the traits in the table. There are no statistically significant differences between the averages shown with the same letters.

The highest green herbage and dry herbage yields were obtained from Hıstal (3419 kg da^{-1} and 716 kg da^{-1}, respectively), while the lowest green herbage and dry herbage yields were obtained from Sevil (1099 kg da^{-1} and 251 kg da^{-1}, respectively) and Salkım (1197 kg da^{-1} and 249 kg da^{-1}, respectively). The average values of green herbage and dry herbage yields of some faba bean varieties were 1950 kg da^{-1} and 388 kg da^{-1}, respectively (Table 2). Geren and Alan (2005) determined the green herbage yield as 4720 kg da^{-1} in their study conducted in the ecological conditions of Ödemiş district of İzmir province. Yıldırım and Özaslan Parlak (2016) found the green herbage and dry herbage yields of faba bean as 2543.5 kg da^{-1} and 455 kg da^{-1}, respectively, in their study conducted in Çanakkale 18 Mart University Dardanos Application area. Coşkun and Topçu (2022) determined the green herbage yield of faba bean as 3406 kg da^{-1} in their study conducted under the ecological conditions of Bornova district of İzmir province.

Table 3. Mean values of ADF, NDF, DDM and DMI ratios and relative feed values of faba bean cultivars

Varieties	ADF (%)	NDF (%)	DDM (%)	DMI (%)	RFV
Emiralem	19.61 de	35.32 cd	73.62 ab	3.40 bc	194.26 b
Filiz 99	27.23 a	43.24 a	67.69 e	2.78 e	145.64 d
Hıstal	20.11 de	35.61 cd	73.23 ab	3.37 bc	191.44 b

Kıtık 2003	21.07 cd	37.36 bc	72.49 bc	3.21 cd	180.59 bc
Luz De Otono	18.27 e	32.35 e	74.67 a	3.71 a	214.72 a
Reina Mora	21.06 cd	34.59 d	72.49 bc	3.47 b	194.98 b
Sakız	22.63 bc	39.56 b	71.27 cd	3.03 d	167.60 c
Salkım	22.86 bc	38.69 b	71.09 cd	3.10 d	170.97 c
Sevil	21.96 bcd	38.71 b	71.80 bcd	3.10 d	172.63 c
Sorgun	23.89 b	39.11 b	70.29 d	3.07 d	167.20 c
Average	**21.87**	**37.45**	**71.86**	**3.23**	**180.01**
CV (%)	**3.89**	**2.02**	**0.92**	**2.13**	**2.95**

Statistically significant differences at 1% level were found between the varieties in terms of the traits in the table. There are no statistically significant differences between the averages shown with the same letters.

Table 2 shows that the highest crude protein content was obtained from Kıtık 2003 (20.41%) and Emiralem (18.77%), while the highest crude protein yield was obtained from Hıstal (130.9 kg da^{-1}). The lowest crude protein rate was obtained from Reina Mora (13.22%), while the lowest crude protein yields were obtained from Filiz 99, Luz De Otono, Reina Mora, Salkım and Sevil varieties. The average crude protein ratios and yields of faba bean varieties were 16.93% and 67.0 kg da^{-1}, respectively. Geren and Alan (2005) reported that crude protein rate in broad bean was 18.95%, Yıldırım and Özaslan Parlak (2016) reported that crude protein rate was 27.40%, Coşkun and Topçu (2022) reported that crude protein rate was 17.91% in their study conducted in İzmir Bornova ecological conditions. Belekoğlu and Kır (2021) determined the crude protein yield of faba bean as 73.1 kg da^{-1} in their study conducted in Karamürsel district of Kocaeli province.

As shown in Table 3, the highest ADF and NDF ratios of different faba bean cultivars were obtained from Filiz 99 (27.23% and 43.24%, respectively), while the lowest ADF and NDF ratios were obtained from Luz De Otono (18.27% and 32.35%, respectively). The mean ADF and NDF ratios of faba bean varieties were 21.87% and 37.45%, respectively. Yıldırım and Özaslan Parlak (2016) determined the ADF and NDF ratios of faba bean plants as 36.23% and 46.99%, respectively, and Coşkun and Topçu (2022) determined the mean ADF and NDF ratios of faba bean varieties as 34.8% and 39.9%, respectively, in their study conducted in Çanakkale 18 Mart University Dardanos Application area.

The highest DDM rate was obtained from Luz De Otono variety with 74.67%, followed by Emiralem (73.62%) and Hıstal (73.23%) varieties which were in the same group statistically. The lowest DDM rate was determined in Filiz 99 variety with 67.69%. The average digestible dry matter of faba bean varieties was 71.86%. The highest dry matter intake rate and relative feed value of faba bean varieties were obtained from Luz De Otono (3.71% and 214.72%, respectively), while the lowest DMI rate and RFV were obtained from Filiz 99 (2.78% and 145.64, respectively). The averages of DMI ratios and RFV of faba bean cultivars were 3.23% and 180.01, respectively.

CONCLUSION

Considering the results of the research; in terms of yield characteristics (plant height, first pod height, green herbage yield, dry herbage yield and crude protein yield) It was determined that Hıstal variety showed superior characteristics and Luz De Otono variety showed superior characteristics in terms of quality traits (ADF, NDF, DDM, DMI and RFV). Therefore, it is recommended that Hıstal and Luz De Otono varieties should be preferred in terms of herbage yield and quality traits in Bingöl ecological conditions.

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Statement of Conflict of Interest
The authors declare that they are no conflict of interest The author(s) should declare that they are no conflict of interest.

Authors' Contributions
Kagan Kokten and Mehmet Ugur Erik designed, analyzed the research and studies arranged. 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.

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Use of Essential Oil in Aquaculture

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ABSTRACT: Plants are one of the indispensable basic sources of life from the existence of humanity to the present day. Essential oils are generally colorless or light yellow compositions obtained from leaves, flowers, bark, seeds and roots of plants, usually in liquid form at room temperature, easily crystallized. Biological activities including antibacterial, antiviral, antifungal and anti-inflammatory effects are well known. It is widely used in pharmacology, agricultural, food industry as a preservative and flavoring, alcoholic beverages, animal nutrition, sanitation, cosmetics and perfumery. The use of spices and natural aromatic plants, which are used as additives to increase the odor and taste properties of seafood, is becoming increasingly important. The short shelf life caused by chemical and microbial spoilage reactions can be stopped with traditional preservation methods, but there is increasing interest in natural preservation methods. Essential oils are natural antioxidants and antimicrobials that can extend the shelf life of seafood, alone or in combination with other techniques. In this study, general information will be given about the properties of essential oils, their production methods and their use in seafood.

Keywords: Aquaculture, Antibacterial, Essential oil, Plant

INTRODUCTION

Plants are one of the indispensable basic sources of life from the existence of humanity to the present day (Turan et al., 2012). Essential oils called aromatic, essential or essential oils, are obtained from various plants with aromatic properties, usually in countries with tropical or temperate climates. Flowers, leaves, roots or bark of plants are used in the production of essential oil. Although there are many extraction methods in production, steam distillation is generally preferred. The use of essential oils dates back thousands of years (Rustamlı, 2020).

Essential oils are present in different proportions in different organs of medicinal and aromatic plants. They are liquid at room temperature, crystallizable, colorless or light yellow, volatile compounds with intense odor (Uçar et al., 2015). In addition, these oils have characteristics specific to the plant from which they are obtained, giving the plant odor, burning taste, consisting of many chemical components, volatile at room temperature, having the property of being entrained with water, and the most distinctive features are that they are volatile and fragrant (Turan et al., 2012). Although they are defined as oil because they are insoluble in water and dissolve in organic solvents, they are different from fixed oils (Bayaz, 2014).

They are mixtures consisting mainly of terpenes that are insoluble in water but readily soluble in organic solvents. They are especially found in flowers and fruits. Its antiseptic, antioxidant, antimicrobial and enzymatic effects are its most important functions (Sutili et al. 2007; Şengözer and Güngör, 2008).

Of the approximately 3000 known essential oils, about 300 are of commercial importance, and some of them or some of the components they contain are used in pharmacology, agricultural applications, food industry, alcoholic beverages, animal nutrition, sanitation, cosmetics and perfumery products, and natural therapeutic folk medicine. Today, some plant essential oils are classified by the US Food and Drug Administration (FDA) as GRAS 'Generally safe-harmless' taste-smell or food additives (Bayaz, 2014; Hanif, 2019).

METHODS OF ESSENTIAL OIL PRODUCTION

Essential oils are usually obtained from the essential oil-bearing parts of the plant. Essential oils can be obtained from the buds, flowers, leaves, stem, branches and roots of the plant. The method to be applied is chosen depending on the plant's resistance to heat, the amount of essential oil, whether it dissolves in water and its components (Kutlular, 2007).

The distillation method was developed in Spain and France at the beginning of the 1300s to obtain essential oil, and in the 1550s, new techniques were started to be applied in order to meet the needs of different branches such as pharmacology (Emir Çoban, 2010).

Essential oils from plants; It is obtained by distillation, extraction and mechanical methods depending on their sensitivity to heat and water, their density and solubility in water (Terzi Gürel, 2022).

1.Distillation Method

Distillation is a separation process performed by making use of the differences in boiling points of liquids (Emir Çoban, 2010).

1.1. Water Distillation

It is a traditional method widely used in obtaining volatile compounds. The basis of the method; It is based on boiling water and plant material for 2-8 hours in a glass balloon connected with the cooler, condensing the oil molecules moving together with the water vapor in the cooler and separating them from the water. The amount of essential oil obtained is expressed in volumetric terms. Water distillation gives the best results in powdered materials (eg root or wood flour) (Asfaw, 2022).

1.2. Steam distillation

It is based on the separation of volatile organic substances using water vapor. Suitable for heat sensitive substances (cinnamon, thyme). In this method, the speed and temperature of the steam can be controlled. It is mostly preferred for essential oil production on a large scale (Kaya and Günç Ergönül, 2015).

1.3. Vacuum distillation

Some compounds have very high boiling points. In order to obtain these compounds, it is more effective to lower the pressure rather than increase the temperature. Once the pressure is reduced below the vapor pressure of the compound, the boiling and distillation process begins (Taştan, 2021).

2. Extraction Method

In simple terms, it is the process done by extracting essential oil into a solvent. There are six different extraction methods. These are: supercritical liquid extraction, solvent extraction, microwave extraction, compressed solvent extraction, solid-phase micro-extraction, and versatile extraction (Sayın, 2019).

2.1. Solvent Extraction

It is the traditional extraction method, where the plant material can be directly immersed in the solvent at room temperature or boiled with organic solvent in a soxhlet. At the end of the extraction, the organic solvent is removed by distillation and recovered (Çoşkun et al., 2021).

2.2. Supercritical Fluid Extraction

Supercritical fluid extraction is essentially a solvent extraction. Substances with supercritical liquid properties are used as solvents instead of organic solvents. While it can dissolve many substances with the dissolving power of liquid solvents, it also spreads the solute rapidly with its diffusion coefficient feature close to gases (Cellat, 2011).

2.3. Microwave Extraction

Microwave technology, which has been used since the Second World War, was used in the analytical laboratory in the late 1970s. Microwaves are electromagnetic radiations varying in the range of 0.3–300 GHz, and extraction of natural products is usually carried out at 2.5–75 GHz. The effectiveness of microwave energy largely depends on the solvent content, plant material and applied microwave power. In the presence of polar molecules and ionic species, a faster energy dissipation takes place. Extraction with the help of microwave is carried out with two different systems. The most common system is closed system extraction, which is done in a closed vessel with temperature and pressure controllable. The other method is carried out in an open container under atmospheric pressure (Yaman and Kulesan, 2016).

2.4. Simultaneous distillation extraction

According to the working principle of the method, the sample is boiled by placing it in a glass balloon filled with water on the left side of the SDE apparatus. The volatiles are evaporated in the solvent on the right side of the SDE apparatus while moving up the left column as steam distillation. The extraction process takes place by condensation of water and solvent vapor on the walls of the cooler located at the top of the apparatus. Condensed water and solvent return to the glass balloons they are in, and volatile compounds are obtained by condensing the water and solvent part separately (Emir Çoban, 2010).

USE OF ESSENTIAL OILS IN AQUACULTURE

The use of spices and natural aromatic plants, which are used as additives to increase the odor and taste properties of seafood, is becoming increasingly important. The use of essential oils as natural additives has become widespread in order to keep seafood meat fresh. The short shelf life caused by chemical and microbial spoilage reactions can be stopped with traditional preservation methods, but there is increasing interest in natural preservation methods. Essential oils are natural antioxidants and antimicrobials that can extend the shelf life of seafood, alone or in combination with other techniques (Kadak et al., 2021). There are several studies on seafood with different essential oils added.

Dimitrijevi ć et al., (2019), it was investigated whether essential oils reduce the microorganism density in fish inoculated with *Listeria monocytogenes* during storage of vacuum packed trout under refrigerator conditions. It has been reported that the microorganism density decreased significantly in the group treated with plain eugenol compared to the groups treated with carvacrol, carvacrol + eugenol.

Oğuzhan Yıldız (2019) determined that the addition of citrus (lemon, orange and bergamot) essential oils at different concentrations (0.5% and 1%) significantly reduced the bacterial count of rainbow trout (*Oncorhynchus mykiss*) fillets and delayed the spoilage of the samples.

Arslan (2020), in his study investigating the effects of black cumin oil (1, 1.5 and 2%) on rainbow trout (*O. mykiss*) fillets, on sensory properties, reported that fillets using 2% black cumin oil were preferred.

Mehtizadeh et al. (2019), in their study where they applied sage essential oil on rainbow trout (*O. mykiss*) fillet, it was determined that the microbial activity of the group in which sage oil was applied significantly decreased.

Bahurmiz et al. (2020) investigated the antimicrobial activity of eight different plant essential oils (cardamom, cinnamon, clove, eucalyptus, lemongrass, linden, nutmeg and rosemary) on tilapia fish and found that cinnamon essential oil had the highest antimicrobial activity. They reported that clove essential oil followed.

Navarro-Segura et al. (2020) reported that the application of thyme essential oil mist on sea bream (*Sparus aurata*) fillets delayed microbiological spoilage and increased shelf life.

Uçar (2021) investigated the effect of fish oil powders obtained by microencapsulation of citrus peel essential oils (orange, lemon, tangerine and grapefruit) added to anchovy oil by spray drying on sensory quality. It was stated by the panelists that the fishy odor observed in the control group was suppressed by the added essential oils.

Statement of Conflict of Interest

The authors have no conflict of interest.

Authors' Contributions

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

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Food Poisoning From Seafood

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ABSTRACT: Due to the increase in the world population, the need for food is increasing day by day. Fishery products have an important place among the foods that meet the animal protein needs in nutrition. In recent years, there has been an increase in the consumption of aquatic products, which have an important place in human nutrition. Seafood contains many important fatty acids, proteins, vitamins and minerals. Its nutritional benefits are mainly rich in polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, if fishery products are caught or grown in biologically and microbiologically contaminated waters, they threaten food safety and human health. Seafood poisoning is a foodborne illness caused by the consumption of fish and shellfish containing toxins. Toxins in fishery products are formed by algae and bacteria and cause great economic losses. In this review, the potential health risks from seafood and food poisoning will be discussed.

Keywords: Poisoning, Seafood, Toxin

INTRODUCTION

Nutrition, It can be expressed as the consumption of the items necessary for the growth, development and maintenance of physiological functions of human beings. In order for people to protect their health, it is important not only to have an adequate and balanced diet, but also not to threaten their health and to be safe in the foods they take (Şahin, 2019).

Due to the increase in the world population, the need for food is increasing day by day. Fishery products have an important place among the foods that meet the animal protein needs in nutrition (İşgöz and Yücel, 1993). In recent years, there has been an increase in the consumption of seafood, which has an important place in human nutrition (Kocatepe et al., 2013). Seafood contains many important fatty acids, proteins, vitamins and minerals (Atar and Alçiçek, 2009). Its nutritional benefits are mainly rich in polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Elal Muş and Çetinkaya, 2017).

In addition to their nutritional properties, the consumption of aquatic products is not completely safe. Although rare, a wide variety of chemical and microbiological hazards may occur However, if fishery products are caught or grown in biologically and microbiologically contaminated waters, they threaten food safety and human health. In poisoning caused by seafood; The aquatic product itself, pathogenic microorganisms, microorganism toxin-infections, algal toxins and heavy metals may be causative agents (Elal Muş and Çetinkaya 2017).

POISONING FROM SEAFOOD

Histamine (Scombroid) Poisoning

Histamine poisoning is a food poisoning caused by ingesting high-dose histamine-containing foods (Taylor and Eitenmiller, 1986). This food poisoning was previously called 'Scombroid poisoning' because it is commonly caused by fish from the Scombridae family (Feng et al., 2016).

In the world, food poisoning caused by fish was most common in this family species. However, this disease is now known as histamine poisoning, since fish not belonging to the Scombridae family, and other foods such as cheese, red meat, wine and beer also cause this poisoning (Ruiz-Capillas and Herrero, 2019). Histamine poisoning can occur as a result of consumption of fresh, canned, salted and dried fish. Symptoms of this disease are severe headache, dizziness, nausea, palpitations, abdominal pain, blood pressure disorder, dry mouth, difficulty in swallowing, diarrhea, cramps, edema, itching, disturbance of heart rhythm and shock effects (İşgöz and Yücel, 1993).

Tetrodotoxin (TTX)

Blowfish, puffer fish, ocean sunfish and similar species in the Tetraodontiformes order mostly contain tetrodotoxin, which is an intoxicating agent, in their tissues, and various neurological diseases occur after the digestion of this toxin. TTX poisoning is unique to these regions due to the frequent presence of pufferfish (fugu) in the culinary culture of Japan, Taiwan, and Southeast Asian countries. Symptoms such as tingling and prickling in the tongue, lips and fingers that occur a few minutes after ingestion of poisonous fish are similar to the symptoms of paralytic shellfish poisoning. In some cases, nausea, vomiting and gastrointestinal pain are observed. Depending on the amount of toxin consumed, loss of corneal reflex and breathing difficulties may occur (Köşker et al., 2015; Elal Muş and Çetinkaya, 2017; Makarova et al., 2019; Özdemir et al., 2021).

Ciguatera poisoning

Ciguatera fish poisoning is seen as a result of consumption of coral rock fish living in tropical and subtropical regions. This poisoning has been known for centuries and is a growing problem in some areas, caused by benthic dinoflagellates such as Ciguatera, Gambierdiscus, and possibly related species of Ostreopsis siamensis, Coolia monotis, Prorocentrum lima and Gambierdiscus toxicus. Toxins are transported through the food chain and reach humans, usually through the consumption of fin fish. There is chemical similarity between ciguatoxin and brevetoxin, and the effects of both toxins are obviously caused by changes in sodium ion flux in the infected organism. Ciguatoxins also cause death in marine mammals. It progresses in the form of gastrointestinal tract and its neurons and lasts about 1-2 days (Withers, 1982; Friedman et al., 2008; Aydın and Uzar, 2019).

Paralytic Shellfish Poisoning (PSP)

Paralytic Shellfish Poisoning is a serious disease that causes gastrointestinal and neurological symptoms in humans as a result of consumption of shellfish fed with toxic dinoflagellates. Paralytic shellfish poisoning is one of the most common types of poisoning that causes severe symptoms in humans. PSP poisoning in humans is a type of poisoning that occurs by consuming seafood containing these toxins or swallowing sea water containing these toxins. Shellfish fed in areas with algae producing PSP toxins accumulate these toxins in their bodies through diet, and people who consume these foods are exposed to the poisons of these toxins (Kao,1993; Mons et al., 1998; Demirel and Çelik, 2013; Gültekin Kuşoğlu, 2020).

Neurotoxic Shellfish Poisoning (NSP)

Neurotoxic shellfish poisoning occurs when brevetoxin produced by Karenia brevis, another dinoflagellate species, is filtered by bivalve mollusks and consumed by humans. Since neurological symptoms are observed in patients who consume contaminated seafood, it is called neurotoxic shellfish poisoning. NSP symptoms; tingling-numbness in the tongue, lips and throat, muscle pain, gastrointestinal disorders and dizziness. These symptoms disappear within a few days (Kocatepe et al., 2013).

Amnesic Shellfish Poisoning (ASP)

Amnesic shellfish poisoning, which causes memory loss in humans, is caused by "domoic acid" produced by diatoms (Pseudo-nitzshia), a single-celled algae. The first domaic acid poisoning was seen in Canada in 1987, and it was reported that more than 150 people were affected and 4 people died as a result of consuming mussels contaminated with toxin. Consumption of various types of mussels, scallops, oysters, and fish such as anchovies and sardines that feed on plankton, contaminated with domoic acid, may cause neurotoxigenic disease symptoms and death in advanced stages. While symptoms of dizziness, nausea, vomiting, abdominal cramps, headache, diarrhea, heart palpitations, agitation are observed in humans as symptoms of acute poisoning; In chronic cases, short-term memory loss, double vision, loss of consciousness, disorientation and coma may occur (Kocatepe et al., 2013; Elal Muş and Çetinkaya, 2017).

Diarrhetic Shellfish Poisoning

It is a symptom that occurs with the consumption of crustaceans contaminated with toxic dinoflegallates. It generally affects the gastrointestinal tract. It usually progresses with acute poisoning symptoms such as diarrhea, vomiting, nausea and abdominal pain within the first three hours and recovery is observed within 3 days. DTX toxins is common in the marine environment and fishery products all over the world. Especially scallops, oysters and mussels are among the risky foods in terms of diarrheal shellfish poisoning (Yasumoto et al., 1985; Aune and, Yndestad 1993; Tailor, 2008).

MICROBIOLOGICAL HAZARDS FROM FISHERY PRODUCTS

Fisheries, like other foods, have a unique natural microbial structure that is affected by external factors in the living spaces of living things. The discharge of waste water and sewage into the sea causes microbial contamination of fishery products, and as a result, bacterial, viral and parasitic infections arise from the consumption of fishery products (İşgöz and Yücel, 1993; Kocatepe et al., 2013; Elal Muş and Çetinkaya, 2017).

Vibrio

Raw and undercooked seafood play a role in causing these pathogens.

Listeria monocytogenes

It is found in rotting vegetables, soil, water, sewage, silage, animal feed, fresh frozen poultry, fresh and processed meat products, raw milk, cheeses, fish, crustaceans and insects. In most patients, listeriosis is mild, often showing symptoms similar to flu and gastroenteritis.

Botulizm

Clostridium botulinum type E spores are widely distributed in both fresh and salt waters in many parts of the world. The first symptoms are gastrointestinal disturbances. Nausea, vomiting and pain may ocur.

Salmonella

Salmonella is a type of bacteria that is frequently reported in food poisoning. Fisheries-borne Salmonella outbreaks are mostly caused by the consumption of contaminated fish, shrimp, oyster and mussel species. Infections occur as a result of raw consumption of shellfish and improper preparation or insufficient cooking of other seafood such as fish.

Statement of Conflict of Interest

The authors have no conflict of interest.

Authors' Contributions

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

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Possibilities of Using Essential Oils against Pests: Review

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ABSTRACT: Due to the increase in the world's population, the need for food has increased and with the rapid increase in industrialization and faulty agricultural practices, the existing agricultural areas have decreased and more yield has been obtained from the unit area. As a result of intensive production with conventional agriculture, faulty practices and changes in climatic events have led to an increase in diseases and pests. In this context, while the most effective struggle in the management of increasing diseases and pests is seen as chemicals, the application of these pesticides causes soil, water, and environmental pollution and residues in food. These residues may cause negative effects on living organisms such as mutations, toxicity, and different morphological or physiological symptoms. However, to minimize chemical inputs in crop production and to minimize their negative effects, one of the alternative control methods has been the use of essential oils against agricultural diseases and pests. Approximately 1/3 of the existing plant species contain essential oil. Considering the rich plant biodiversity due to the geopolitical location of our country, it is of great importance to include these plants containing essential oils in the control of diseases and pests to ensure sustainability in agriculture. In this study, information about the possibilities of using essential oils against pests in agriculture and the results obtained are given.

Keywords: Essential oil, Alternative control, Acaricide, Herbicide, Nematicide, Insecticide

INTRODUCTION

Essential oils are volatile, strong-smelling, and oily mixtures, usually colorless or light yellow, volatile, strong-smelling and oily mixtures obtained from plant sources or parts of plants such as roots, leaves, fruits, stems, bark, and flowers by various methods, which are liquid at room temperature, freeze in some cases, crystallize easily. They are called "essential oil" because they can evaporate even at room temperature if left uncovered; "etheric oil" because they volatilize like ether; and "essential oil" because they are fragrant and used in perfumery. Essential oils are aroma substances of plants and have been used for many years for various purposes, especially in scientific and commercial fields. These include cosmetics, pharmaceuticals, the food industry, dentistry, oral care products, perfumery, dyeing, aromatherapy, and phytotherapy. The area of use of essential oils is very wide and therefore has recently attracted the attention of many scientists (Yaylı, 2013).

In the last decade, interest and curiosity in the application of traditional medicine have been awakened in the world. In Turkey, there are 347 species collected from nature and sold both domestically and abroad. Thirty percent of them are used in foreign trade. Of the world's commercial shares of medicinal and aromatic plants, 50% is used in food, 25% in cosmetics, and 25% in the pharmaceutical industry (Faydaoğlu and Sürücüoğlu, 2011). Medicinal and aromatic plants are used for food, spices, and medicinal purposes. There are medicinal plants used in the treatment of some diseases and disorders. For kidney diseases, ayrıkotu, altınotu (immortelle), and horsetail; for indigestion, allspice, galangal, aniseed, dill, chamomile, cardamom, cumin, fennel, and ginger; thuja, ginger, yarrow, rose hips, sultanas for hemorrhoids; cinnamon, flax, fennel, cinquefoil seeds for constipation; hawthorn, mistletoe for heart disease; ginger, fern root, mint for nausea and stomach pains; rosemary, thyme, anise, horsetail, chamomile, clove, lavender, lemon balm for rheumatic pain; echinacea, juniper, mallow, ginger, linden, licorice, mint, clove, eucalyptus, chamomile for colds, colds and coughs; valerian, aniseed, evening primrose, fennel, lemon balm, chamomile, and hops are used in sleep disorders (Faydaoğlu and Sürücüoğlu, 2011). This study examines the possibilities of using essential oils against agricultural pests.

Properties and Chemical Structure of Essential Oils

Essential oils are also called essential oils, organic oils and ether oils. Essential oils are composed of hydrocarbons and are generally expressed by the simple formula C_5H_8. Most essential oils are a mixture of many compounds. Önel and Akbay (2022), as a result of a study on essential oils, which are generally colorless, observed that the colors of essential oils exposed to air and sunlight darken. Therefore, it was determined that it would be appropriate to store them in amber glass bottles in dry and cold environments Önel and Akbay (2022), Essential oils, produced by plants, generally have a fragrant, oily and viscous (liquid or resinous) structure. All essential oils have their characteristic odors. Essential oils can be formed from parts of plants such as branches, bark, roots, fruits, flowers, etc. or from a single organ of the plant. The essential oil of the same type of plant varies according to the geographical location where the plants live, the age of the plants, the methods of obtaining and many other factors. Ertaş et al. (2019), defined essential oil obtained from aromatic plants as volatile, complex, natural compounds in their studies. Therefore, the strong aroma of essential oil consists of terpenes, terpenoids, and various aromatic and aliphatic compounds. Terpenes constitute the largest group among chemical structures. Terpenes are categorized into four groups according to the number of isoprenes: monoterpenes, sesquiterpenes, diterpenes and triterpenes. The simplest terpenes are monoterpenes containing two isoprenes. Sesquiterpenes contain three isoprenes and diterpenes contain four isoprenes. Each isoprene molecule has five carbon atoms. Although the components found in essential oil vary from species to species, many compounds such as vanillin, menthol, oxide, kuminil, nerol, and kuminil alcohol isomentone can be given as examples.

Methods of Obtaining Essential Oils

The yields of essential oils vary according to the harvest time, the part of the plant, environmental factors, the vegetation period of the plant, the structure of the material and analytical methods (Taştan, 2021). To determine the essential oil method, the region of the plant from which the essential oil will be extracted, the type of plant and the amount of oil should be well known. It is generally possible to examine the methods of obtaining essential oil under three groups. These are the distillation method, extraction method and mechanical method (pressing).

1. Distillation

Distillation is the oldest and simplest method of obtaining essential oils and the most preferred method. With this method, a volatile liquid is separated from a non-volatile substance. In the distillation method, the distillation temperature, working pressure, type, and quality of the plant material change the amount of essential oil. Different distillation methods are used to obtain essential oils. These are; Water distillation method, Steam distillation method, Water-Vapor distillation method, Dry distillation method, and Hydrodiffusion method (Taştan, 2021). The method of distillation is determined by the volatility of the essential oil, the sensitivity of the essential oil to the effect of heat and water, and the solubility of the essential oil in water.

Water distillation (hydrodistillation): It is a widely used method for obtaining volatile compounds. It can be applied to plant materials that do not deteriorate when boiled. Water and plant materials are boiled together, evaporated and then cooled and condensed (Deniz, 2015).

Steam distillation: This widely used method is a method of separating volatile organic substances with water vapor. It is generally suitable for heat-sensitive substances. The steam distillation method is preferred for large-scale oil production (Gülgen, 2016).

Water vapor distillation: It is a combination of steam and water distillation. In this method, water and material do not directly contact the walls of the container and vaporization occurs with the transmitted heat. The vapor produced is passed through the cooler to condense. Water and oil phases are separated by phase separation.

Dry distillation: It is not a very preferred method. In this method; after the plant is dried and turned into small particles, it is placed in the boiler and heat is applied and dry distillation is provided. This method, which is not used for most plants, partially breaks down the volatile elements in some plants and distillation occurs (Taştan, 2021).

Vacuum distillation: The boiling point of some compounds is quite high. Despite this, it can deteriorate even at temperatures below the boiling point. To prevent deterioration, it is preferred to reduce the pressure instead of increasing the temperature (Cellat, 2011).

Hydrodiffusion: In this method, steam is sent to the boiler from the top and separated. This method is used for plant materials that are at boiling temperature and are not damaged when dried. After diffusion processes, the essential oils in the interior reach the surface and are then entrained by steam. Steam distillation yields more efficient essential oils. However, the disadvantage of this method is that water-soluble compounds such as coumarin, lipids, fats, chlorophyll, and fatty acids, which have little or no volatility, pass into the oil. Therefore, its industrial use is not very common (Gülgen, 2016).

2. Extraction Method

Extraction is a method used to purify or isolate a product. This method requires a long time. In the extraction method, the way the oils are extracted from the plant is very important. An inappropriate extraction method disrupts the chemical structure of the essential oil and causes events such as color changes and odor changes. As a result of his studies, he showed that the quality of the product obtained from plants depends on factors such as the choice of extraction method, the effect of the surfactant before extraction and during excitation, mixer type, humidity, material porosity, particle size, pressure, mixing speed and time (Başer, 2010). The basis of the extraction method is the removal of volatile oil with solvents. It is possible to classify the extraction method into two groups as traditional method and the modern method. Although the low cost of traditional methods is seen as an advantage, it also has many disadvantages. The disadvantages include hydrolysis and heat-induced degradation of the processes and residues in essential oils (Taştan, 2021). Extraction methods can be generally classified as Solvent extraction, Super Critical Liquid extraction (SFE), Microwave vapor extraction (MWE), Microwave vapor diffusion method and Solid phase microextraction (SPME) (Deniz, 2015). In traditional methods, the process takes a long time and the solvents used cause environmental pollution, so methods using supercritical fluids are preferred. Microwave and supercritical extraction methods are modern, fast and efficient methods.

3. Mechanical Method (Pressing)

This is one of the best methods for extracting essential oils. Cold pressing is used to extract essential oils from citrus peels such as citrus, grapefruit, bergamot and lemon. The peels are separated from the fruit, ground or chopped and then pressed. The disadvantage of this method is that the extracted oils deteriorate in a short time (Taştan, 2021). Oils are obtained by squeezing citrus peels with a cold hydraulic press by placing them in a cloth bag.

Utilization of Essential Oils in Agricultural Pests

1. Use as herbicide

Recently, realizing the importance of environmentally friendly measures in weed control, many researchers have focused on these methods, especially herbal extraction and essential oil control methods. Essential oils are an alternative to herbicides due to their allelopathic effect on plant growth and their degradability compared to herbicides. The use of essential oils as herbicides should not affect the crop but should inhibit the growth and development of weeds. It has been observed that essential oils used in the germination of weed seeds cause weakening with increasing dosage and completely inhibit germination. Essential oils, rated as nano-herbicides, are considered as a modern method of weed control and reduce the toxic effect and environmental pollution caused. In this sense, we see that essential oils used in weed control are increasing (Nohutcu et al., 2021). Nepeta transcaucasica Grossh. (Lamiaceae) essential oil (nepetalactone content 93. 75%) of cultivated plants (Poaceae; *Hordeum vulgare*, *Zea mays*, *Triticum aestivum*) and weeds (*Onopordium acanthium* (Asteraceae), *Cynodon dactyloni* (Poaceae), *Amaranthus retroflexus*) on seed germination and seedling growth. It was reported that all concentrations (0, 2, 5, 10, and 20 µL/L) delayed or even significantly inhibited germination by affecting α-amylase activity in weeds. It was concluded that cultivated plants were slightly inhibited and the essential oil of N. transcaucasica Grossh species could be used as a natural herbicide (Karakuş et al., 2019). Monoterpenoids, which have a high potential to be used as herbicides among terpene compounds, have been the most studied group since they inhibit germination and growth. Some of the important monoterpenes that can be used as herbicides are thujone, camphor, myrcene, citronellol, eugenol, carvacrol, linalool, linalyl acetate, geraniol, limonene, menthol and 1,8-cineole (Aydın and Tursun, 2010; Baydar, 2013).

2. Use as an insecticide

The mechanism of action of essential oils on pests is by direct spraying (contact effect) or inhalation, and the fumigation method is preferred especially for storage pests (Backer et al., 2003; Göktürk et al., 2020). Nicotine, whose use as an insecticide dates back to ancient times, is highly toxic and is used in the control of pests such as thrips, aphids, and mites. Şanlı et al. (2020), reported that rosemary essential oil killed 46.3% of nymphs in the control of greenhouse whitefly nymphs. Erler et al. (2009), determined that *Teucrium divaricatum* Sieber (Lamiaceae), *Melissa officinalis* L. (Lamiaceae), *Inula viscosa* L. (Asteraceae), *Origanum onites* L., *Pimpinella anisum* L. (Apiaceae) species had toxic effects against the mushroom fly. It was revealed that *Melia vollkensii* (Meliaceae) showed a toxic effect against Coleoptera, Diptera and Lepidoptera pests thanks to the limonoid active substance it contains (Şanlı et al., 2020). In Yiğit et al., 2021 examined the effects of three different doses of three different thyme oils on *T. confusum* with two different applications, directly on the insect and by spraying on the food medium, and observed the presence of the insecticidal effect of thyme oils on *T. confusum* at increasing doses (Yiğit et al., 2021). In the same year, Çiftçi and colleagues investigated the effects of lavender, juniper seed and cedarwood oils, including thyme oil, on Tribolium castaneum and found that thyme oil had the highest fumigant effect (Çiftçi et al., 2021).

Plants belonging to the same genus often contain similar substances (Mohammadhosseini et al., 2017; Polatoğlu et al., 2013). Therefore, considering the results of the studies, it is possible to say that different plants belonging to the same genus may have similar effects on insect species (Ateyyat et al., 2009; Çakır et al., 2016). Durna and Kayahan (2022) determined the effects of the herbal insecticide Azadirachtin and four entomopathogenic fungi, which are commercial preparations with the active ingredients *Verticillium lecani*, *Metarhizium anisopliae*, *Beauveria bassiana*, *Paecilomyces fumosoreus*, against *T. castaneum*. According to the results, it was determined that the biological preparations (especially fungi) applied on *T. castaneum* started to be effective on the said pest from the 14th day and the most effective preparations were reported to be bioinsecticides with the active ingredients Azadirachtin and *M. anisopliae*.

Bozhüyük and Kordali, (2019), Satureja cilicica P.H. Davis, *Satureja cuneifolia* Ten, *Satureja hortensis* L., *Satureja spicigera* (C. Koch) Boiss, *Satureja thymbra* L. and *Satureja montana* L. showed toxic effects on potato beetle adults and larvae by 2.22-100% in petri and desiccator experiments at doses of 10, 15 and 20 mg/mL depending on the concentration increase and exposure time. In petri trials, the highest mortality rate was recorded as 100% in the second larval stage at a dose of 20 mg/mL of *S. spicigera* ethanol extract 96 hours after application. In the desiccator trials, the highest toxicity rate was 100% for the first larval stage at a dose of 20 mg/mL of S. thymbra ethanol extract 96 hours after treatment. The results of this study suggest that the ethanol extracts of the tested *Satureja* L. species can be used as bio-larvicides and insecticides for *L. decemlineata* larvae and adults. Cotton, flax, sesame, poppy, and olive were mixed with water and soap and water and used directly to kill pests and get effective results. In the studies, the effects of garlic oil on *Trigoderma granarium* (Hypocreaceae) and eucalyptus, anise and cumin oils on *Aphis gossypii* (Aphididae) were observed (Nohutcu et al., 2021).

3. Use as an acaricide

Mites prevent photosynthesis in plants by sucking the sap from the leaves, causing the breakdown of chlorophyll, and often causing damage resulting in the drying of the plant (Kumral and Kovancı, 2004). Due to the short life cycles and rapid reproduction of these pests, they show rapid resistance to synthetic acaricides compared to other pests. In addition, the non-selective nature of the selected acaricides eliminates predator species and causes the pest to reach high populations and economic losses (Topuz and Madanlar, 2006). The emergence of resistant populations due to the intensive use of chemical acaricides has led to the emergence of alternative methods and the control with essential oils has come to the fore (Whalon et al., 2023). In their study, Topuz and Madanlar (2006) used *Foeniculum vulgare* Miller, *Mentha pulegium* L., *Vitex agnus-castus* L. (Lamiaceae), *Schinus molle* L. (Anacardiaceae), *Pistacia terebinthus* L. (Anacardiaceae) were investigated for their repellent effect against *Tetranychus cinnabarinus* Boisd. (Tetranychidae) mite. The repellent effect was reported to be 85% at a dose of 1 ml/l of V. agnus-castus species. Kesdek et al. (2019), *Artemisia dracunculus* L., *Satureja hortensis* L., *Thymbra spicata* L. (Lamiaceae), *Tanacetum argyrophyllum* C. Koch (Asteraceae) and *Tanacetum balsamita* L., at doses of 5, 10 and 20 µL/petri, caused 62.6% to 100% mortality on *T. cinnabarinus* adults after 96 hours. It was reported that the essential oils of *Thymus* spp. (Lamiaceae), *Hyssopus officinalis* L. (Lamiaceae), *Syzygium aromaticum* L. (Myrtaceae), *Eucalyptus globulus* (Myrtaceae), *Mentha piperita* (Lamiaceae) were effective in the control of mites, especially in Varroa (Varroidae) (Demirel et al., 2019). The effect of thyme and clove essential oils on *V. destructor* was investigated and effective results were obtained (Sabahi et al., 2017). The effects of binary combinations of thymol, eucalyptol, carvacrol, phellandrene and myrcene essential oil compounds on the development of V. destructor were investigated and it was revealed that thymol-fellandrene combination was lethal in mites (Brasesco et al., 2016).

4. Use of Essential Oils as Nematicides

Chemical control methods are generally used and preferred for nematode pests that cause significant losses in quality and yield in agricultural activities. Nematodes are plant parasitic nematodes of 4000 species and cause 35-40% crop losses in our production (Maggenti, 1991; Williamson and Gleason, 2003). Their chemical compounds are geranial, thymol, anethole, limonene, carvacrol, pulegon, and artemisia ketone as nematicides from essential oils (Oka et al., 2000). It was determined that o-margarine, thione mon 2- butenyl, Furano-triterpenoid, acetylenic thiophene 5-(3-button-1inyl)-2 active ingredients of *Azadirachta indica* species caused very positive results in *Tylenchulus semipenetrans* and *Meloidogyne javanica* nematodes (Ahmad et al., 2004; Oka et al., 2007; Cristobal Alejo et al., 2006). Jardim et al. (2018) tested the essential oil of *Cinnamomum cassia* L. on *Meloidogyne incognita* nematode and found that it was more effective on *Meloidogyne incognita* larvae and eggs than carbofuran, which is used effectively on the nematode at a concentration of 173 pg ml. (E)-cinnamaldehyde, one of the components of the oil, was found to be effective against nematodes by GC-MS. The effect of *Melia azedarach* essential oil on *Meloidogyne incognita* nematode was reported (Ntalli et al., 2009). The effect of *Boswellia carterii*, *Paeonia noutan*, *Perilla frutescens*, *Thymus vulgaris*, *Syzygium aromaticum*, and *Boswellia inter* folia essential oil against nematodes has been determined (Choi et al., 2007; El-Badri et al., 2008). The effects of essential oils against nematodes differ depending on the root lesion nematode. *Foeniculum vulgare* Miller in *P. penetrans* species, *Ferulago cassia* Boiss in *P. thorny* species, and *Coriondrum sativum* L. essential oils in *P. neglectus* species provided a great benefit. Avato et al. (2017) reported that the mortality rate of *Pratylenchus vulnus* increased to 75% after 96 hours with 15 mg/ml *Rosmarinus officinalis* essential oil.

CONCLUSION

With the increasing level of awareness in the fight against diseases and pests that significantly affect yield and quality in agricultural production, the use of chemical methods should be reduced and the fight with environmentally friendly plant-based inputs should be supported. Today, natural resources are rapidly depleting due to the damage caused by synthetic products. Due to the harmful effects of chemical products, there is a tendency towards alternative methods in the fight against diseases and pests. In alternative methods, essential oils have properties such as insecticide, fungicide, nematicide, and herbicide. However, their applicability in practice is very low. Commercial production is restricted due to legal difficulties in obtaining licenses due to problems arising from standardization. Existing studies carried out today are mostly laboratory trials. They could not be integrated into cultivation areas in practice. Importance should be given to studies on the formulation of essential oils in application doses and application methods for their practical use. The studies conducted are mostly focused on antimicrobial, herbicidal and insecticidal effects and have been limited in laboratory areas such as acaricides, viricides and nematicides. Due to the geopolitical position of our country and the four seasons, plant diversity and the number of endemic plants are high. While many of these plants have the potential to be used as biopesticides, very few of them are utilized. With the orientation of researchers to these areas, the use of essential oils in alternative control methods is important in terms of reducing the use of chemicals.

Statement of Conflict of Interest

The authors have no conflict of interest.

Authors' Contributions

Fatih DADASOGLU, Muhammed TATAR, Elif EMIR, Zeynep DOGAN and Sumeyye CETIN designed the research. Muhammed TATAR edited the article according to the template. All authors contributed to the writing of the article, participated in the publication process of the article, read and approved it.

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Food Safety and Aquaculture

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ABSTRACT: Today, the greatest need of societies is to provide safe foodstuffs. The rapid increase in the world population, environmental pollution due to developing technology, economic weakness and lack of education deepen the nutritional problems and make it difficult to obtain safe food. Developing food technology and consumer awareness increase the efforts to improve product quality today. The production of foods, which are the basic needs of consumers for their lives, in line with current technological requirements is an important service in ensuring healthy nutrition. Food safety is the whole of the measures taken to eliminate all kinds of physical, chemical and biological damage that may occur in foods. Efforts to ensure food safety and quality assurance are also of great importance in terms of consumer and public health. Seafood is among the perishable food products. For this reason, they must be handled very carefully and kept under control during the period from hunting to processing. In this review, the relationship between food safety and aquaculture is examined.

Keywords: Aquaculture, Food Safety, Health

INTRODUCTION

Nutrition is a consumption situation that includes food security, which is one of the basic needs that human beings cannot give up, and which is necessary for the continuation of health and life. The basic principle in safe food production is the supply of raw materials away from all kinds of hazards (physical, chemical and biological), making them ready for consumption under good hygiene and production conditions and delivering them to the consumer. Reaching healthy and safe food is the most basic need of human beings (Ünal Şengör, 2021)

Food safety; It is concerned with the presence of food-borne hazards in food at the time of consumption or (when used by the consumer). The entry of food safety hazards can occur at any step of the food chain, so effective control throughout the food chain is essential. Therefore, food safety is a phenomenon provided by the contribution of all units in the food chain (Anonymous, 2023a).

Since seafood has a high protein structure, it creates a suitable living environment for microorganisms, so the risk of spoilage increases, and when consumed in this way, it causes various diseases and deaths. It also causes significant financial losses (Cengiz Tuzlu, 2015).

FOOD SAFETY

Nutrition and a healthy life, which are among the most basic needs of human beings, are possible on the basis of food safety. The emergence of many new diseases in the world has shown the importance of food safety. Foodborne diseases have negative consequences on human health in both developed and developing countries.

These diseases pose serious dangers especially to children, the elderly and pregnant women. The dangers posed by such diseases cause people to lose their health and life (Anonymous, 2023b).

The terms food safety and food quality are sometimes confused. Food safety refers to chronic or acute hazards that can harm consumer health. Quality, on the other hand, is a criterion that provides the qualities and price advantage that will create satisfaction for the producer and the consumer, but it can also be defined as all the features that affect the value of a product for the consumer. These; negative effects such as spoilage, contamination, loss of color, bad odor and positive effects such as origin, color, aroma, texture and food processing methods. This distinction between safety and quality is also influential on public policies and ensures the creation of the most appropriate food control systems to meet predetermined national targets (Yeşilsu and Özyurt, 2013).

The regulations put into effect with the start of the harmonization negotiations with the European Union on food legislation in Turkey have enabled our products to compete with the food products of the countries all over the world, especially the European Union, in terms of food safety (Yeşilsu and Özyurt, 2013). 'Quality control and management systems' have been established in order to ensure competition and competition and sustainability of competition regarding food safety. The main ones of these systems are the 'ISO 9000 Quality Standards' created by the International Organization for Standardization and the 'Turkish Standard' created by the Turkish Standards Institute (TSE) established in 1960. The TS 13001 standard, created by TSE regarding food safety, is based on the internationally accepted 'Hazard Analysis at Critical Control Points (HACCP)' principles (Giray and Soysal, 2007).

FOOD SAFETY AND AQUACULTURE

Food safety is the whole of the measures taken to eliminate all kinds of physical, chemical and biological damage that may occur in foods. In ensuring food safety, water and soil management, plant and animal production, production, storage, processing and waste control of foodstuffs are of great importance within the scope of the concept from farm to table, and for this purpose Good Manufacturing Practices (GMP), Good Hygienic Practices (GHP), Good Veterinary Various applications such as Medical Practices (GVP) are made (Anonymous, 2023b).

Today, the world is facing the problem of hunger. It is very important to ensure the sustainability of food resources and to prevent food waste (Okocha et al., 2018). The increase in the world population causes people to be malnourished and not to benefit enough from their animal protein needs. In this sense, seafood is one of the foods that will fill the animal protein deficit. High-protein seafood meets 40% of the world's protein needs, and contains a significant level of omega-3 polyunsaturated fatty acids. In addition, they contain nutrients such as vitamins A, D and E, B group vitamins, iodine, calcium, zinc and selenium (Kırım vd., 2015; Berat Torusdağ and Meral, 2021).

Aquatic products, which are so valuable, are among the food products that can perish very quickly. For this reason, they should be handled very carefully and kept under control during the period from hunting to processing (Anonymous, 2023c).

It is seen that the fishery products processing plant have developed their applications and infrastructures in terms of ensuring food safety and adapted them to today's requirements (Mol, 2015). Aquaculture processing assessment instruction; It tries to implement it with many legal and administrative regulations such as However, despite all this organization, legal regulations and administrative audits, our country; it is understood that fishery products are behind the developed countries and the EU in terms of complying with food safety and hygiene rules. Because it is stated that the most difficult chapters in the negotiations with the EU will be on the determination of common agricultural and fisheries policies (Savaş, 2010).

Fisheries consumed as human food in the world and in our country are obtained in two ways as hunting and aquaculture. The products obtained from both ways come from two different water sources, fresh waters and seas. These products, which are obtained through hunting and aquaculture, go through many stages until they reach the table of the consumer (Anonymous, 2023d).

Implementation of hygiene rules: "Health measures to be taken at all stages of aquatic products, starting from aquaculture and hunting, to processing, production, transport and consumption in detail in the Regulations on Places, and the health rules to be followed by the personnel working in the facility, and the criteria related to their application procedures and principles" means (Savaş, 2010).

Statement of Conflict of Interest

The authors have no conflict of interest.

Authors' Contributions

Fatih DADASOGLU, Muhammed TATAR, Emre ERDEN, Mustafa OZGERIS, Kenan KARA and Oktay OZBAY designed the research. Muhammed TATAR edited the article according to the template. All authors contributed to the writing of the article, participated in the publication process of the article, read and approved it.

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Research of the LED Emission Mode Impact on the Productivity and Physicochemical Marks of Japanese cabbage in the Tiered Hydroponic Module Conditions

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ABSTRACT: Growth rates, plant biomass and the concentration of beneficial compounds largely depend on the quality and intensity of illumination. Plants of the ‘Mizuna Red’ variety were grown using a low-volume technology in a regulated agroecosystem of a tiered hydroponic module produced by VIM (Russia). The plants were illuminated by specially designed LED lamps manufactured by VIM (Russia) with a dy-namically controlled spectral composition in 4 channels. For experimental researches, the design of the lighting system included several modes of emission: continuous, pulsed and scanning with a radiation intensity of 15000 lux and a total PAR of 321 μmol $m^{-2}s^{-1}$: blue – 97 μmol $m^{-2}s^{-1}$; green – 84 μmol $m^{-2}s^{-1}$; red – 122 μmol $m^{-2}s^{-1}$; far red – 18 μmol $m^{-2}s^{-1}$ (Proportions B: G: R ~ 30:26:44). The aim of the study was to assess the effect of different modes of emission on productivity, physi-cochemical indicators and to develop technological methods for obtaining highquality commercial products of Japanese cabbage variety 'Mizuna Red' grown in a longline hydroponic module. The use of a pulsed emission mode made it possible to increase the photosynthetic activity of ‘Mizuna Red’ plants, which eventually influenced the growth of the aboveground mass and its quality indicators with a strong correlation between these indicators. The concentration of photosynthetic pigments was dependent on the emission mode.

Keywords: *Brassica rapa* L. subsp. *Nipposinica*, hydroponic, productivity, LED, photosynthetic pigments

INTRODUCTION

Considering the priority of the issues of nature saving and environmental sustainability, the need to minimize the outlays of energy and other resources in the conditions of modern high-intensity agricultural production, an important task is to develop technologies and technical means for the controlled cultivation of vegetables in urban conditions (Sturiale et al., 2019; Rouphael et al., 2020; Diacono et al., 2021). Greenhouse and indoor growing modules not only allow for significantly higher yields compared to open field cropping systems, but also they can facilitate out of season production and substantial manipulation of the chemical composition and bioactive profile of the final product (Rouphael et al., 2018; Butturini and Marcelis, 2020).

Currently, one of the current trends in the development of urban agriculture is the creation of energy-saving LED irradiators, designed for growing plants in light culture (Loi et al., 2021; Santin et al., 2021). The advantage of LED light sources (Light-emitting diode – LED) is the ability to flexibly adjust the intensity and spectrum of lighting (Lobiuc et al., 2017), and also due to the minimum energy costs through the high efficiency, in addition to a long service life, low heat dissipation, lower volume/weight (Polzella et al., 2020) and close location to plants makes LED lighting a cost-effective solution for multilevel cultivation technology without natural light in indoor spaces (Beacham et al., 2019).

Artificial lighting is one of the main parameters that significantly affects the growth and quality of plants grown under controlled conditions of vertical indoor farms (PFAL) (Kumar et al., 2020; Orsini et al.,2020). Numerous studies (Loconsole et al., 2019; Pennisi et al., 2020) confirm that adjusting the spectrum and intensity of LED lighting has a positive effect on the productivity and quality of *Lactuca sativa* L. Frąszczak and Kula-Maximenko (2021) observed that the blue spectrum stimulated leaf growth in five lettuce plants with different leaf colors. Red light negatively affected the length of the leaves in varieties, but contributed to an increase in the quantitative content of chlorophyll and an increase in biomass in varieties containing anthocyanins. According to Korean scientists research Nguyen et al. (Nguyen et al., 2021), white (W) LED stimulated biomass accumulation and certain phenolic acids and flavonols in lettuce and can be used as an effective emission source for commercial vertical farming. When treated with blue LEDs, the levels of sucrose, glucose and fructose increased compared to treatment with white and red LEDs in *Brassica oleracea* var. acephala plants, indicating more efficient photosynthetic activity (Metallo et al., 2018).

MATERIAL AND METHOD

Experimental installation. In order to conduct experimental studies, a tiered hydroponic module produced by VIM (Russia) was developed for growing vegetables under controlled conditions. The appearance of the module is shown on Figure 1. The module includes two tiers with trays for hydroponic growing. Modern LED lights with variable spectral com-position are mounted above each tray. The module has a plant nutrition system, lighting system and intelligent control system of climatic parameters with preset characteristics in automatic mode with subsequent storage of data archive.

Figure 1. Tiered hydroponic module produced by VIM (Russia) for 'Mizuna Red' growing

Light emission. The illumination of the plants was carried out by specially designed LED lamps manufactured by VIM (Russia) with a dynamically controlled spectral composition in 4 channels. For experimental studies, the design of the lighting system included several modes: continuous, pulsed and scanning with an emission intensity of 15000 lux and a total PAR 321 μmol $m^{-2}s^{-1}$: blue – 97 μmol $m^{-2}s^{-1}$; green – 84 μmol $m^{-2}s^{-1}$; red – 122 μmol $m^{-2}s^{-1}$; far red – 18 μmol $m^{-2}s^{-1}$ (Proportions B:G:R ~ 30:26:44). During the research, the same parameters in terms of emission intensity and spectral composition were set for all modes in the range from 380 nm to 780 nm (Figure 2). Measurements of the photon flux and spectral composition of emission were performed using an MK350D Compact Spectrometer (UPRtek Corp. Miaoli Country, Taiwan).

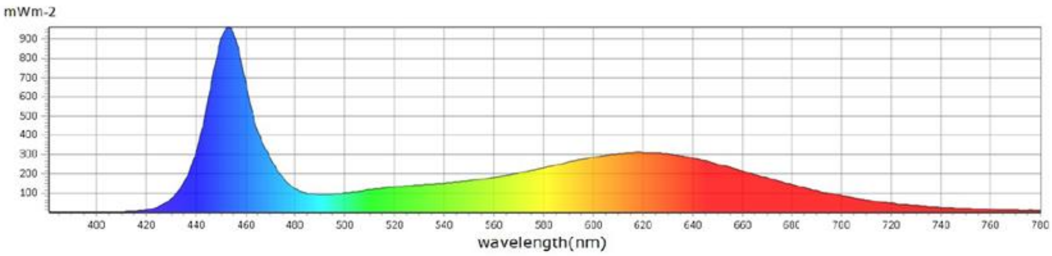

Figure 2. Spectral composition of illumination in a tiered hydroponic module when growing 'Mizuna Red'

Continuous light is the control variant and provides a continuous, stable luminous flux from three lamps, evenly distributed over the entire growing area of the plants. The pulsed emission also provides a uniform light flux over the entire plant growth area due to three lamps. However, the function of intermittent, impulse glow according to the scheme has been added to their control algorithm (Figure 3).

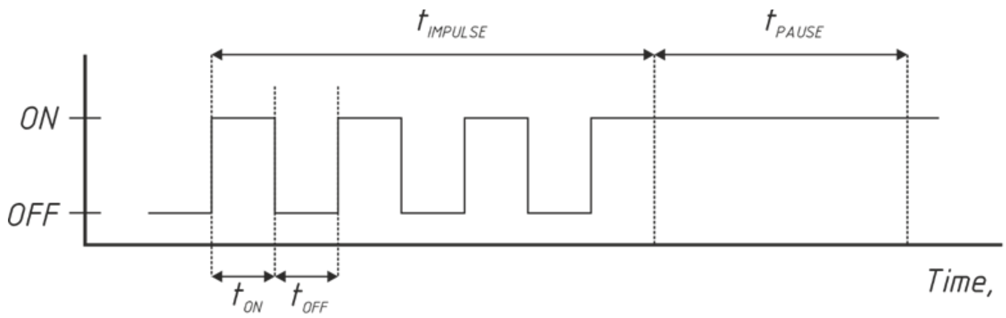

Figure 3. Scheme of pulsed emission when growing 'Mizuna Red' plants in a tiered hydroponic module

Lighting operation time is divided into two ranges: $t_{impulse}$ – pulse range (30 seconds), t_{pause} – pause range (15 seconds). In the range of pulses, the lighting worked in modulation mode with a frequency of 1 Hz and a duty cycle of 50%. The duration of lamps operation with pulsed emission was carried out in the t_{ON}/t_{OFF} ratio – 0.5 seconds. In the pause range, the light was turned off. The duration of one cycle was calculated using the formula: $t_{sum} = t_{impulse} + t_{pause}$.

Morphobiological indicators. Scanning lighting involves the use of a single luminaire mounted on movable rails with an electric drive by means of a stepper motor. Along the guides, the lamp moved over the growing tray from one row of the plant to another. The light/dark cycles were 50/70 seconds. The duration of the full cycle of illumination of all plants in the tray was 120 seconds.

The leaf surface area, wet and dry weight, shoot length and parameters of photosyn-thetic activity of the plants leaf apparatus were examined twice on days 15 and 30 during the growing season. The efficiency of the photosynthetic activity of the 'Mizuna Red' plants was assessed by the volume of work of the leaves (photosynthetic potential) and the increase in dry matter per 1 m^2 per day (pure productivity of photosynthesis). The leaf area is one of the important features characterizing the activity of the photosynthetic apparatus of plants. The leaf area in the study of the photosynthetic apparatus was measured using a LI-COR-LI-3100C photoplanimeter device (USA). The determination of the dry substance was carried out on an Ohaus EX224/AD analytical balance (Switzerland).

Quantification of photosynthetic pigments. The quantitative content of the main pigments (chlorophyll *a*, *b* and carotenoids) and anthocyanins in the leaves of Japanese cabbage was determined on the 30th day of vegetation by the spectrophotometric method using a Speks SSP-705M device (Russia).

The pigment concentration was calculated for 100% acetone using the Holm-Wettschnein equation (1)-(4):

Chl a = 9.784D662 – 0.990D644 (1)

Chl b = 21.426D644 – 4.650D662 (2)

Chl a + b = 5.134D662 + 20.436D644 (3)

Ccar = 4.695D440,5 – 0.268Chl a + b (4)

where D is the value of the optical density of the extract at the corresponding wavelength.

Statistical analysis. The results obtained were processed statistically using ANOVA analysis of variance. Significant differences between the means were calculated using the Duncan test at a probability level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Growth rates, plant biomass and the concentration of useful compounds in them largely depend on the quality and intensity of illumination. Japanese cabbage 'Mizuna Red' plants were grown using a low-volume technology in a regulated agroecosystem of a tiered hydroponic module produced by VIM (Russia) for 30 days.

Changing growing conditions affects the response of plant growth and development. Evaluating the effect of different modes of emission on the morphobiological parameters of mizuna plants, it was found that at the beginning of the growing season on the 15th day of observation, the greatest daily growth (1.3 cm·day^{-1}) (Figure. 4A) with a shoot length of 18.75 cm (Figure. 4B) was observed with pulsed emission. The scanning mode of emission contributed to a decrease in the daily growth of plants – 0.7 cm·day^{-1} at a plant shoot length of 10.52 cm, which is lower than the control values.

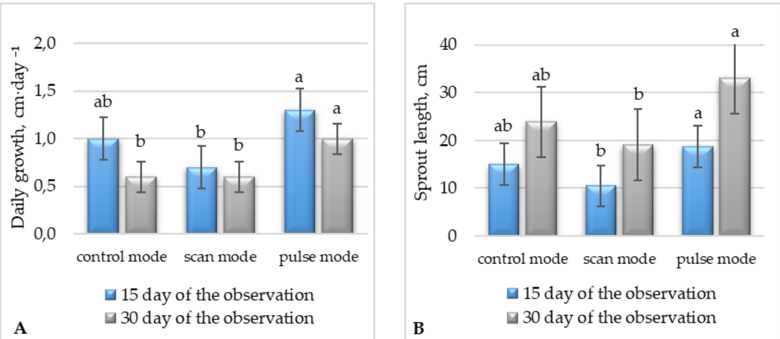

Figure 4. Growth and development dynamics of Mizuna Red' plants depending on LED light mode (n = 25): A – daily growth; B – sprout length. Statistical analysis was carried out separately on days 15 and 30 of observation. The different letters indicate significant differences among radiation modes according to Duncan's test ($p \leq 0.05$). The bars represent the standard deviation

On the 30th day of observation, in general, the growth rate of plants decreases to 0.6 cm·day^{-1} (control and scanning modes of emission), while maintaining the tendency of a larger increase with pulsed emission – 1.0 cm·day^{-1}. Pulsed emission contributed to the better development of plants throughout the growing season, the growth rate of plants ex-ceeded the control by 38.5%. Thus, it can be noted that the growth and development of plants between the studied radiation options were comparable with the control through-out the growing season. As can be seen from Figure 4, the most pronounced significant differences were observed between the scanning and pulsed radiation modes.

The productivity of plants depends on the efficiency of the leaf apparatus. The use of different emission modes made it possible to reveal the regularities of the parameters of the photosynthetic activity of the leaf apparatus of 'Mizuna Red' plants grown under con-trolled conditions. We assessed the indicators of plant photosynthesis activity: leaf index, volume of work of leaves (photosynthetic potential), increase in dry matter per 1 m² per day (net productivity of photosynthesis), these characteristics are presented in Figure 5A-C.

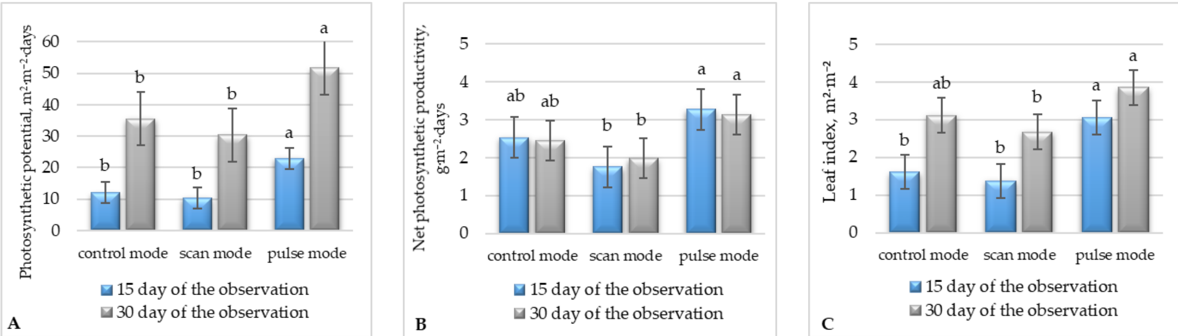

Figure 5. Photosynthetic activity indicators of the leaf apparatus of 'Mizuna Red' (n = 25): A – photosynthetic potential; B – net photosynthetic productivity; C – leaf index. Statistical analysis was carried out separately on days 15 and 30 of observation. The different letters indicate significant differences among radiation modes according to Duncan's test ($p \leq 0.05$). The bars represent the standard deviation

Photosynthetic potential ranged from 10.35 m²·m⁻²·days (scan mode) to 22.90 m²·m⁻²·days (pulse mode) on day 15 of observation. Visually, it can be seen that the pulsed mode of radiation contributed to the increase in the photosynthetic productivity of 'Mizuna Red' plants, which is confirmed by the results in Figure 5A ($p \leq 0.05$). As the Japanese cabbage plants grew, the photosynthetic potential indicator by the end of the growing season (30 days of observation) increased to 30.40-51.77 m²·m⁻²·days with the maximum values under the action of the pulsed emission mode on the plants. The increase in dry matter per 1 m² per day during the growing season was at the level of 1.76-3.27 g·m⁻²·days depending on the variant of the experiment Figure 5B. The use of a pulsed mode of emission made it possible to increase the photosynthetic activity of 'Mizuna Red' plants, which ultimately influenced the growth of the aboveground mass and its quality indicators. The mode of scanning emission had some negative effect on the work of the photo-synthetic apparatus of plants, reducing the indicator of the net productivity of photosynthesis and thereby influencing its productivity. It can be seen from the Figure 5C that the leaf surface index of the studied plants was at the level of 1.38-3.05 m²·m⁻² with the highest indicator in the variant with a pulsed radiation mode. It is important to note that the pulsed radiation mode improved the photosynthetic activity of the ‘Mizuna Red’ leaf apparatus compared to scanning radiation.

In the course of the research, changes in the concentrations of the main photosynthetic pigments in mizuna leaves were analyzed when plants were grown under different lighting conditions (Table 1). We considered three groups of pigments: chlorophylls, carotenoids and anthocyanins. It was found that the pulsed mode of emission contributed to the maximum accumulation of pigments (chlorophylls *b*, chlorophylls (*a*+b) and anthocyanins). The main conclusion is that the accumulation of photosynthetic pigments by 'Mizuna Red' plants grown under pulsed radiation was similar or higher compared to other radiation regimes.

Table 1. Content of the main photosynthetic pigments (n = 6) in the leaves of 'Mizuna Red'. Values represent mean ± standard deviation. The different letters indicate significant differences among radiation modes according to Duncan’s test ($p \leq 0.05$).

Emission mode	Quantity, $mg \cdot g^{-1}$				
	Ch. *a*[1]	**Ch. *b*[2]**	**Ch. (*a*+*b*)[3]**	**Carotenoids**	**Anthocyanins**
Control (continuous)	2.67±0.57ab	0.92±0.26b	3.59±0.26b	0.72±0.21ab	2.13±0.31b
Scanning	1.95±0.41b	0.57±0.16b	2.52±0.16b	0.50±0.14b	0.61±0.09c
Pulsed	3.52±0.75a	1.87±0.53a	5.39±0.53a	1.06±0.30a	3.65±0.54a

[1]Chlorophylls *a*, [2]Chlorophylls *b*, [3]Chlorophylls (*a*+*b*)

The emission modes used had different effects on the accumulation of photosynthetic pigments. The lowest values were observed in the 'Mizuna Red' plant bio-mass under scanning emission. Plants had practically no anthocyanins under this mode. This is more likely due to the speed of movement of the luminaire, which depended on the light flux falling on the plants. Pigments are able to absorb light only in a certain part of the spectrum in a long light mode. In our study, the scanning mode of emission provided a duration of the light phase of 50 seconds, which could be insufficient and lead to a violation of the absorption process of the spectrum. Despite the same parameters of light emission applicable to all studied modes (intensity and spectral composition of emission).

CONCLUSION

Our results showed that the use of different modes based on LED lighting is very promising for growing leafy vegetables in a vertical farming system. We studied the effect of different radiation regimes on the quantitative content of photosynthetic pigments (chlorophyll a, b and carotenoids) and anthocyanins in the leaves of 'Mizuna Red'. We evaluated the indices of photosynthetic activity of the leaf apparatus of plants. Cultivation of Japanese cabbage 'Mizuna Red' by low-volume technology under conditions of regulated agroecosystem of tier hydroponic module at pulse treatment, showed a positive effect on growth, development and quality of plants compared to the control conditions of continuous lighting.

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Statement of Conflict of Interest
The authors declare no conflict of interest.

Authors' Contributions
Conceptualization, I. K.; methodology, I. K., O. V. A. G.; software, formal analysis, valida-tion, A. G., I. K., O. V., A.T.; investigation, I. K., O. V., A.T.; resources, A. G.; writing-review and editing, I. K., O. V.; All authors have read and agreed to the published version of the manuscript.

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Nutritional Potential of Freshwater *Cladophora Glomerata* Macroalgal Biomass for Growing Rabbits

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ABSTRACT: Considering today's challenges in livestock, *C. glomerata* biomass is an ecologically and commercially important green macroalga that could potentially reduce the usage of traditional feed raw materials. The aim of this study was to analyse the impact of *C. glomerata* biomass on rabbit performance. The trial was carried out with Californian rabbits, which were assigned to two dietary treatments (*n*=10 rabbits/diet) and fed standard compound diet (SCD) and SCD + 4% *C. glomerata* (CG4). During the feeding trial (52-122-days), rabbits' individual weight, ADG, DFI, and FCR were recorded. At the end of the trial, 12 rabbits (*n*=6 rabbits/diet) were randomly selected and euthanized; dissection procedures of the carcasses adhered to WRSA guidelines. During the trial, all rabbits grew evenly, with growth rates of 1.45 in CG4 and 1.46 in SCD. However, during each trial period, the weight of rabbits fed CG4 was slightly lower compared to SCD and had higher FCR, ADG, and DFI during almost all periods ($p>0.05$). The slaughter performance was not affected by CG4. While *C. glomerata* had no significant impact on rabbit growth or slaughter performance, it did not cause adverse effects and is safe to use as an alternate material to partly replace traditional ones.

Keywords: Macroalgae; Sustainability; Feed alternatives; Growth performance; Slaughter performance; Rabbits

INTRODUCTION

As the demand for animal products increases, it's becoming more challenging to feed livestock. That's why scientists are exploring alternative feed materials to make animal production more efficient and sustainable. These materials include insect meal, algae, by-products from food processing, and many others (Messyasz *et al.*, 2018; Wan *et al.*, 2019; Altmann *et al.*, 2020). Using these alternative raw materials can improve not only the quality of animal feed but also reduce its impact on the environment and improve animal health. However, there are some challenges to using these materials, like safety concerns or dosage regulations.

Algal biomass is a valuable alternative source material that can be utilised in various industries such as agriculture, chemicals, cosmetics, pharmaceuticals, feed, and food due to its advantageous properties (Korzeniowska, Łęska and Wieczorek, 2020). More specifically, one of these green filamentous algae is *Cladophora glomerata* (*C. glomerata*), which is found in marine and freshwater habitats worldwide. It forms dense mats and produces high amounts of oxygen during photosynthesis and its biomass has potential uses in bioremediation, biofuel production, wastewater treatment, and feed production (Ahmad *et al.*, 2022). In particular, *C. glomerata* biomass can be a valuable source of protein and other nutrients for animal feed, potentially reducing reliance on traditional feed ingredients and contributing to sustainable agriculture practises (Nutautaitė *et al.*, 2021). The protein content in *C. glomerata* biomass can vary depending on environmental factors, but it has been shown to be a suitable protein source for various animal species.

Compared to more traditional meat production, rabbit meat consumption has been increasing worldwide due to its nutritional value, low fat content, and environmental sustainability (Kumar *et al.*, 2022). Rabbits are highly efficient converters of feed into meat and can be raised on various types of feed, including agricultural by-products and grasses. The demand for rabbit meat has grown steadily in recent years, especially in developing countries where there is a need for affordable sources of animal protein. Additionally, rabbit meat is considered a healthier alternative to other types of meat due to its lower cholesterol and fat content (Siddiqui *et al.*, 2023). The global rabbit meat market is expected to continue growing as consumers become more health-conscious and seek out sustainable food sources. To produce high-quality meat, it is essential to ensure optimal growth performance in animals. Moreover, from a sustainable livestock development perspective, it is important to replace traditional raw materials with alternative ones that can meet all the nutrient requirements of animals, support steady growth performance, and lead to higher efficiency and product quality. So, the aim of this study was to analyse the impact of freshwater *C. glomerata* macroalgal biomass on rabbit growth and slaughter performance.

MATERIAL AND METHOD

The study was conducted at an indoor rabbit breeding farm with proper housing conditions and facilities to ensure optimal health and performance. The trial involved 20 weaned male Californian breed rabbits (52 days of age) of similar weight (*n*=10 rabbits/treatment), which were randomly assigned to two dietary treatments and fed twice daily with a standard compound diet (SCD) and the same diet supplemented with 4% freshwater *C. glomerata* biomass (CG4). The standard compound diet was formulated and analysed to meet the nutrient requirements, including vitamins and minerals, as recommended by the National Research Council. The rabbits were housed according to the standards set by Council Directive 98/58/EC of July 20, 1998, concerning the protection of animals kept for farming purposes. The indoor temperature was maintained at 19±2°C using a heating system in the building in the separate cages (*n*=1 rabbit/cage).

The freshwater macroalgal biomass used in the production of the experimental feed was collected from the Šventoji River in Lithuania. The chemical characteristics, nutritional profile, and antioxidant capacity of the biomass have been thoroughly assessed and published in our previous research (Nutautaitė *et al.*, 2021; Nutautaitė, Vilienė, Racevičiūtė-Stupelienė, Bliznikas, Karosienė and Koreivienė, 2022; Nutautaitė *et al.*, 2022)

During the feeding trial (52-122 days), periodically (at 52–66; 66–80; 80–94; 94–108; 108–122 days of age) rabbits' individual weight, growth rate, average daily gain (ADG), daily feed intake (DFI), and feed conversion ratio (FCR) were recorded.

Upon completion of the feeding trial, 12 rabbits (*n*=6 rabbits/treatment) were chosen randomly, weighed, fasted for a night, and subsequently euthanized in compliance with established farming practises. The rabbit slaughter occurred in a farm slaughterhouse in compliance with the Republic of Lithuania's laws and regulations (Order No. B1-866 of October 31, 2012, of the Director of the State Food and Veterinary Service regarding the requirements for the care, management, and utilisation of animals for educational and scientific purposes). The rabbit carcasses were processed according to the techniques reported by Blasco and Ouhayoun (2010) and cooled in a ventilated space at 4°C for 24 hours. The dissection of warm and chilled carcasses adhered to the World Rabbit Science (WRSA) recommendations, and slaughter efficiency was evaluated.

Statistical analysis. The data obtained during the study were analysed using SPSS for Windows, version 25.0 (IBM Corp., released in 2017; Armonk, NY, USA). Prior to data analysis, the normality of the data was determined using the Kolmogorov-Smirnov test. For normally distributed data, a parametric independent T-test was performed on all data obtained during each trial period to identify any differences among the different treatments. Statistical significance was defined as a *p* value less than 0.05 (p<0.05).

RESULTS AND DISCUSSION

Rabbit growth performance is important as it determines the efficiency of rabbit production and can impact the profitability of the enterprise (Mukaila, 2023). To ensure growth performance, it is first necessary to fully satisfy the animal's nutritional needs. For example, *Cladophora* species, in terms of their diversity and concentration of proteins, amino acids, lipids, and essential elements, are similar in composition to other plants that are used in feed production (Heiba, Al-Easa and Rizk, 1997). The results of *C. glomerata* biomass studies reveal a valuable profile of essential fats and amino acids (Messyasz *et al.*, 2015; Nutautaitė *et al.*, 2021; Nutautaitė, Vilienė, Racevičiūtė-Stupelienė, Bliznikas, Karosienė and Koreivienė, 2022). Specifically, amino acids, especially exogenous ones, can increase the digestibility of animal nutrients, compensate for the lack of nutrients, and improve the quality of feed (Konkol *et al.*, 2018), which can further lead to more efficient growth performance in the animal. The growth performance of rabbits was evaluated during a feeding trial, and steady growth was observed throughout the trial, as illustrated in Figure 1. However, statistical analysis revealed no significant differences between the treatment groups at any stage of the trial, as the weight differences observed were minimal ($p>0.05$). According to previously published research, *C. glomerata* was more often used to supplement aquaculture and poultry feed, and research on rabbits is extremely limited. An experiment conducted by Anh et al. (2018) investigated the use of *Cladophora* spp. biomass as an alternative protein source for post-larval tiger shrimp (*Penaeus monodon*) to replace fishmeal. The study found that a dosage of 10–20% of *Cladophora* spp. biomass resulted in optimal shrimp productivity and feed conversion. In addition, other research conducted on broiler chickens found that supplementing their feed with 15% *C. glomerata* biomass resulted in higher growth rates compared to standard feed (Abid and Abid, 2006).

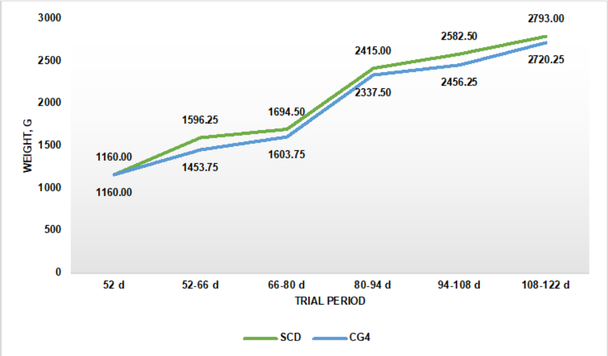


Figure 1. Impact of freshwater *C. glomerata* biomass on rabbits' growth performance during different trial periods

Growth performance in rabbits is important for several reasons. Firstly, it is a key indicator of the efficiency of the rabbit production system. Efficient growth rates can help to maximize the productivity and profitability of a rabbit farming operation, by reducing the time and resources required to produce marketable rabbits. Additionally, rapid, and steady growth is important for ensuring that rabbits reach their target weight and size for commercial purposes, such as meat production, at the appropriate time. Moreover, growth performance is closely related to the overall health and welfare of the rabbits (Sayers, 2010). Slow or inconsistent growth rates can be a sign of health problems or nutritional deficiencies and may lead to reduced reproductive performance, increased susceptibility to disease, and higher mortality rates. On the other hand, fast and consistent growth rates can indicate that the rabbits are receiving a balanced and adequate diet and are in good health. In a recent study, the growth rates of rabbits fed a standard compound diet and a SCD supplemented with 4% *C. glomerata* biomass (CG4) were evaluated, and no significant difference was observed between the two treatments (Table 1). The growth rate of SCD-fed rabbits was 1.46, while CG4-fed rabbits had a growth rate of 1.45, which was only 0.01 less than SCD-fed rabbits ($p>0.05$). Therefore, it can be concluded that the addition of 4% *C. glomerata biomass* to the SCD did not significantly impact the growth rate of the rabbits.

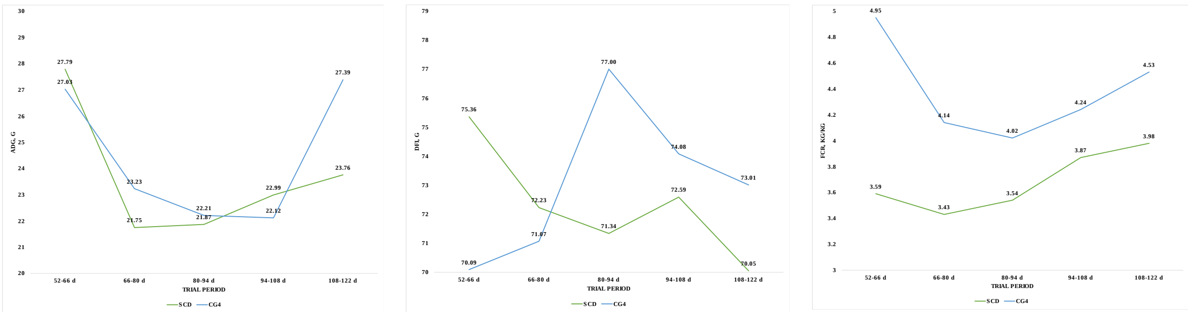
Table 1. Impact of freshwater *C. glomerata* biomass on rabbits' growth rate (52-122 days of age)

Item	Treatment		p-value*
	SCD	CG4	
Growth rate	1.46	1.45	n.s.

*n.s., means not significant (*p*>0.05).

The feeding trial involved the calculation of average daily gain (ADG), daily feed intake (DFI), and feed conversion ratio (FCR) indicators (Figure 2abc). ADG is an important factor that impacts the overall growth and development of rabbits (Fang *et al.*, 2020). It refers to the average weight gain of the rabbits per day over a specific period of time. A higher ADG indicates that rabbits are growing at a faster rate and are gaining weight more quickly. This can lead to an increase in body size, muscle development, and overall growth; on the contrary, a lower ADG indicates slower growth, which may result in a smaller body size and lower muscle development. Therefore, monitoring ADG is crucial in evaluating the effectiveness of a particular diet or feed supplement in promoting the growth and development of rabbits. The highest ADG was observed at the beginning of the trial (52–66 days of age), with SCD-fed rabbits having a 0.76 g higher ADG compared to those fed with SCD supplemented with *C. glomerata* biomass (CG4) (*p*>0.05; Figure 2a). ADG changed similarly in both groups thereafter, with the largest non-significant difference found in the final trial period (108–122 days of age), where SCD-fed rabbits had an ADG lower by 3.63 g compared to those fed with CG4 (*p*>0.05).

Another essential indicator of growth performance is the DFI. A sufficient amount of feed intake is necessary to meet the animal's nutritional requirements and maintain optimal health (Kumar, Soni and Sahoo, 2022). Insufficient feed intake can lead to malnutrition, reduced growth rates, and overall poor performance. Conversely, excessive feed intake can result in obesity and other health problems. Therefore, monitoring and adjusting feed intake to match an animal's individual needs is critical to achieving optimal growth performance in rabbits. The DFI between the treatments was analysed throughout the feeding trial, as shown in Figure 2b. The largest differences between the treatments were observed at the beginning (52–66 days of age), middle (80–94 days of age), and end (108–122 days of age) of the trial. Specifically, a 5.27 g lower DFI was observed in CG4-fed rabbits at the beginning of the trial, while in the middle and end periods, the DFI of CG4-fed rabbits was 5.66 g and 2.96 g higher, respectively, compared to SCD-fed rabbits (*p*>0.05). However, these differences between the DFI of SCD and CG4 treatments were not found to be statistically significant (*p*>0.05).



(a) (b) (c)

Figure 2. Impact of freshwater *C. glomerata* biomass on rabbits': **(a)** average daily gain (ADG), **(b)** daily feed intake (DFI) and **(c)** feed conversion ratio (FCR) during different trial periods.

FCR is a widely used metric that assesses the efficiency of feed utilisation by animals. It is calculated by dividing the amount of feed consumed by an animal by its body weight gain during a specific period. A lower FCR value indicates a more efficient use of feed by the animal, resulting in better weight gain efficiency. Specifically, in rabbits, a lower FCR signifies their ability to convert feed into body weight gain more effectively, thereby leading to faster growth rates and higher productivity levels. In our case, the FCR of rabbits fed with *C. glomerata* biomass (CG4) was consistently higher compared to those fed with a standard diet (SCD) throughout the trial periods, as shown in Figure 2c. Despite FCR values ranging from 4.02 to 4.95 for CG4 and 3.43 to 3.98 for SCD, no statistically significant differences were found between the two treatments (p>0.05).

Table 2. Impact of freshwater *C. glomerata* biomass on rabbits' slaughter performance (122 days of age)

	Treatment		
Item	**SCD**	**CG4**	**p-value**
Pre-slaughter weight, g	2765.67	2753.00	n.s.
Warm carcass, g	1369.94	1330.44	n.s.
Chilled carcass, g	1317.74	1280.08	n.s.
Head, g	224.90	223.41	n.s.
Fore part, g	145.22	133.27	n.s.
Hind part, g	534.69	527.49	n.s.
Thighs with bone, g	370.65	365.95	n.s.
Thighs without bone, g	308.76	310.25	n.s.
Shin with bone, g	127.18	127.67	n.s.
Shin without bone, g	96.63	97.40	n.s.
Femur bone length, cm	9.03	9.27	n.s.
Tibia bone length, cm	9.93	9.53	n.s.
Longissimus dorsi muscle, g	201.08	201.20	n.s.
Perirenal fat, g	114.38	115.19	n.s.
Abdominal fat, g	26.03	26.83	n.s.
Drip loss, %	3.83	3.80	n.s.
Dressing-out percentage, %	49.55	48.33	n.s.
Carcass yield, %	60.64	59.95	n.s.
Hind leg muscle yield, %	30.89	31.91	n.s.
Longissimus dorsi muscle yield, %	15.30	15.72	n.s.

*n.s., means not significant (p > 0.05).

C. glomerata biomass contains various nutrients, including amino acids, carbohydrates, proteins, phenolic compounds, and vitamins, which can have positive effects on rabbit development (Nutautaitė *et al.*, 2021; Ankita and Santanu, 2021; Nutautaitė, Racevičiūtė-Stupelienė *et al.*, 2022). Some of the specific mechanisms by which *C. glomerata* biomass can affect rabbit muscle development are as follows: (1) *protein content:* it is rich in protein, which is an essential nutrient for muscle growth and repair. The amino acids in protein are the building blocks of muscle tissue, and a diet that is high in protein can support muscle development and maintenance; (2) *fibre content:* it also contains high levels of fibre, which can improve rabbits' digestive health. Good digestive health is important for optimal nutrient absorption, which can support muscle development and other aspects of growth and development; (3) *vitamins and minerals:* it is a rich source of vitamins and minerals, including B vitamins, vitamin C, calcium, and iron. These nutrients are essential for various physiological processes in the body, including muscle function and development; (4) *antioxidants:* it contains antioxidants, such as phenols and flavonoids, which can reduce oxidative stress in the body. Oxidative stress can damage cells and tissues, including muscle tissue, and may impair growth and development; (5) *immune-boosting properties:* it has been shown to have immune-boosting properties, which can support the overall health and well-being of rabbits. A healthy immune system can help prevent infections and diseases that may negatively impact growth and development. However, as the weight gain and growth rates of rabbits fed the standard diet (SCD) and the SCD supplemented with *C. glomerata* biomass (CG4) were almost the same, the dissection parameters and the development of muscles and other body parts were also similar. Statistical analysis revealed no significant differences between the two treatment groups (p>0.05). Moreover, these results suggest that the addition of *C. glomerata* biomass up to 4% in the standard diet did not have any adverse effects on rabbit growth performance or slaughter performance.

CONCLUSION

In conclusion, the results of this study indicate that the addition of 4% *C. glomerata* biomass to the standard diet had no significant impact on the growth or slaughter performance of rabbits. Although some differences between both treatments were observed in ADG, DFI, and FCR, statistical analysis revealed that they were non-significant. These findings suggest that *C. glomerata* has the potential to be a safe and effective alternative material to partially replace traditional ones in rabbit diets without any adverse effects. However, further studies are necessary to fully evaluate the potential benefits and limitations of *C. glomerata* as a feed supplement for rabbits. Overall, this study provides important insights into the potential use of *C. glomerata* as a sustainable and eco-friendly alternative feed source for livestock production.

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Statement of Conflict of Interest

The authors declare that they are no conflict of interest.

Authors' Contributions

MN, ARS and VV designed and analyzed the research, MN, ARS and VV studies arranged. MN worked on the preparation of VV 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.

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Effect of Increased DL-α-Tocopherol Dose on Rabbit Productive Performance and Meat Qualities*

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ABSTRACT: Worldwide, animal breeders are constantly looking for high-quality, inexpensive additives, natural or artificial, to use in the production of novel meat products, seeking to satisfy consumers' demands. So, the aim of this study was to determine the effect of an increased DL-α-tocopherol dose on the productivity of rabbits and meat quality. A total of 30 Californian rabbits (42-days-old) were split into two groups (n=15 rabbits/group) and fed either the standard compound diet + 50 mg/kg DL-α-tocopherol (Control), or the standard compound diet + 100 mg/kg DL-α–tocopherol (Experimental). During the trial (42-112 days), the growth performance of rabbits was recorded, and at the end of the trial, the meat quality of the longissimus dorsi (LD) and hind leg (HL) was determined. DL-α-tocopherol in the feed had a positive impact on the weight of the internal organs, α-tocopherol accumulation in LD and HL muscles, and the cholesterol level in HL muscles ($P<0.05$). Moreover, DL-α-tocopherol in HL muscles increased fat content, yellowness (b*), and MDA content in stored LD muscles ($P<0.05$). In conclusion, increased levels of DL-α–tocopherol can lower cholesterol levels and increase α-tocopherol accumulation in muscles; however, they can also negatively increase MDA levels.

Keywords: DL-α-Tocopherol, Increased dose, Rabbits, Productivity, Meat quality

INTRODUCTION

According to projections, the world population is expected to increase to 10–11 billion individuals by 2050, leading to an increase in per capita wealth and demand for food (FAO, 2017; The Eat-Lancet Commission, 2019). To meet this demand, more food must be produced, and rabbit meat, with its distinct nutritional characteristics, could be considered an ideal source of nutrition (Dalle Zotte, 2002). Rabbit meat has a high biological value in terms of protein content, making it a valuable source of nutrition. Additionally, rabbit meat is a good source of minerals such as potassium, phosphorus, and selenium, as well as polyunsaturated fatty acids (PUFAs), which can make up to 35–40% of all fatty acids and are crucial for maintaining human health (Para et al., 2015; Mattioli et al., 2017; Ali and Kunugi, 2020). Rabbit meat is also low in salt and cholesterol. Among different meat species, rabbit meat is known to be one of the richest sources of vitamin B12 (Dalle Zotte and Szendrő, 2011).

In general, animal meat and its products are susceptible to oxidation and quality deterioration, which can have negative impacts on the health, productivity, and welfare of farm animals and the profitability of the domestic animal business (Hendawy et al., 2019). Due to the inherent endogenous antioxidant's failure to fight free radicals produced during the process, quality deterioration typically starts during the antemortem stage and continues through the post-mortem condition (Falowo, Fayemi and Muchenje, 2014). Free radicals strongly induce intracellular membrane lipid oxidation in animal muscles, including rabbit meat, which contains a high amount of PUFAs (Ali and Kunugi, 2020). For example, due to the high content of PUFAs in rabbit meat, it is prone to lipid oxidation, which shortens its shelf life and is indicated by rancidity and colour deterioration (Dalle Zotte and Szendrő, 2011). However, adding antioxidants like vitamin E can help decrease the susceptibility of animal products to oxidation and quality degradation.

The cell walls of animal muscles contain fat-soluble vitamin E, which plays a critical role as an antioxidant in increasing the oxidative stability of the muscle tissue (Roselló-Soto et al., 2018). This is particularly important in preventing lipid oxidation, which can lead to quality deterioration and affect the shelf life of animal products. Moreover, supplementation of livestock diets with vitamin E has been demonstrated to exhibit antioxidant properties that reduce oxidation and extend meat shelf life (Possamai et al., 2018; Ahmadian et al., 2019). When rabbits consume feed containing vitamin E, it can be efficiently incorporated into the lipids of their meat, resulting in improved shelf life and quality characteristics such as colour, flavor, and texture, while also increasing its nutritional value as a source of vitamin E for consumers (Dalle Zotte et al., 2020). Despite a growing interest in the use of vitamin E in animal husbandry, there is still a lack of research on the synthetic form of vitamin E, DL-α-tocopherol. Thus, this study aimed to investigate the impact of increased doses of DL-α-tocopherol on rabbit productivity and the quality of the resulting products.

MATERIAL AND METHOD

Animals and experimental design

The research was conducted in compliance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and with the Commission’s recommendation of 18 June 2007 concerning the protection of animals kept for farming purposes.

The experiment was carried out on thirty weaned Californian rabbits aged 42 days, with all 30 rabbits randomly assigned to 2 treatments (n=15 rabbits per treatment) and fed twice a day (*ad libitum*) with a standard compound diet supplemented with 50 mg/kg vitamin E (**Control**), while the experimental group feed was composed of standard compound feed supplemented with 100 mg/kg vitamin E (DL-α-Tocopherol) (**Experimental**). The regular compound diet was created and examined to include all the vitamins and minerals that growing rabbits need, as recommended by the National

Research Council (Arrington et al., 1977; Table 1). The main ingredients of the composite feed were hay, corn, oats, wheat, sunflower, vegetable oils, and minerals.

Table 1. Chemical composition of a standard compound diet (42-112 days of age)

Item	Value
Digestive energy, kcal	2370.70
Metabolized energy, kcal	2257.20
Crude protein*, %	16.40
Crude fibre*, %	16.39
Moisture*, %	10.65
Starch*, %	9.56
Sugar, %	4.38
Total lysine, %	0.65
Methionine + cysteine, %	0.65
Tryptophan, %	0.20
Linolenic acid, %	1.04
Threonine, %	0.61
Total methionine, %	0.39
Available phosphorus, %	0.37
Calcium*, %	1.29
Phosphorus*, %	0.59
Sodium, %	0.25
Chlorine, %	0.54

Contents of the premix: vit. A – 10.08 TV, vit. D3 – 1.14 TV, vit. K3 – 0.99 mg/kg, vit. B1 – 3.71 mg/kg, vit. B2 – 2.80 mg/kg, vit. B5 – 9.80 mg/kg, vit. B12 – 0.01 mg/kg, nicotinic acid – 20.40 mg/kg, folic acid – 0.22 mg/kg, choline chloride – 170.00 mg/kg, magnesium – 76.28 mg/kg, iron – 317.00 mg/kg, zinc – 110.89 mg/kg, copper – 19.16 mg/kg, cobalt – 0.29 mg/kg, iodine – 0.67 mg/kg, selenium – 0.31 mg/kg

Rabbits were raised indoors in individual cages to guarantee their best health and efficiency. The animals were kept in wire cages with a grid floor that measured 34 x 34 x 61 cm, with one rabbit per cage, and they had unrestricted access to individual bowls filled with food and water.

Growth performance and carcass evaluation

Individual rabbit weight and feed intake were recorded during the feeding study (42–112 days), after which the average daily weight gain (ADG), daily feed intake (DFI), and feed conversion ratio (FCR) were calculated.

After the feeding trial (112 days of age), 14 rabbits (n=7 rabbits per treatment) were randomly chosen, weighed, starved for the following night, and then put to death in accordance with standard farming procedures. The carcasses were prepared in accordance with Blasco and Ouhayoun's reports (2010). The skin, genitalia, urinary bladder, gastrointestinal tract, and distal portions of the legs were cut off post-mortem after rabbits had been bled. The carcasses were placed in a ventilated room and kept at 4°C for 24 hours. The *longissimus dorsi* and hind leg muscles were separated from the reference carcass. Warm and cold carcasses were dissected in accordance with WRSA guidelines (Blasco and Ouhayoun, 2010).

Meat chemical and physical quality

From each of the two treatments, seven samples of *longissimus dorsi* muscle and seven samples of hind leg muscle were obtained from rabbit carcasses. The samples were then analysed to determine various chemical-physical and quality properties of the meat, such as physicochemical indicators, cholesterol levels, accumulation of vitamin E, and a fatty acid profile. Fresh meat samples were evaluated for these properties, while malondialdehyde (MDA) analysis was carried out on both fresh and stored (at -18°C for three months) meat samples.

To evaluate colour changes, a freshly cut *longissimus dorsi* and hind leg slice were left to oxygenate at 4°C for 1 hour after slaughter and were then subjected to colour measurements according to the lab system (L*: lightness; a*: redness; and b*: yellowness), using a Minolta colorimeter CR-300® (Minolta Camera Co., Osaka, Japan) with illuminant D65 and a 0° observer.

Chemical analysis of the muscles was conducted using standard methods: dry matter was determined by heating the samples at 105ºC until reaching a constant weight; fat content was determined through extraction using a Soxtec System with petrol ether at 40–60°C; protein content was determined by the Kjeldahl method (King-Brink and Sebranek, 1993); ash content was determined through incineration of the samples in a furnace at 600°C (AOAC, 2006). The cholesterol content in the muscles of the *longissimus dorsi* and hind legs of rabbits was assessed using the high-performance liquid chromatography (HPLC) method described by Polak et al. (2008). The degree of lipid oxidation, measured by malondialdehyde (MDA) content, in the *longissimus dorsi* and hind leg muscles of rabbits was evaluated 24 hours post-mortem and three months after storage in a refrigerator at -18°C by the high-performance liquid chromatography system Varian ProStar (Varian, Inc., Palo Alto, CA, USA) with a ProStar 363 fluorescence detector and method described by Mendes et al. (2009). Vitamin E accumulation in rabbit meat was determined according to the method of Hewavitharana et al. (2004). To determine vitamin E homologues (a-tocopherol, g-tocopherol), a high-pressure gradient HPLC system, Varian Pro Star (Varian, Inc., Palo Alto, USA) was used. The fatty acid composition was analysed according to the methodology of Folch et al. (1957), and the content of fatty acids as a percentage of the total content of fatty acids in rabbit meat was determined using a Shimadzu GC-2010 gas chromatography system (Shimadzu Corp., Kyoto, Japan) using an Rt-2560 column (100 m × 0.25 mm ID, 0.20 µm film) (Restek, Bellefonte, PA, USA).

Statistical Analysis

The data obtained during the study were analysed using SPSS for Windows, version 25.0 (IBM Corp., released in 2017; Armonk, NY, USA). Prior to data analysis, the normality of the data was determined using the Kolmogorov-Smirnov test. For normally distributed data, a parametric independent T-test was performed on all data obtained during each trial period to identify any differences among the different groups. Statistical significance was defined as a P value less than 0.05 ($P<0.05$).

RESULTS AND DISCUSSION

Growth performance and carcass traits

The effect of increased DL-α-tocopherol dose on rabbits' growth performance and carcass evaluation is presented in Table 2. The increased dose of DL-α-tocopherol did not show any impact on rabbits' initial body weights (42 days of age), average daily weight gain (ADG), daily feed intake (DFI), and feed conversion ratio (FCR) during the whole feeding trial (42–112 days), compared with the control group (P>0.05). However, the increased dose of DL-α-tocopherol significantly reduced the weight of the rabbits on the last day of the experiment (112 days of age), compared to the control group (P<0.05). Our research findings are in agreement with Dalle Zotte et al. (2022), who also found that vitamin E had no effect on rabbit growth characteristics except for FCR, which was significantly improved when vitamin E was added to rabbit feed. However, in contrast, Cardinali et al. (2015) found that after 80 days of feeding trials, adding 150 ppm of vitamin E to the diet of New Zealand White rabbits significantly increased feed intake, FCR, ADG, and live weight. Moreover, according to Ebeid et al. (2013), supplementing the feed of California rabbits with a vitamin E additive significantly increased feed intake, final body weight, and daily weight of rabbits but decreased the FCR compared to the control group. It is possible that the different results observed in these studies were due to variations in the breeds of rabbits used, their ages, the duration of the trial, and the different doses of vitamin E used.

Upon examination of the rabbit carcass evaluation data presented in Table 2, it was observed that the increased dose of DL-α-tocopherol had a statistically significant effect on certain carcass traits. Specifically, it resulted in a reduction of slaughter weight and hind leg muscle yield in the experimental group while increasing the weight of internal organs compared to the control group (P<0.05). However, no significant differences were observed for other carcass traits. These findings are consistent with those reported by Castellini et al. (2001), who did not observe any significant differences in the carcass traits of rabbits fed diets supplemented with vitamin E at either 50 mg/kg or 200 mg/kg. Similarly, Dalle Zotte et al. (2020) did not find any effect of vitamin E on slaughter data or carcass traits in rabbits.

Table 2. Effect of an increased DL-α-tocopherol dose on rabbits' growth performance (n=30; 42–112 days of age) and carcass evaluation (n=14; 112 days of age)

Indices	Groups	
	Control	Experimental
Body weight (42 days of age), g	842.00±0.01	840.13±0.02
Body weight (112 days of age), g	2697.86±0.08[a]	2511.13±0.06[b]
ADG, g	26.58±0.00	23.01±0.00
DFI, g	69.57±6.48	70.16±7.69
FCR, kg/kg	3.58±0.16	3.50±0.10
Weight before slaughter, g	2634.00±73.69[a]	2448.20±57.55[b]
Warm carcass, g	1345.25±34.62	1263.80±33.56
Chilled carcass, g	1293.10±39.36	1225.58±31.08
Dressing out percentage, %	50.83±2.03	50.74±0.61
Carcass yield, %	72.62±3.80	72.84±0.80
Hind leg muscle yield, %	20.71±0.63[a]	19.21±0.29[b]
Longissimus dorsi muscle yield, %	10.41±0.33	10.00±0.55

Internal organs weight, g*	93.20±5.64[a]	94.40±8.42[b]

[a, b] – means within each row with different superscripts are significantly different at P<0.05;
*- heart, liver, spleen, lung, kidneys.

Chemical-physical characteristics and quality of muscle

Meat quality is a crucial aspect of evaluating the nutritional value and overall quality of meat products. One of the key indicators is the chemical composition of the meat portion of the carcass, which includes protein, fat, moisture, and other elements. In this study, we investigated the meat quality of the *longissimus dorsi* and hind leg muscles of rabbits by analysing their chemical and physical properties. The results, as presented in Table 3, showed no significant effect of increased supplementation with DL-α-tocopherol on the chemical composition of the rabbits' muscles, including dry matter, protein, fat, and ash (P>0.05). However, a higher amount of fat was found in the hind leg muscles of the experimental group of rabbits (P<0.05). These findings suggest that the increased DL-α-tocopherol dose did not significantly affect the chemical composition of rabbit muscles, except for the higher fat content observed in the hind leg muscles.

Dietary manipulation with vitamin E supplementation can have an additional impact on rabbit carcass quality parameters, such as meat or fat colour, which enhances the overall appearance and marketability of rabbit meat products. Compared to other animal species used for meat production, rabbit meat is typically classified in the group with the highest lightness of meat, alongside poultry (Hernández et al., 2006; Metzger et al., 2006). Our study analysed the L*, a*, and b* colour values of the *longissimus dorsi* and hind leg muscles, revealing a significant interaction (P<0.05) only for the b* value of the both muscles (Table 3). The increased DL-α-tocopherol dose in rabbit feed reduced the b* value in the *longissimus dorsi* muscle but increased it in the hind leg muscle compared to the control group (P<0.05). Similar to our findings, Corino et al. (2007) found that vitamin E additives in feed had no significant effect on the colour intensity of rabbit meat. However, these studies also discovered that the impact of vitamin E supplementation was more pronounced in thigh meat, enhancing the lightness of the meat, the intensity of the yellowness, and the chromaticity. Other studies, such as those conducted by Dalle Zotte et al. (2020), did not determine the impact of vitamin E on the colour intensity of rabbit meat.

Table 3. Effect of an increased DL-α-tocopherol dose on rabbits' *longissimus dorsi* and hind leg muscle chemical-physical properties (n=14)

Item	**Groups**	
	Control	**Experimental**
	Longissimus dorsi	
Dry matter, %	24.39±0.62	25.12±0.50
Protein, %	21.24±0.57	21.72±0.42
Fat, %	1.81±0.12	2.00±0.25
Ash, %	1.34±0.02	1.40±0.05
L*	58.35±0.38	57.48±0.79
a*	10.32±0.60	10.95±0.46
b*	5.71±0.22[a]	4.93±0.29[b]

Item	**Groups**	
	Control	**Experimental**
	Hind leg	
Dry matter, %	26.99±0.19	26.81±0.22
Protein, %	22.29±0.32	21.70±0.26
Fat, %	3.62±0.21[a]	4.06±0.26[b]
Ash, %	1.08±0.05	1.04±0.10
L*	54.41±1.75	55.81±2.50
a*	12.99±1.16	12.37±1.36
b*	3.76±0.58[a]	4.12±0.39[b]

[a, b] – means within each row with different superscripts are significantly different at P<0.05.

Rabbit meat is known for its nutritional benefits, being lean, protein-rich, and containing low levels of cholesterol. However, the cholesterol content in rabbits can be influenced by genetic and environmental factors as well as dietary supplements such as DL-α-tocopherol (Dalle Zotte and Szendrő, 2011). The study data presented in Table 4 investigated the effect of an increased DL-α-tocopherol dose on meat quality, specifically the cholesterol content in the rabbits' muscles. The results showed a significant reduction in cholesterol content in the hind leg muscle of the experimental group compared to the control group (P<0.05). This reduction in cholesterol can be attributed to the antioxidant properties of DL-α-tocopherol, which can scavenge free radicals and reduce oxidative stress in cells. Additionally, DL-α-tocopherol can inhibit the activity of HMG-CoA reductase, an enzyme involved in cholesterol synthesis. By inhibiting this enzyme, DL-α-tocopherol can reduce cholesterol synthesis and, consequently, lower the cholesterol content in tissues (Pal et al., 2003). Therefore, the observed reduction in cholesterol content in the hind leg muscles of rabbits in the experimental group is likely due to the antioxidant and HMG-CoA reductase inhibitory properties of DL-α-tocopherol.

Malondialdehyde (MDA) is a biomarker for lipid peroxidation, a process that can lead to cellular damage when free radicals react with unsaturated fatty acids in living organisms (de Zwart et al., 1999). In a recent study, the content of MDA in the *longissimus dorsi* muscles was lower compared to hind leg muscles in both fresh and stored meat. It was also found that the content of MDA in the experimental group significantly decreased in fresh meat in the hind leg muscle and increased in stored meat samples in the *longissimus dorsi* (P<0.05) (Table 4). This indicates that lipid peroxidation occurs during storage and can negatively impact meat quality. However, increased DL-α-tocopherol supplementation in rabbit feed had a protective effect against lipid peroxidation, as evidenced by significantly lower MDA content in the hind leg muscles of the experimental group compared to the control group. A similar trend was observed by Minardi et al. (2020), who reported that dietary supplementation with different doses of vitamin E led to decreased MDA content in rabbits' muscles compared to the basal diet-treated group. The reduced MDA content in hind leg muscles of the experimental group in this study can be attributed to the increased DL-α-tocopherol dose, which may have prevented or reduced lipid peroxidation. These findings suggest that DL-α-tocopherol can enhance the quality and shelf life of rabbit meat by reducing lipid peroxidation and oxidative stress.

The present study investigated the impact of an increased dosage of DL-α-tocopherol in rabbit feed on the accumulation of α-tocopherol in rabbit muscles and its effect on antioxidant capacity (Table 4). The results showed that the increased DL-α-tocopherol dose led to a higher accumulation of α-tocopherol in the muscles of experimental rabbits, which correlated with an increase in antioxidant capacity ($P<0.05$). This was attributed to the direct scavenging of free radicals generated during lipid peroxidation by α-tocopherol, thereby preventing the propagation of oxidative damage. The increase in antioxidant capacity was further corroborated by the reduction of MDA levels observed in the hind leg muscles of rabbits receiving the increased DL-α-tocopherol dose. MDA is a by-product of lipid peroxidation and serves as an indicator of oxidative damage. These findings are consistent with previous studies by Lo Fiego et al. (2004), Dalle Zotte et al. (2020), and Minardi et al. (2020), which reported that an increase in the amount of vitamin E in rabbit feed resulted in a higher accumulation of α-tocopherol in rabbit meat. Taken together, these results highlight the importance of dietary vitamin E supplementation in enhancing the antioxidant capacity of rabbit meat and reducing oxidative damage.

Table 4. Effect of an increased DL-α-tocopherol dose on rabbits' *longissimus dorsi* and hind leg muscle quality properties (n=14)

Item	**Groups**	
	Control	**Experimental**
	Longissimus dorsi	
Cholesterol, mg 100 g^{-1}	38.98±1.46	35.90±2.93
MDA fresh, µmol kg^{-1}	0.70±0.09	0.90±0.13
MDA after 3 months, µmol kg^{-1}	0.82±0.07^{a}	1.15±0.16^{b}
α-tocopherol, mg kg^{-1}	2.34±0.11^{a}	4.09±0.20^{b}
γ-tocopherol, mg kg^{-1}	0.43±0.02	0.49±0.02
ΣSFA	14.98±1.14	15.75±1.28
ΣMUFA	37.39±0.51	37.78±0.46
ΣPUFA	47.63±0.73	46.47±1.20
ΣPUFA/ΣSFA	3.18±0.10^{a}	2.95±0.26^{b}
	Hind leg	
Cholesterol, mg 100 g^{-1}	46.62±1.68^{a}	41.11±3.18^{b}
MDA fresh, µmol kg^{-1}	1.88±0.17^{a}	1.60±0.15^{b}
MDA after 3 months, µmol kg^{-1}	2.38±0.19	2.21±0.25
α-tocopherol, mg kg^{-1}	3.36±0.18^{a}	6.39±0.85^{b}
γ-tocopherol, mg kg^{-1}	0.60±0.04	0.68±0.07
ΣSFA	18.53±2.04	19.32±1.88
ΣMUFA	37.61±1.42^{a}	36.86±2.47^{b}
ΣPUFA	43.86±1.56	43.82±1.49
ΣPUFA/ΣSFA	2.36±0.10	2.26±0.13

[a, b] – means within each row with different superscripts are significantly different at $P<0.05$.

Fatty acids are important components of meat, as they play a crucial role in determining the meat's quality and its nutritional value. The fatty acid profile of our study rabbit meat (total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and the ratio of polyunsaturated to saturated fatty acids (ΣPUFA/ΣSFA)) was very similar when analysing both *longissimus dorsi* muscles and hind leg muscles (Table 4). However, the experimental group receiving an increased dose of DL-α-tocopherol supplementation exhibited a lower ΣPUFA/ΣSFA ratio in the *longissimus dorsi* muscle and a lower MUFA content in the hind leg muscle of rabbits ($P<0.05$). This effect may have been due to the inhibition of delta-6 desaturase activity by DL-α-tocopherol, leading to a decrease in PUFA synthesis and a subsequent reduction in the ΣPUFA/ΣSFA ratio in the *longissimus dorsi* muscle.

Additionally, DL-α-tocopherol may have increased stearoyl-CoA desaturase activity, promoting MUFA synthesis and resulting in a higher conversion of SFA to MUFA. This, in turn, caused a lower SFA and higher MUFA content in the hind leg muscle. While previous studies have examined variations in the fatty acid composition of different types of rabbit muscles, only a few have explored these variations comprehensively. For instance, Mordenti et al. (2010) reported some differences in the fatty acid composition of the entire thigh and loin muscles, while Minardi et al. (2020) found similar MUFA and PUFA levels in rabbit muscle with different vitamin E inclusions. However, the latter study also reported higher SFA levels and ΣPUFA/ΣSFA ratios, which differed from our findings.

CONCLUSION

In conclusion, the study indicated that the elevated dosage of DL-α-tocopherol in rabbit feed did not impact the initial body weight, ADG, DFI, or FCR of rabbits. However, it led to a decrease in the weight of rabbits on the final day of the experiment and a significant reduction in the slaughter weight and hind leg muscle yield of the rabbits. Furthermore, the increased dose of DL-α-tocopherol affected the cholesterol content and fatty acid composition of the meat, resulting in a lower ΣPUFA/ΣSFA ratio in the longissimus dorsi muscle and a decrease in MUFA content in the hind leg muscle of rabbits. The study findings suggested that the elevated dose of DL-α-tocopherol had a notable impact on meat quality and the accumulation of α-tocopherol in the muscles.

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Not applicable

Statement of Conflict of Interest

The authors declare that they are no conflict of interest.

Authors' Contributions

V.V., A.R.S. and V.Š. designed and analysed the research, V.V., A.R.S. and M.N. studies arranged. S.B. worked on the preparation of M.N. and A.B. 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.

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Selenomethionine Utilisation in Fattening Pig Diets and Impact on Productivity and Final Production Quality

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ABSTRACT: Feed supplementation with Se not only improves livestock performance and quality, but also stimulates the enrichment of Se in animal tissues, resulting in functional livestock products. The aim of this study was to investigate the impact of selenomethionine on fattening pig productivity and meat quality. The trial was performed with 52 45-day-old pigs, which were weighed and randomly assigned to 2 dietary treatments: standard compound diet + 0.50 mg/kg Se (from sodium selenite; **SS**) and standard compound diet + 0.50 mg/kg selenomethionine (**SM**). During the feeding trial (45–153 days), productivity traits of pigs were recorded; after slaughter, meat quality traits (chemical composition, MDA, accumulation of Se) were determined. The results revealed that the addition of 0.50 mg/kg of selenomethionine to fattening pig feed had no effect on productivity traits, fat and muscle thickness, or meat chemical composition ($p>0.05$). However, selenium accumulation in the *M. longissimus dorsi* muscle under SM treatment increased to 0.956 µg/g and increased MDA after 24 hours, which was 2.673 µmol/ kg higher compared to SS treatment ($p<0.05$). Based on the findings, feeding fattening pigs feed enriched with selenomethionine can improve selenium deposition in the muscles, which can have a beneficial impact on the customer's health.

Keywords: Organic selenium, Malondialdehyde; Se accumulation; Fattening pigs, Growth performance; *Longissimus dorsi*

INTRODUCTION

In recent years, there has been a significant increase in pork consumption globally, which has led to an increased demand for high-quality pork products (Mote and Rothschild, 2020). In this context, enhancing the production efficiency and meat quality of fattening pigs has become a focus of the swine industry. One potential approach to improving these aspects is through the supplementation of selenium in pig diets, as selenium is an essential trace element that has been shown to have positive effects on growth performance, immune function, and meat quality in pigs (Silva et al., 2019; Falk et al., 2020; Liu et al., 2021).

Selenium is an essential trace element for humans and animals, and its deficiency leads to various health problems, including muscular dystrophy, exudative diathesis, necrotic liver degeneration, severe heart disease, and neuronal cell damage (Kumar and Priyadarsini, 2014). In general, Se is reported to be a critical trace mineral that plays a crucial role in regulating the growth and physiological functions of animals (Ferro et al., 2021). However, excessive Se levels can lead to both acute and chronic toxicity in the body, despite its essential role in physiological growth and function (Chantiratikul et al., 2018).

Optimal selenium supplementation is critical for improving livestock productivity and the quality of animal products, as it facilitates selenium enrichment in animal tissues. However, excessive selenium intake can lead to the oxidation and cross-linking of vital protein thiol groups, ultimately resulting in the generation of reactive oxygen species and apoptosis. But not only the dose of this trace element is crucial; its form can play a vital role in animal nutrition as well. Selenium can be supplemented to diets as inorganic mineral salts, typically as sodium selenite (Na_2SeO_3), or as organic forms like selenomethionine (Se-Met) and Se-enriched yeast (SY) (Shini et al., 2015; Calvo et al., 2017). In the animal industry, it is generally believed that organic Se is more efficient in absorption, anti-oxidation, and tissue accumulation and leads to less environmental pollution than inorganic Se. However, the production process of SY requires a specific strain and a culture medium with Na_2SeO_3 (Surai and Fisinin, 2014). Furthermore, the application of Se-Met is limited due to its complicated production process and high cost (Shini et al., 2015). Therefore, it has become of interest to find efficient and safe Se sources. So, the aim of this study was to investigate the impact of organic selenomethionine on fattening pig productivity and meat quality, including Se accumulation in muscle tissues.

MATERIAL AND METHOD

The pigs for fattening were kept in stalls, and their keeping conditions were in accordance with Council Directive 2008/120/EC of 18 December 2008, which lays down minimum standards for the protection of pigs. The feeding trial was conducted using fifty-two Landrace x Yorkshire (mother) and Pietrain x Duroc (father) pigs for fattening (45-day-old), which were individually weighed and randomly assigned to two dietary treatments with four replicate stalls of twenty-six pigs each. The pigs were fed *ad libitum* with a standard wheat-barley-soybean meal compound diet supplemented with 0.5 mg/kg of selenium from sodium selenite (inorganic selenium) (SS) and 0.5 mg/kg of selenomethionine (organic selenium) (NRC, 2012). The data recorded during the feeding phase were live weight (LW) at 45, 61, 89, 121, and 153 days from the start of the study, average daily gains (ADG), and feed conversion ratio (FCR) during the periods 45–61, 62–89, 90–121, 122-153, and from the start of the study (45–153 days).

Before the pigs were slaughtered, their fat thickness and muscularity were measured using ultrasound equipment called Piglog-105 (Piglog 105, 1991). The measurements included two assessments of fat thickness and the thickness of the *M. longissimus dorsi*. At the end of the trial (153 days), 5 pigs for fattening were selected and slaughtered from each group, totalling 10 pigs. The slaughter was conducted according to standard procedures, and samples for the analysis of MDA, selenium, and chemical composition were taken from the *M. longissimus dorsi* between the 12th and last rib.

The chemical analysis of the muscle samples was carried out using standard methods. The dry matter was determined by heating the samples at 105ºC until they reached a constant weight. The fat content was extracted using a Soxtec system with petrol ether at 40–60 °C. The protein content was determined by the Kjeldahl method, as described by King-Brink and Sebranek (1993). The ash content was determined through incineration of the samples in a furnace at 600°C, following the procedure outlined in AOAC (2006). The degree of lipid oxidation in the pork was assessed by measuring the malondialdehyde (MDA) content. MDA content was evaluated 24 hours post-mortem and three months after storage in a refrigerator at -18°C, using the high-performance liquid chromatography system Varian ProStar (Varian, Inc., Palo Alto, CA, USA) with a ProStar 363 fluorescence detector. The method used for MDA analysis was described by Mendes et al. (2009). Selenium accumulation in pork was determined using a ThermoFisher iCE 3000 atomic absorption spectrophotometer with a GFS 35Z graphite furnace; the background correction system used was based on the Zeeman opto-magnetic effect (Thermo Fisher Scientific Inc., Altrincham, United Kingdom).

Statistical analysis. The data obtained during the study were analysed using SPSS for Windows, version 25.0 (IBM Corp., released in 2017; Armonk, NY, USA). Prior to data analysis, the normality of the data was determined using the Kolmogorov-Smirnov test. For normally distributed data, a parametric independent T-test was performed on all data obtained during each trial period to identify any differences among the different groups. Statistical significance was defined as a p value less than 0.05 ($p<0.05$).

RESULTS AND DISCUSSION

The growth performance of pigs for fattening fed a wheat-barley-soybean meal compound diet supplemented with various forms of selenium active substances is presented in Figure 1 and Table 1. The group of pigs fed with organic selenium additive (SM treatment) showed a 2-3% increase in weight gain from day 89 of age compared to the group fed with inorganic selenium ($p>0.05$) in the study. This result may be attributed to the higher bioavailability of organic selenium, which is incorporated more efficiently into proteins, enzymes, and other molecules involved in various physiological functions in the body. Additionally, organic selenium has antioxidant properties that can help reduce oxidative stress and improve the immune response of animals. These findings suggest that using organic selenium as a supplement in pig feed can potentially enhance growth performance and improve overall health outcomes. However, it is important to note that the obtained live weight results were statistically unreliable ($p>0.05$). The efficacy of organic selenium as a supplement in improving animal weight and productivity has been well-established in various studies. Organic selenium, in contrast to inorganic selenium, provides a predetermined amount of protein, amino acids, vitamins, and other nutrients in addition to selenium sources. This nutrient combination aids in fat production within the body. The objective of the Zhang et al. (2020) study was to determine whether organic selenium supplementation could accelerate the development of pigs used for fattening, and the results supported this hypothesis.

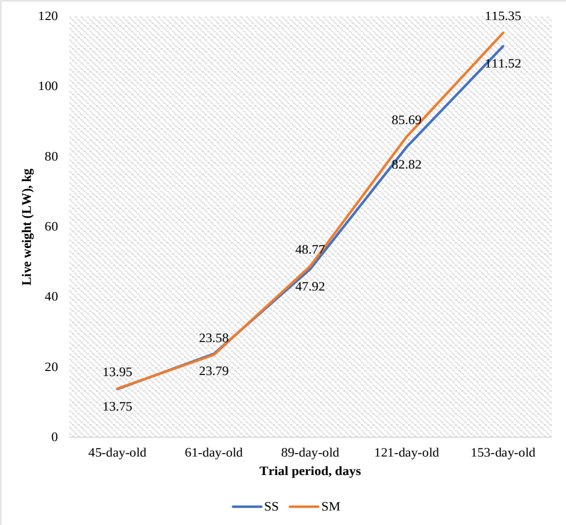

Figure 1. Effect of selenomethionine on the live weight (LW) of fattening pigs during a feeding trial (45-153 days)

In this recent study, the average daily gain (ADG) and feed conversion ratio (FCR) of pigs for fattening were evaluated and presented in Table 1. During the first period of the study (45–61 days of age), pigs that received the inorganic selenium treatment (SS) had a 4% lower ADG than those in the selenomethionine group (SM). However, from day 61 onward, pigs fed with SM showed a faster growth rate, gaining 3% to 6% more weight per day than those in the SS treatment. Over the entire study period (45–153 days), the ADG of pigs fed with organic selenium (SM treatment) was 4% higher than that of those fed with inorganic selenium (p>0.05). Another study by Zhang et al. (2018) also found that supplementing pig diets with selenium-enriched yeast improved ADG and showed a rising trend in average daily feed intake (DFI). The observed increase in ADG with organic selenium supplementation could be attributed to the fact that selenomethionine provides a more bioavailable form of selenium to the pigs, leading to better utilisation of the nutrients in their diet and improved growth. The increased availability of selenium in the form of selenomethionine may also help optimise thyroid hormone metabolism, leading to better feed conversion efficiency and higher growth rates. However, the results of this study did not show any statistically significant effects of selenium supplementation on ADG and FCR during all feeding periods. Further studies are needed to confirm the mechanisms of action and potential benefits of organic selenium supplementation in pig diets.

Table 1. Effect of selenomethionine on the pigs' average daily gain (ADG) and feed conversion ratio (FCR)

	ADG, g		FCR, kg/kg	
Treatment	**SS**	**SM**	**SS**	**SM**
Feeding period				
I feeding period (45–61 days of age)	0.628	0.602	1.78	1.81
II feeding period (62–89 days of age)	0.862	0.900	2.24	2.07
III feeding period (90–121 days of age)	1.091	1.154	2.59	2.45
IV feeding period (122–153 days of age)	0.897	0.927	3.60	3.36
Whole feeding period (45–153 days of age)	0.639	0.663	2.72	2.56

Table 2 displays the impact of selenomethionine on the muscularity of fattening pigs. The thickness of the fat (bacon) and muscle, as well as the percentage of muscularity, were determined by measuring the pigs at 153 days of age. The results indicated that pigs in the SM treatment had thicker bacon, ranging from 8% to 13%, and slightly thinner muscle by 2% compared to those in the SS treatment. Additionally, the percentage of muscularity in the SM treatment was 1.59% lower. These findings suggest that organic selenium may have a positive effect on fat deposition in pigs, leading to thicker bacon. However, the underlying mechanisms behind this effect require further investigation.

Table 2. Effect of selenomethionine on the pigs' fattening muscularity (153 days of age)

Treatment	Weight, kg	Fat thickness, mm		Muscles thickness, mm	Muscularity, %
		1 point	2 point		
SS	103.50±1.44	13.00±0.76	12.88±0.47	55.63±2.87	58.79±0.45
SM	106.78±1.07	14.00±1.06	14.56±0.75	54.67±2.95	57.20±0.92

The chemical composition of pork is important because it provides information about the nutritional quality and safety of the meat. It also affects the flavour, texture, and overall eating experience. Analysing the chemical composition of pork can help ensure that it meets the necessary quality standards and regulations for consumption and can provide valuable In our study, the addition of selenomethionine to pig feed resulted in a slight decrease in dry matter and fat by 0.13% and 0.31%, respectively, and a slight increase in protein and ash by 0.23% and 0.04%, respectively, compared to the SS group (p>0.05; Table 3). However, we did not observe any significant differences in the pork's chemical composition between the two treatments (p>0.05). Other studies have reported that the addition of selenium-enriched yeast (SY) and a combination of SS and Se-Met can significantly decrease moisture content and increase crude protein content in the longissimus thoracis of pigs compared to only SS group (Zhang et al., 2020). Additionally, the fat content of pork fed with organic selenium sources may increase. However, the ash content of *longissimus thoracis* did not show any significant impact when the fattening pigs were treated with various Se sources as in our recent research.

Table 3. Effect of selenomethionine on pork chemical composition, lipid oxidation (MDA) levels, and selenium accumulation in muscles

Items	Treatment	
	SS	**SM**
Dry matter, %	30.02±15.01	29.89±14.94
Protein, %	23.72±11.86	23.95±11.97
Fat, %	5.11±2.55	4.80±2.40
Ash, %	1.06±0.53	1.10±0.55
MDA after 24 hours, µmol/kg	0.354±0.022	3.027±1.028*
MDA after 3 months, µmol/kg	0.532±0.061	3.176±0.893
Selenium, µg/g DM (95%)	0.430±0.081	1.383±0.145*

* means within each row are significantly different at p<0.05

Selenium is a natural antioxidant that has the ability to reduce fat oxidation in tissues. In our study, we measured the malonildialdehyde (MDA) levels in the *M. longissimuss dorsi* muscle of fattening pigs 24 hours after sampling and found that the MDA content of the selenomethionine (SM) treatment was significantly higher than the control treatment (SS) (*p*<0.05; Table 3). After three months of storage at -18°C, the MDA content in the SM treatment was 1.64 μmol/kg higher than the SS treatment, but the difference was not statistically significant (*p*>0.05). In general, selenomethionine is the major organic form of selenium found in selenium-enriched yeast, and it acts as a powerful oxidant scavenger of peroxynitrite, which is a by-product of nitric oxide and superoxide that can oxidize a wide range of biomolecules (Calvo et al., 2017). Additionally, MDA is an excellent marker of the onset of oxidative stress caused by heat stress and can also be utilized as a marker of cell membrane damage caused by lipid peroxidation (Jócsák et al., 2020). These studies suggest that inorganic selenium supplementation (SS) can help reduce MDA accumulation in pork, which can lead to improved meat quality and shelf life. However, the optimal level of selenium supplementation may vary depending on various factors such as the age, breed, and diet of the pigs. Therefore, it is important to consult with a veterinarian or animal nutritionist to determine the appropriate level of selenium supplementation for a particular herd. Additionally, our study found that inorganic selenium, as a natural antioxidant, can reduce fat oxidation in tissues in fresh *M. longissimus dorsi* muscles of pork.

Selenium is an essential mineral that has many important functions in the body, including acting as an antioxidant and supporting the immune system. Adequate selenium intake has been associated with a lower risk of several chronic diseases, including heart disease and certain types of cancer. Moreover, selenium plays a vital role in thyroid hormone metabolism and helps protect against oxidative stress and inflammation. However, it is important to note that excessive intake of selenium can be toxic and lead to adverse health effects. Therefore, it is crucial to maintain a balance in selenium intake to achieve its benefits without causing harm. In our study, we found that selenomethionine can increase selenium accumulation in the *M. longissimus dorsi* muscle of pigs. Specifically, the SM treatment resulted in a significant increase in higher selenium accumulation by 0.956 μg/g compared to the inorganic selenium supplemented treatment SS (p<0.05). These findings are consistent with the results obtained by Grossi et al. (2021), who found that a selenium-enriched diet led to a greater accumulation of organic forms of selenium in animal muscle tissue compared to inorganic forms. Overall, these results suggest that the use of organic selenium sources, such as selenomethionine, can lead to higher levels of selenium accumulation in animal tissue, which may have important implications for human health and nutrition.

CONCLUSION

In conclusion, organic selenomethionine supplementation in pig feed has potential benefits for growth performance and overall health due to its higher bioavailability and antioxidant properties. However, our study did not find statistically significant differences in productivity outcomes, and further research is necessary to determine the underlying mechanisms. Additionally, our findings suggest that organic selenium sources may increase fat deposition in pigs, leading to thicker bacon, but further research is needed to fully understand the impact on pork composition. Nevertheless, the addition of selenium, particularly inorganic selenium, can reduce fat oxidation in fresh *M. longissimus dorsi* muscles of pork, thereby improving meat quality and shelf life. Furthermore, we observed that selenomethionine can increase selenium accumulation in the *M. longissimus dorsi* muscle of pigs.

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Statement of Conflict of Interest

The authors declare that they are no conflict of interest.

Authors' Contributions

ARS, VV and MN designed and analyzed the research, ARS, MN and VV studies arranged. VŠ and EŽ 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.

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Possibilities of Using Essential Oils Against Diseases

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ABSTRACT: In the world and our country, the use of chemicals in the fight against weeds, diseases and pests in crops has increased considerably to increase agricultural production and quality in production. Chemicals, which are used with the habits of the past without considering the economic damage threshold, cause residues and environmental pollution in the products produced due to misuse, non-compliance with waiting periods and usage doses. Since these residues can remain in nature and the environment for a long time, they can be carcinogenic to living organisms. To reduce the use of chemicals in agricultural production and the possible damages that may occur, studies are carried out on different control methods and one of these control methods is essential oils obtained by utilizing herbal drugs. Due to the high plant diversity in our country, the possibilities of using essential oils and extracts against weeds, diseases and pests become even more important. In this study, information about the use of essential oils and extracts against diseases in agricultural production was given.

Keywords: Essential oil, Alternative control, Bactericide, Fungicide, Herbicide

INTRODUCTION

Considering the changing world population, the need for nutrition, which is one of the basic needs of humanity, is increasing day by day. In agricultural activities, the fight against plant diseases and pests gains importance in this sense. Most of the struggles are carried out with the use of pesticides due to their rapid effect and ease of use. This situation poses a great threat to human health and the environment. As a result of intensive and unconscious chemical use, the pesticide itself or different substances in its content remain in water, air, soil, and food. These residues cause irreversible and perhaps irreversible damage to all living things and nature. Today, sustainable agriculture comes to the fore. Considering the excessive use of synthetic pesticides in conventional agriculture, it is seen that good agricultural practices gain more importance. In addition to synthetic pesticides against weeds, plant diseases, and pests, it has been observed in studies that bioagents and herbal pesticides are used (Belgüzar et al., 2016, Dadaşoğlu et al., 2016; Aktepe, 2019). In this study, the use of essential oils against weeds and plant diseases was investigated.

Use of Essential Oils Against Weeds

In our country and the world, different control methods such as cultural, physical, mechanical, biological and chemical are used to control weeds. Before chemical control of weeds in agriculture became so widespread, physical control, which is important for sustainable agriculture, was carried out. Due to the excessively increasing world population, the growth of agricultural areas and the decrease in manpower used in agriculture, physical weed control has been replaced by chemical control (Kitiş, 2019). The use of pesticides has become an indispensable practice to reduce and eliminate the density of weeds in agriculture to reduce and eliminate crop losses to meet the food needs of the world's growing population every year. The excessive use of herbicides worldwide has serious negative consequences for the health of living beings and the future of the soil on which we produce. In addition, synthetic herbicides used in high doses for control have caused environmental pollution and the formation of herbicide-resistant weeds (Yarnıa, 2019).

1. Use of Allelopathy in Weed Control

One of the principles established for the sustainability of world agriculture is to control the germination and growth of weed seeds by utilizing the allelopathic effects between plants. Many researchers have been investigating the allelopathic effects of each plant secreting a variety of different chemicals to protect itself and attract insects, and the allelopathic effects of these secretions between different plant species. These secretions can produce different effects under different climatic and soil conditions. These effects can be effective on the germination of weed seeds and shell permeability (Arıkan et al., 2015). Işık et al. (2018), investigated the control of vetiver (*Chenopodium album* L.) with some essential oils. By drying the vetiver seeds in the laboratory environment and placing them in petri dishes with a volume of 9 cubic centimeters. The essential oils obtained from thyme, mint, lavender, sage and coriander in doses of 0.25, 0.5, 1, 2, 4, 8 and 16, which were previously prepared, were given to the vinegar seeds with the help of 5 ml of pure water and left to incubation for 21 days. When this study was compared with the control study, it was observed that germination, root and stem development were promoted at low doses and decreased at high doses.

Cunedioğlu et al. (2018) investigated the effects of essential oils obtained from Sütçüler Thyme (*Origanum minutiflorum* O.) and Rosemary (*Rosmarinus officinalis* L.) on the germination of some weed seeds. The essential oils prepared from Rosemary and Sütçüler Thyme were applied on weeds such as *Sinapis arvensis*, *Urtica urens*, and *Solanum nigrum* (2.0, 4.0, 8.0, 16.0, 32.0 µl/petri) at certain intervals. As a result of this study, it was observed that essential oils prevented the germination of the weed seeds between 31.5% and 100% at the lowest and highest doses. Efil et al. (2019) tried to determine the bioherbicide potential of essential oils obtained from Mountain Thyme (*Origanum syriacum* L.) and Marjoram (*Origanum majorana* L.) plants by applying them to some weed species. The essential oils they obtained. They applied the essential oils to weed seeds such as dog grape (*Solanum nigrum* L.), red-rooted foxtail (*Amaranthus retroflexus* L.), lantern grass (*Physalis angulata* L.) and purslane (*Portulaca oleracea* L.) and examined the effect on their germination. And as a result, they observed that it prevented their germination at a rate of 16% at the lowest and 80% at the highest.

Use of Essential Oils Against Pathogenic Bacteria

Alternative control methods include the use of herbal essential oils and herbal extracts obtained from medicinal plants. Positive results have been obtained in the use of these essential oils and extracts obtained by certain methods against plant pathogenic bacteria. The antimicrobial substances obtained can be used directly in disease control, or their active ingredients are produced synthetically and have led to the emergence of new pesticides with herbal ingredients. In the study conducted by Belgüzar et al. (2016), the essential oil obtained from thyme (*Thymus vulgaris* L.) was used against *Clavibacter michiganensis* subsp. *michiganensis*, the pathogen causing bacterial cancer and wilt disease in tomatoes, and its antibacterial effect were investigated. In these studies, methods such as adding essential oil to the medium, well method and impregnation of essential oil on the paper glued to the lid were used and the method of impregnating essential oil on the paper glued to the lid was the most effective method among these methods. The method with the lowest effect among these methods was the good diffusion method applied to agar.

The antibacterial activity of the essential oil obtained from *Ferula communis* was tested against all 18 bacterial isolates obtained from different organs of the plants and it was observed that it formed an inhibition zone at varying rates. The largest inhibition zone was 14 mm and the lowest inhibition zone was 10 mm (Dadaşoğlu et al., 2016). The effect of essential oil extracts from *Mentha piperita*, *M. spicata*, *M. dumetorum*, *Lippia citriodora*, *Origanum vulgare*, *O. onites* and *O. syriacum* on the growth of *Clavibacter michiganensis* subsp. *michiganensis* was investigated. The essential oils of *Lippia citriodora*, *Origanum syriacum*, *O. vulgare* and *O. onites* were found to be 100% effective, *Mentha piperita* 78.1%, *M. spicata* essential oil 78.9% and *Mentha dumetorum* 66.7% (Yanar et al., 2016).

The most important inoculum source of the pathogen is known as *Pseudomonas syringae* pv. *pisi*, which causes bacterial leaf blight disease in peas, is seeds. Umarusman et al. (2019) reported positive results of some plant extracts used against this pathogen in their study. When applied to pea seeds in pot trials, the disease was detected in 51% of the plants treated with *Cistus creticus* extract, 33% of the plants treated with *Allium sativum* extract and 3% of the plants treated with *Syzygium aromaticum* extract. In field trials, when *C. creticus* extract was applied to the seeds contaminated with the pathogen, 12% of the plants, 1% of the plants developing from the seeds treated with *S. aromaticum* extract and 8% of the plants treated with *Allium sativum* extract showed the disease. According to this study, the most effective method to be applied for the control of this pathogen is the application of *Syzygium aromaticum* (Spice Clove) essential oil to the seeds before sowing (Umarusman et al., 2019).

Mentha arvensis (Japanese mint), *Allium sativum* (garlic), *Thymus vulgaris* (thyme), *Cinnamomi ceylanici*, *Syzygium aromaticum* (clove), *Cymbopogon citratus* (lemongrass) and *Lavandula officinalis* (lavender) plant extracts applied against the fire blight disease pathogen called *Erwinia amylowora* were found to inhibit the development of the pathogen under in vitro conditions. Except for *Cymbopogon* *citratus*, all of these essential oils showed even stronger antibacterial activity than the antibiotic called streptomycin (Aktepe et al., 2019).

In another study, commercially available *Eucalyptus citriodora* (eucalyptus), *Hypericum perforatum* (St. John's wort), *Pimpinella anisum* (aniseed), *Brassica nigra* (mustard) and *Dianthus caryophyllus* (clove) oils were used against *Clavibacter michiganensis* subsp. *michiganensis*. According to the results, *Hypericum perforatum* (St. John's wort), *Dianthus caryophyllus* (clove), *Pimpinella anisum* (aniseed) and *Brassica nigra* (mustard) essential oils did not create any inhibition zone in bacterial growth, and the most effective oil was *Eucalyptus citriodora* (eucalyptus), which created a 20 µl inhibition zone. As a result of the studies, studies were carried out to determine the most effective dose of *Eucalyptus citriodora* (eucalyptus) and when different amounts of essential oil were applied on bacterial isolates, 5 µl and 10 µl essential oil amounts did not form an inhibition zone. The maximum inhibition zone was at 25 µl of oil and no increase in the inhibition zone was observed at higher amounts (Elçi et al., 2019).

In the study conducted by Tekiner Aydın et al. (2023), antibacterial trials of the essential oil of *Saturaje hortensis* (Koç grass), which grows naturally in Erzurum province, were carried out against some soft rot agents and it was stated that the highest inhibition zone among pathogenic bacterial isolates was determined as 33.5 mm against *Pectobacterium carotovorum* subsp. *carotovorum* and the lowest inhibition zone was measured as 20 mm in Enterobacter cloacae pathogen.

Psedomonas syringae pv. *tomato* pathogen causes bacterial spot disease in tomato. In a study by Yağız et al. (2017), essential oils obtained from some *Galium* species were used against the pathogen and it was observed that essential oils obtained from *Galium incanum*, *G. diecpathogenine* kii and *G. aladaghense* formed an inhibition zone of 4.3-12.3 mm (Yağız et al., 2017).

In the study conducted by Zhang et al. (2023), thyme oil and clove oil applications were made against the pathogen *Pectobacterium carotovorum* subsp. *carotovorum*, which causes blackleg disease in potatoes, and Minimum Inhibitory Concentration (MIC) values were examined and when the results were examined, thyme oil was determined as 100 µL and this ratio was determined as 400 µL in clove oil applications. The pathogen is more sensitive to oregano oil than clove oil. The results of this study show that essential oils obtained from thyme and clove oils have an antibacterial effect against *Pectobacterium carotovorum* subsp. *carotovorum*.

Essa et al. (2020) used thirteen essential oils (fenugreek, lemon, cinnamon, basil, onion, garlic, nagarmotha, jasmine, camphor, lavender, peppermint, cranberry and black seed) against *Xanhomonas campestri* pv. *vesicotoria* pathogen and the maximum antibacterial activity of these essential oils was determined to be lavender and cranberry essential oils.

This study evaluates the chemical compositions and antioxidant and antipathogenic properties of commercial orange (*Citrus sinensis* (L.) Osbeck) essential oils obtained using the cold press method

(COP) and cold press method followed by steam distillation (EOPD). The chemical compositions of the volatile fractions determined by gas chromatography-mass spectrometry were similar in both samples. Relatively large amounts of γ-terpinene were found in EOPD (1.75%) compared to EOP (0.84%). Monoterpene hydrocarbons formed the major phytochemical group, with limonene (90.4-89.8%) followed by myrcene (3.2-3.1%) as the main compounds. Non-volatile phenolics were eight times higher in EOP than in EOPD. Several assays with different levels of specificity were used to study antioxidant activity. Although both essential oils presented similar reducing capacities, the radical elimination ability was higher for EOP. Regarding antipathogenic properties, EOs inhibited the biomass and cell viability of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Biofilms. Furthermore, both EOs similarly attenuated the production of elastase, pyocyanin and quorum sensing auto-inducers as assessed using Gram-negative bacteria. EOP and EOPD showed significant antioxidant and antipathogenic properties, thus they may offer natural alternatives to extend the shelf life of food products by preventing oxidation and contamination caused by microbial spoilage (Manzur et al., 2023).

This study examined the susceptibility of the bacterial plant pathogen *Pseudomonas syringae* pv. *syringae* by three methods, including disk diffusion, well diffusion and vapor phase tests. Gas chromatography (GC) and gas chromatography coupled to a mass spectrometer (GC-MS) were used to identify and quantify essential oil compounds. The largest growth inhibition zones for disk diffusion, well diffusion and vapor tests were observed to be 0.925 cm, 1.75 cm and 1.45 cm for the highest essential oil volume (50 μL) of *Z. clinopodioides*, respectively. Regarding the EOs obtained from *C. copticum* seeds, the diameters of the growth inhibition zones were 0.775 cm, 1 cm and 0 cm, respectively. According to the results obtained, the highest antibacterial activity belonged to 50 μL EOs of *Z. clinopodioides* with a growth inhibition zone of 1.75 cm. On Luria-Bertani (LB) agar medium. The minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) for EOs of Z. clinopodioides were 8 μL mL -1 and 1 μL mL -1 on LB agar medium. The MBC and MIC values for the EOs of C. copticum were 16 μL mL -1 and 1 μL mL -1 on LB agar medium, respectively. According to the chromatography results, 16 different compounds were identified in the EOs of *C. copticum*, of which thymol (54.6%) was the major component. In addition, 27 different compounds were identified in *Z. clinopodioides*. EOs containing pulegone (23%) and 1,8 cineole (20.3%) were the main essential oil components. The results showed that EOs of *Z. clinopodioides* plants had higher chemical diversity and exhibited stronger antibacterial activities against *P. syringa* pv. (Feizi et al., 2023).

The effects of *Thymus vulgaris* essential oil on selected virulence factors (growth, fixed cell survival, swimming, swarming and exopolysaccharide production) were evaluated in phytopathogenic *Pseudomonas syringae* strains isolated from soybean fields in Argentina; the reference strains *Pseudomonas savastanoi* pv. *glycinea* and *Pseudomonas aeruginosa* are responsible for bacterial blight, a disease affecting crops worldwide. Plant bacterioses are often treated with antibiotics and copper compounds, which can contribute to the development of resistance in pathogens and damage the environment. For these reasons, environmentally friendly alternatives are needed. Although aromatic plants are a natural source of antimicrobial substances, their effects on phytopathogenic bacteria remain largely unexplored. Sub-inhibitory concentrations of the oil significantly reduced the slope and rate of bacterial growth. In addition, biofilm and exopolysaccharide (EPS) production was inhibited by affecting swimming and swarming patterns at all tested oil concentrations (Carezzano et al., 2023).

Use of essential oils against pathogenic fungi

Nowadays, due to the protective effects of essential oils against diseases and due to the increase in the economic damage threshold of chemical control, scientific research has started to shift in this direction. In recent years, essential oils have been used against plant diseases of bacterial, viral, and fungal origin due to their antimicrobial components (Viuda-Martos et al., 2010). In the study conducted by Tükmen (2019), the antifungal activity of essential oils obtained from medicinal plant species such as myrtle (*Myrtus communis* L.), laurel (*Laurus nobilis* L.) and fennel (*Foeniculum vulgare* Mill.), their blends and their microemulsion (µE) forms against fungal root rot and white mold disease agent *Sclerotinia sclerotiorum* was investigated under in vitro and in vivo conditions. The antifungal activity of the essential oils and their blends on mycelial growth was determined. According to the results obtained, essential oils inhibited mycelial growth at increasing rates in parallel with the increase in their concentrations. According to in vitro antifungal activity results, fennel essential oils showed higher antifungal effects compared to essential oils obtained from myrtle and laurel plants. Mycelial growth of *S. sclerotiorum* was completely inhibited at concentrations of 0.3 µg/ml of fennel essential oil, 1 µg/ml of laurel essential oil and 2 µg/ml of myrtle essential oil. When the effects of mycelial growth of essential oil mixtures obtained from laurel (D), myrtle (M) and fennel (R) plants were examined, a fungicidal activity was shown by 100% inhibition of mycelial growth at 0.4 µg/ml from R¾D¼ and R¾M¼ mixtures.

In the study conducted by Demirkol (2021), the in vitro antifungal effects of essential oils and ITC compounds against fungal disease agents harmful to tomato plants were shown. Considering the volatile activity of essential oil components with antifungal activity (MITC and especially oils obtained from different thyme species), it was determined that plant essential oils and ITC compounds can be used as an environmentally friendly preparation that can be used as an alternative to chemical control against pests. The different mechanisms of action of essential oil compounds show antifungal activity, and it is difficult for disease agents to gain resistance to this form of control (Demirkol, 2021).

In the study conducted by Oğuz (2022), low-dose essential oil applications showed a positive effect on drought stress, while high-dose essential oil applications showed a suppressive and inhibitory effect. It was determined that low levels of essential oil applications had a significant effect on wheat lines under *Fusarium culmorum* stress. The most accurate high-dose essential oil applications on disease rate.

In the study conducted under field conditions, 0.05% of essential oil applications had a positive effect on the examined traits. The effect of essential oil applications varied according to wheat lines, essential oil type and doses. Positive results were obtained that will contribute to biologically based applications needed especially in organic wheat farming (Oğuz, 2022).

CONCLUSION

Due to the negative effects of synthetic pesticides used against weeds, diseases and pests, which significantly affect agricultural production and product quality, on the natural environment and human health, alternative control methods should be supported to reduce the use of chemical control. The use of herbal essential oils, one of the alternative control methods, is difficult to apply because it is difficult to control in open areas, although it can be controlled in closed areas. Therefore, most of the studies conducted are in vitro conditions. Transfer to production areas has not been achieved. In order to increase the use of essential oils with herbal ingredients, application methods and formulation doses should be emphasized. Considering the plant diversity in our country, the herbal drugs used in the studies conducted are very few. It is thought that researchers will reduce the use of synthetic chemicals by focusing more on unused plant species.

Statement of Conflict of Interest

The authors have no conflict of interest.

Authors' Contributions

Fatih DADASOGLU, Muhammed TATAR, Emre ERDEN, Mustafa OZGERIS, Kenan KARA and Oktay OZBAY designed the research. Muhammed TATAR edited the article according to the template. All authors contributed to the writing of the article, participated in the publication process of the article, read and approved it.

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Foliar Boron Applications in Wheat Agriculture Usage Potential

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ABSTRACT: As the interest in agriculture and agricultural production increases in the world, the need for micro and macro nutrients also increases. Today, it is not enough to think of plant nutrients only in terms of Nitrogen, Phosphorus and Potassium, which are widely used, but other macro and micro elements should also be evaluated. Boron, which is a micronutrient among the nutrients, is a micronutrient that many plants need in different scales. In the researches conducted, it has been determined that wheat *(Triticum aestivum L. ssp.)*, like all cultivated plants, needs a certain amount of boron for its normal development. Considering that plant nutrient reserves are depleted day by day in areas where agricultural production is intensively practiced, it has become extremely important to make plant nutrition correctly and effectively in cereals, which are the main source of food, especially wheat, in order to cope with agronomic and economic problems. For this purpose, it is very important to consider boron, which provides a significant increase in the formation of hormones affecting growth, root development, bud and flower formation, yield and product quality in wheat, which is of strategic importance. In this study, the effects of foliar boron applications on wheat agriculture were investigated and the potential for use was determined and compiled.

Keywords: Wheat, Boron, Yield

INTRODUCTION

According to studies conducted in cereal farming areas around the world, it is known that the availability of micronutrients such as boron and zinc is decreasing day by day. These minerals have a significant impact on the yield and quality of wheat. Boron, which is one of the most important plant nutrients in wheat agriculture, is used in many different fields today in addition to its usage opportunities in the agricultural sector. From glass and paper industry to ceramic industry, cleaning and bleaching industry, nuclear applications, photography and paint industry.

The largest boron deposits in the world were formed in lake environments as a result of chemical precipitation. Borate formations are also found in salt deposits formed in the marine environment as well as in lake environments. In addition, B minerals can also be formed as a result of crystallization of under ground magma as it rises towards the earth. Contaminated waters, residues from B mines and chemical wastes are among the most common sources of B in soil (Anonymous, 2015).

Boron deficiency is common in calcareous soils with increasing pH (Marschner, 1995). Boron deficiency in soils can cause decreases in yield and quality of plants (Yarnia et al., 2013). Boron plays an important role in pollination (Marchner, 1995), cell wall synthesis and sugar transport in many plants

(Güneş et al., 2011). However, excessive boron application may cause toxic effects in plants (Dell and Huang, 1997). Many studies have been conducted on the effects of boron applications on grain yield and quality in cereals, especially in recent years (Alpaslan et al., 1996; Soylu et al., 2004; Kaur and Nelson, 2015; Koca, 2016; Konuşkan et al., 2017 and 2019). It is known that there is a decrease in the amount of micronutrients such as boron, zinc and magnesium in agricultural lands (soil) where cereals are grown in our country.

Boric acid and its derivatives borax, which can be used as inorganic fertilizers in agriculture, are forms that are readily soluble in soil and easily taken up by plants. On the other hand, water-soluble suda-boron, which dissolves much faster than boric acid and borax, is usually the form of B applied directly to plant leaves (Perica, S et al., 2001). Boron is a micronutrient that plants need in trace amounts, and when deficiency and toxicity limits are compared, these values are very close. Boron must be present in sufficient amounts in order to provide the desired productivity in plants. Boron plays an important role in protein and carbohydrate metabolism, phenol and auxin metabolism, tissue differentiation, membrane permeability, pollen germination and pollen tube growth (Barut H. and et al 2018). Boron deficiency primarily causes damage to the growth points in the plant and therefore the growth of plants slows down a lot. For this reason, the boron applications needed by the plant should be given to the plant organically or inorganically or through soil and leaves.

Considerable work has been done describing the effects of the presence or absence of boron on cereal crops. According to the available literature, a large number of studies have been conducted on the activities of boron in vegetation, its uptake and management in plants, its effects on grain content and its role in nutrient structure. In many countries around the world, boron deficiency has led to agronomic and economic problems.

In cases where plants cannot find the plant nutrients they need in sufficient and appropriate ratios in the soil or cannot utilize the plant nutrients in the soil sufficiently for any reason, they cannot develop normally and therefore the quantity and quality of plant nutrients and yield rate decrease. Plant nutrition is more important than in other countries, especially in countries such as Turkey, Ukraine and Russia, whose nutrition is based on plant products in general and cereals in particular.

For this purpose, in our research, the strengths and weaknesses of B were revealed and compiled with reference to the studies conducted in the literature in recent years in order to reveal the relationship between the B element needed by the plant and the plant, its use in plant nutrition, the uptake of B by the plant and its transport within the plant, and the potential of foliar B applications in wheat agriculture.

BORON UPTAKE AND TRANSPORT IN CROPS

As a result of the researches, the vital importance of B, which is the only nonmetal plant nutrient among microelements, as a plant nutrient for plants was determined about 200 years ago. However, it is known that the activities and functions of B in plants are still not fully understood. Plant uptake of B from soil is influenced by factors such as 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, soil organic matter content, soil wetting and drying, and soil/water ratio (Goldberg 1997, Keren et al. 1985).

Many researchers have shown that B is an essential nutrient for the growth of coniferous, dicotyledonous and monocotyledonous plants, ferns, most diatom (seaweed) species and nitrogen-fixing cyanobacteria (Shelp 1993, Loomis and Durst 1992). In general, some monocot species, such as oats and wheat, have lower B requirements than dicot and other monocot species, such as maize and lilies (Marschner 1995).

Although there is no general consensus among researchers on the uptake of B by plants, it is believed to be taken up mainly through passive absorption in the form of undissociated boric acid [B(OH)3]. It has been reported by several researchers that B uptake by plants is a passive process occurring at root plasma membranes that allows easy passage of boric acid [B(OH)3] and subsequently formed cis-diol complexes (Brown and Hu 1994, Shelp 1993, Seresinhe and Oertli 1991). It has been reported that B uptake by plants is presumed to occur via passive absorption in the form of boric acid (H3BO3) and that B taken up by plants forms B complexes very rapidly in the cell walls and cytoplasm; however, the formation of B complexes in the plant decreases the concentration of boric acid in the cell walls, which increases the plant's uptake of B from solution, and the concentration of B in plant tissues may increase excessively due to the concentration of free boric acid from solution (Hu and Brown 1997).

B uptake by plants is primarily influenced by the B concentration of the medium from which B is taken up and the transpiration capacity of plants (Marschner 1995). The B uptake potential of plants varies among plant species growing in the same soil. Generally, B requirements of plants may reflect the marked differences of plant species. Studies have reported that differences in B uptake among plant species are due to differences in membrane permeability, the amount of B complex formation inside and outside the root, the amount of organic compounds that determine B complex formation, and some currently unidentified mechanisms (Hu and Brown 1997).

As a result of experimental and field studies conducted by researchers, it was found that B uptake varies greatly among plant species, even in plants grown under similar environmental conditions (Hu and Brown 1997). In a study exemplifying the large differences in B uptake among plant species and even among cultivars of the same species, it was found that the B concentration and total B content in all organs of barley and wheat cultivars grown in water culture to determine the resistance of 5 barley and 6 wheat cultivars to B toxicity were not the same. It was reported that B toxicity resistant varieties always accumulated less B than susceptible varieties (Nable 1988).

It is known that water functions as a carrier in plants and long-distance transport of nutrients takes place in xylem and phloem conduction bundles. Although there is no consensus among researchers about the mobility of boron in the plant, the opinions that its transport in the plant is in the xylem conduction bundles are gaining intensity. The upward transport from the plant roots to the green parts takes place in the non-living cells of the xylem and B transport takes place in the plant depending on the water potential gradient as a result of water losses from the green parts during the day. Initial B transport in the xylem usually occurs towards older leaves (high transpiration zones) that do not require much nutrients (Pate 1975). In contrast, phloem transport within the plant is not transpiration-dependent, but occurs both upward and downward. Moreover, in plant organs that do not experience continuous water loss, such as young leaves, fruits and seeds, most nutrient requirements are met by phloem transport.

Some researchers have attributed the decrease in the upward (acropetal) concentration gradient due to aging of plants to B transport in the xylem and reported that this is in line with water loss in the green parts (Shelp et al. 1987). It has also been determined that a sudden gradient difference in B concentration, even in a single leaf, can cause excessive B accumulation and in the case of a gradient difference, the B concentration in leaves is higher than in phloem sap (Shelp et al. 1996, Marentes et al. 1997; Oertli and Roth 1969, Oertli 1994). In green parts, B deficiency was typically observed in meristematic tissues (terminal buds and young leaves, etc.), while B excess symptoms were usually observed where water leaves the leaf as a result of transpiration, i.e. mostly at the margins of older leaves. This suggests that B is transported within the plant in the xylem (Oertli 1994, Marschner 1995).

In the light of the results obtained from these studies; B is classified as an immobile element in the plant, while some research results reveal the possibility of B mobility in the phloem. For example, in a study showing that boron is not inert in the phloem, a comparison of the relative percentages of all elements by weight to determine the amount of B transported from the phloem of broccoli plants grown under adequate B conditions to the growth organs showed that B was transported to the growth parts of the plant in amounts equal to or greater than elements such as N, P, K, which are classified as mobile in the phloem, and it was suggested that the phloem is more dominant than the xylem in meeting the B requirement in the growth organs of the plant (Shelp 1987).

WHEAT AND BORON CORRELATION

For optimum growth and economic productivity in wheat *(Triticum aestivum L. ssp.)*, Boron must be present in sufficient amounts in the plant. According to significant studies in the literature, the movement of B in the plant is very limited. It is stated that transpiration is effective in the upward transport of boron in the plant. This indicates that boron plants are rather weak and monocotyledons need less boron than dicotyledons (Marschner, 1995).

In order to increase the efficiency of boron utilization, it is very important to determine the stage and time of application according to the critical growth periods in wheat agriculture where B is most needed. Because the amount of B needed for growth periods that vary from plant to plant varies. For example, in cereals such as wheat, rice, rye, maize, millet, oat, barley, bamboo and small grain crops such as canola, the need for B during generative growth periods is more important and necessary than vegetative growth periods (Rerkasem and Jamjod, 1997). It has also been reported that there are large differences in boron sensitivity among plant species as well as among cultivars of the same species and the reason for these differences is that plants are not physiologically affected by B toxicity to the same extent (Paul et al., 1988; Nable, 1988). For example, wheat varieties resistant to B toxicity did not show a significant reduction in yield when grown in soil treated with 150 mg B kg-1, while B-sensitive varieties grown at the same concentration showed severe yield losses. These varieties also showed yield reduction at 25 mg B kg-1 (Paul et al., 1988). Although the response of wheat cultivars to B is different, B application decreases dry weight in most of the cultivars (Barut, 1997).

In some studies on bread and durum wheat seedlings tolerant to B toxicity, toxic levels of B element were measured by physiological parameters such as plant height, % dry matter content and proportional water content and biochemical parameters such as free proline and soluble carbohydrate. In the light of the data obtained, under B toxicity, seedling height of both cultivars decreased and B content increased with increasing B concentration applied. The differences observed in percentage (%) dry matter content and proportional water content were not statistically significant (Başalp, A. et al., 2014).

In studies conducted to determine the effect of different levels of foliar boric acid application on the physical properties of wheat, it was found that boron had a very limited and insignificant effect and no significant correlation could be established between the amount of boric acid application and the physical properties of the plant. In order to observe the effect of boric acid on wheat quality more clearly, it was reported that a positive improvement was achieved in 1000 grain weight, hectoliter weight and flour yield values as expected (Dizlek H., Konuşkan Ö., 2021).

In another study conducted to determine the effect of N, P, K and microelements on infertility in Janak and Sonalika wheat varieties, it was found that the yield was very low and infertility was present, especially in Janak wheat variety without fertilizer, When only N, P, K or N, P, K with farmyard manure were applied, both varieties were beneficial, but when Zn, B and Mo were applied in addition to N, P, K, the most significant effect was found against B in Janak wheat variety and against Mo in Sonalika wheat variety (Ali and Monoranjan 1989).

In another study, as a result of a greenhouse experiment in which wheat plants were grown in a Zn-deficient soil by applying 0, 2.5, 5.0, 7.5 and 10 mg B kg-1 and 0, 10 and 20 mg Zn kg-1; it was reported that the amount of dry matter decreased with the increase of all applied Zn and B levels, increasing B application levels increased B concentrations and uptake in the plant, whereas it decreased with increasing Zn applications. In the same study, it was found that P, K, Cu, Ca, Mg, Fe and Mn uptake decreased with increasing B application levels (Singh et al 1990).

In a study conducted to determine the effect of toxic B concentrations on the development of 7 wheat genotypes at organ and cellular level using root culture technique, it was reported that root elongation and lateral root development of genotypes were different depending on toxic B concentrations, wheat genotypes classified as resistant by field studies had more lateral roots and longer root axis than genotypes classified as sensitive and medium sensitive, but there was no difference between genotypes in axis elongation (Huang and Graham 1990). When the issue was examined at the cellular level, it was reported that genotypes resistant to B toxicity produced more callus than sensitive and medium sensitive genotypes and this difference between genotypes in resistance to toxic B concentrations may be due to the relationship between B and cell membrane permeability.

The amount of boron required by plants is quite small. As with boron deficiency, even a small excess of the required boron has a negative effect on plant growth and development often stops. Considerable work has been done to determine the effect of boron applications on the development of wheat cultivars and boron distribution in the above-ground parts.

Studies in the literature show that cereals are sensitive plants to boron. Wheat tolerates boron up to 2 mg kg-1 in the growing environment and is adversely affected by boron above this dose (Gupta et al., 1985). Monocotyledonous plants such as barley and wheat contain 20-70 mg kg-1 boron (Syworoktin, G.S., 1958). There are large differences in boron sensitivity among plant species as well as among cultivars of the same species, and the reason for these differences is that plants are not physiologically affected by boron toxicity to the same extent (Paull, J.G. et al, 1988; Nable R.O. et al, 1991). While wheat cultivars showing resistance to boron toxicity do not show a significant reduction in yield when grown in soil treated with 150 mg B kg-1, boron-sensitive cultivars grown at the same concentration show severe yield losses and even these cultivars show yield reduction at 25 mg B kg-1 (Paull, J.G. et al., 1988). Although the response of wheat varieties to boron is different, boron application reduces dry weight in most of the varieties (Nable. R.O. et al, 1988; Barut H. et al, 1997).

The reason why cereal species and varieties are affected by B toxicity is attributed to the fact that cereals, especially wheat and barley, are more sensitive to excess B in soil and tissues than other plant species. While B toxicity damage in most crops occurs at the level of 100000 mg/kg B, it can be seen in wheat and barley at the level of 50 mg/kg B (Gupta, 1985; Bergmann, 1992).

In another study conducted to determine the relationship between wheat and boron, it was reported that grain and stalk N, P, K and Ca concentrations increased with pre-wetting application and were highest in IAA application and Na and B concentrations decreased with pre-wetting application in pre-wetting and non-pre-wetting sowings with 0.55, 1.10, 1.60, 3.0 and 4.8 mg B kg-1 and 200 mg kg-1 indole acetic acid (IAA) and 3% Na2SO4 before sowing. In the same study, it was reported that plant height, yield, plant P, K and Ca concentrations decreased with B applications above 1.10 mg B kg-1, while Na and B concentrations increased in the plant, and IAA and Na2SO4 soaking applications reduced the negative effects of B except the highest B applications (Chhipa and Lal 1992).

In another study conducted in a greenhouse environment to determine the relationship between wheat and boron, WL-711 wheat variety was grown with 0, 0.5, 1.0 and 2.0 mg kg-1 B and Mo application before sowing in a sandy loam soil with very low B and Mo content; grain and stem yield increased in applications up to 0.5 mg B kg-1 and 1.0 mg Mo kg-1. In 5 mg B kg-1 and 1.0 mg Mo kg-1 applications, the highest grain yield was obtained in 0.5 mg B kg-1 + 1.0 mg Mo kg-1 application, and when B and Mo were applied together, the highest stem yield was obtained in 1.0 mg B kg-1 + 1.0 mg Mo kg-1 application. In the same study, it was reported that B uptake increased with B applications up to 1.0 mg Mo kg-1 (Singh et al.1992).

POSSIBILITIES OF USE OF LEAF BOR APPLICATIONS IN WHEAT AGRICULTURE

The geographical, ecological, climatic and tapographic structure of the world allows for the cultivation of a high diversity of crops in agricultural production. Wheat production is carried out in many regions of the world and wheat ranks first among field crops in terms of cultivation area and production amount. In our country, which is self-sufficient in terms of wheat production and the diversity of genotypes obtained through breeding, there are some problems in wheat agriculture and wheat quality due to reasons such as unfavorable environmental conditions and the use of chemical inputs. In order to minimize these problems, boron applications, the amount of which has been increasing day by day in recent years, have been emphasized. As a result of the studies, it has been determined that boron has positive effects on reducing Na uptake from soil and increasing K+ uptake, as well as improving the K+ /Na+ ratio in favor of plants. In foliar applications of boron, significant increases were observed in physical traits such as grain yield, tillering, number of grains in spike and thousand grain weight.

In a study where foliar boron application was carried out to determine spike sterility caused by B deficiency in barley and wheat, grain yield was 2913, 906, 1144, 1325, 838, 863 and 1238 kg ha-1 , number of spike per m2 was 290, 272, 290, 312, 312, 312, 312, 304, 314 and 316, respectively, and harvest index was 44% with 1.0 kg B ha-1 soil and 50 g B ha-1 21 (0.005% borax solution) foliar B application. 8%, 19.2%, 22.3%, 22.3%, 22.1%, 22.1%, 17.1%, 16.8% and 20.2%, and the decrease in grain yield and harvest index were statistically significant (Rerkasem and Jamjod 1989).

As a result of a 3-year field trial conducted by growing wheat with 10, 20 and 30 kg B ha-1 borax application together with NPK in an acid soil with a B content of 0.34 mg kg-1; while 0.76 t ha-1 grain yield was obtained with only NPK at the control level, 2.31, 2.65 and 2.33 t ha-1 grain yields were obtained depending on the increase in B levels. In the same study, with 50% sowing, 50% two and four times foliar B application, grain yields were 1.98, 2.11 and 1.94 t ha-1, respectively, and tillering, number of grains per spike and thousand grain weight increased significantly with B application (Mitra and Jana 1991).

In order to determine the effect of B on grain filling physiology in durum wheat (cv. DWL-5023), 5, 10, 20 and 40 mg kg-1 boric acid was applied twice at flowering and 1 week after flowering to determine the effect of B on grain filling physiology; It was reported that boric acid increased invertase and starch synthesis activity in the developing grain and decreased amylase activity compared to the control (water) application, in addition, foliar boric acid application increased the sugar and starch content of the grain compared to the control application and 20 mg kg-1 boric acid application was reported to be the most appropriate level (Sharma et al. 1991).

Studies involving these foliar boron applications have enriched the literature. It is known that the use of chemical fertilizers in wheat agriculture causes significant pollutant emission to the environment. It is thought that the widespread application of important plant nutrients such as B in wheat agriculture will eliminate the negative effects of chemical fertilizers on increasing salinity in the soil, heavy metal accumulation and eutrophication in water.

CONCLUSIONS AND RECOMMENDATIONS

In crop production, not only macronutrients but also micronutrients, which are equally important and functional, should be sufficiently present in the soil or applied as much as the plant needs. However, since nitrogen, phosphorus and potassium fertilizers are given more importance in the world, micro elements and especially Boron (B), which is among them, are not taken into consideration in fertilization. The function of boron in plants is as important as macronutrients such as nitrogen, phosphorus and potassium. For this reason, it is extremely important for plants to find these micronutrients in the environment where they grow, to take them in sufficient quantities and to use them as much as necessary in order to obtain quality and abundant crops.

The fact that wheat is the main source of food in all countries of the world makes wheat a strategic product. Wheat and its by-products provide some of the protein and calories needed in human and animal nutrition. Today, wheat products are diversifying and consumer demands are changing due to changing consumption habits of people and developing technology. The most common forms of wheat consumption are bread, pasta, semolina, biscuits and bulgur. Apart from these products, wheat is also consumed for traditional products, desserts, starch, etc. in the world and in Turkey.

In addition to the elements that are present to a greater or lesser extent in all plants and that ensure their survival, some other elements are needed in minimal quantities. So much so that today much more is needed than just fertilizing cereals. The main factors influencing the technological quality and yield of wheat are the genetic characteristics of the variety and the growing conditions (climate, soil properties, fertilization, agronomic practices). In addition, cereal diseases and pests (such as the common fly and the common gall midge) also affect the quality of wheat both during the vegetation period and during storage (Dizlek and Gül, 2007). Among these factors, the characteristics of the soil in which wheat is grown and, accordingly, the fertilization process and agronomic practices have a very important function in improving wheat quality. Boron deficiency in crop production is the second most important micronutrient in the world after zinc deficiency (Ahmad et al., 2012). In addition, the combination of boron with other plant nutrients often leads to antagonistic effects, making their uptake by plants difficult (Gupta, 1993).

Chemical fertilizers used intensively to increase yields cause pollution of natural resources. The basic condition for ensuring sustainability in agriculture is not to pollute natural resources. For this purpose, in order to prevent pollution caused by chemical fertilizers B is needed for the transport and placement of calcium, which has positive effects such as nucleus formation, wheat grain formation, set and cell division.

Nowadays, since climatic conditions, especially the amount of precipitation, have emerged as a limiting factor in the effect of boron fertilization, it is considered that foliar B application can be used as an alternative method to increase the yield characteristics of wheat varieties when soil B applications are limited by various factors such as soil moisture and climate. In cereal-based countries such as Turkey, determining the responses of cereals, especially wheat varieties, to B and knowing their effects on yield and quality traits will help to increase yield and quality in wheat agriculture in our region. Therefore, soil and foliar B fertilization and dose studies will be beneficial for our country's agriculture.

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The authors declare no conflict of interest.

Authors' Contributions

The authors contributed equally to the preparation of the manuscript.

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Effects on Enzymes of Oxidative Stress, Histopathology and Bioaccumulation in Earthworm *Aporrectodea caliginosa* Induced by TSP Fertilizer*

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ABSTRACT: Intensive use of agrochemicals definitely caused soil pollution. Among soil biota earthworms play a more significant role in soil ecosystem by taking part in organic matter cycle and modifying the soil structure. Earthworms are also used as an indicator species to diagnose environmental pollution in soil ecosystem. The present study was conducted to assess the potential hazards of a Triple superphosphate (TSP) fertilizer, commonly used in agriculture, on adults of *Aporrectodea caliginosa* Savigny, 1826, an abundant species in the region of Tebessa (Northeast Algeria). The fertilizer was tested at two doses (LD_{25}, LD_{50}) under laboratory conditions during a four-week exposure period; biomarkers of oxidative stress were assessed. Furthermore, enzymatic assays revealed a stimulation of the detoxification system traduced by an increase in glutathione peroxidase and lactate dehydrogenase compared to control series.Histological study reveals changes in the intertegumentsand intestinal wall compared to control series. Lastly, TSP fertilizer has been accumulated in earthworm with low bioaccumulation capacity. These obtained results could be useful as a biomarker for the sassessement of contaminated terrestrial ecosystems using *A.caliginosa* as a biomonotor for terrestrial pollution and assessing ecological risks of fertilizers in soil.

Keywords: Fertilizer, Ecosystem engineers, Oxidative stress, Histopathological effects, Bioaccumulation

INTRODUCTION

Earthworms are important organisms in soil communities and are known for sustaining the life of the soil (Miglani and Bisht, 2019). Because of the capacity to accumulate and concentrate large quantities of inorganic and organic pollutants, earthworm species are widely used as a model organism in environmental risk assessment of chemicals and soil toxicology (Peijnenburg and Vijver, 2009). The importance of earthworms to test the potential adverse effects of chemicals on soil organisms has been recognized by various environmental organizations and resulted in a set of standard test guidelines involving earthworm species (OECD, 1984, 2004 ; ISO, 2005). Earthworms are continuously exposured to soil chemicals through their alimentary surfaces and skins. Thus, they are dependent on efficient detoxification systems for their survival (Maity et al., 2008). The use of biomarkers is becoming increasingly important in the evaluation of the effects of contaminants in earthworms. Histopathological responses in earthworms have also been reported as valuable markers of toxicity in previous studies

(Amaral et al., 2006 ; Giovanetti et al., 2010 ; Kiliç, 2011). The term ‘‘biomarker’’ or ‘‘biomarker response’’ often refers to cellular, biochemical, molecular, or physiological changes that are measured in cells, body fluids, tissues, or organs within an organism and are indicative of xenobiotic exposure and/or effect (Lam and Gray, 2003).

Few researchers also determined the negative effects of chemical fertilizers on earthworms (Bhattacharya and Sahu, 2014). Our objective was to assess the effects of a fertilizer (TSP), widely used in agriculture, against *Aporrectodea caliginosa* an abundant earthworm species in Tebessa area (Northeast Algeria) (Bouazdia and Habes, 2017) and used as bioindicator of soil contamination. Thus, the fertilizer was tested on biomakers of oxidative stress (GPx and LDH). The histopathological effects of TSP and its bioaccumulation in the earthworm tissue were also determined.

MATERIAL AND METHOD

Earthworm collection

Earthworm populations were sampled at the end of autumn. Population samples of the endogenic adult’s earthworm’s *A. calliginosa* . were collected by hand from the region of El Merdja, located at 4.5 Km northeast of the city of Tebessa (northeastern Algeria). 40 cm-depth block of soil was excavated and earthworms were hand-sorted according to the method of Bouché (1972). After collection, the earthworms were cultured for 7 days of adaptation (Mekahlia et al., 2016) at their native soil. The experiment was maintained at 20 ± 2% soil moisture and 25 ± 2°C soil temperature.

Fertilizer and treatment

Triple superphosphate (*TSP) an* inorganic fertilizer was supplied from Agricultural Services Directorate of Tebessa area.The studies carried out in plastic containers of 20 × 10 × 7 cm (length × width × depth), were set up each with 500 gm of native soil. Before the initiation of the exposure test, earthworms were rinsed in tap water and moved to Petri dishes on moist filter paper to void their gut contents.

Two doses of the dry powder (500 and 600 mg) corresponding to LD_{25} and LD_{50} obtained in the adult earthworm (Halaimia, 2022), were applied. These were added to the soil surface and then mixed thoroughly with enough water to ensure a homogeneous mixture. For the control treatment, distilled water was used.

Biomarker assays

Glutathione peroxidase assay (GPx)

The same sample preparation protocol was followed for the biomarkers assay. GPx assay was performed according to Flohe and Gunzler (1984). Fragments of the body (50 mg) of control and treated earthworm (LD_{25} and LD_{50}) were homogenized in 1 ml of phosphate buffer (pH 7.8). The homogenate was centrifuged (3000 rpm for 10 min), and the supernatant was recovered as an enzyme source. The assay was performed on an aliquot of 200 µl of supernatant added to 400 µl of the GSH solution (0.2 mM, pH 10). Absorbance reading was performed after 5 minutes at 412 nm. Data were expressed in µM per min per mg of protein and assays conducted with 3 replicates per treatment.

Lactate dehydrogenase assay (LDH)

The determination was based on the conversion of lactate to pyruvate or pyruvate to lactate. The assay of LDH was conducted according to the method of Hill and Levi (1954) as previously described (Halaimia *et al.*, 2021) using NAD (nicotinamide adenine dinucleotide) as substrate. Fragments of the body (50 mg) of control and treated earthworm (LD_{25} and LD_{50}) were homogenized in 1 ml of Tris/HCl (0.1 M, pH 7.2). The homogenate was centrifuged (3000 rpm for 5 min) and then the supernatant recovered for use as enzyme source. The assay was performed with 50 µl of supernatant added to 675 µl of substrate buffer (0.2 M, pH 10) and 50 µl of NAD solution. The absorbance reading was done every minute for 5 min at 340 nm. Data was expressed in µM per min per mg of protein and assays conducted with 3 replicates per treatment.

Histological procedure

The histological procedure was performed according to the method of Hould and de Shawinigan (1984). In brief, earthworms from each control and treated series (D2) were randomly collected at 4weeks after treatment. Each whole-body earthworm was fixed in formalin solutions (30%) for 24 h, and dehydrated in baths of alcohol with increasing concentrations (70, 80, 90 and 95%). Then, specimens were cleared in xylene and embedded in paraffin wax. Serial sections (3 µm) were prepared by a Leitz microtome and stained using the hematoxylin and eosin. Sections were then mounted and covered with glass cover. Histological sections were examined under the light microscope (Leica DM LB2).

X-ray fluorescence spectrometry Analysis of residues

The analyses were carried out at the Research Center for Physico-Chemical analysis (CRAPC) in Bou-Ismail (Tipaza, Algeria). The control and treated earthworms were selected and lyophilized by a Christ model dryer (50 hours). Once these samples have been ground using an electric shredder "Fox Pulverisette 2", we proceed to the formation of 32 mm pellet with a "SPECAC" Pelleter. Then the obtained pellet was analysed by X-ray fluorescence spectrometry.

Statistical analysis

Data are presented as a mean value ± SEM (standard error mean) in each treatment group. The normality of data was verified using the Kolmogorov-Smirnov test, and the homogeneity of variances was checked by Levene's test. Comparison of the experimental groups was tested by analysis of variance (ANOVA), and means were tested for statistical significance by a post hoc Tukey's honestly significant difference test. The statistical tests were performed using GraphPad Prism, version 7.00 (GraphPad Software, San Diego, CA, USA), where $p<0.05$ indicates a statistically significant difference.

RESULTS AND DISCUSSION

Effects on Biomarkers

Results of the specific activities of enzymes of oxidative stress (GPx, and LDH) in *A. caliginosa* are summarized in Figures 1 A, B.

Results of the specific activity of GPx in *A. caliginosa* exposed to two doses of TSP fertilizer showed a significant increase in GPx activity at the second week ($F_{2,6}$=12,63 ; p=0.0071) and the fourth week ($F_{2,6}$=7,581 ; p=0.0228) with two tested doses as compared to control series.

Our results showed a significant increase in LDH activity in the treated series with the highest dose at the fourth week ($F_{2,6}$=7,064 ; p=0,0265) as compared to control series.

Recently, the use of biomarkers to estimate the exposure or resulting effects of chemicals has received considerable attention (Dawood et al., 2017). The use of biomarkers in earthworms is progressively relevant for assessing the impact of pesticides on the soil organism (Tiwari et al., 2016). Various classes of enzymes are used as biomarkers in earthworms because of their crucial role in neurocholinergic transmission and cellular homeostasis to prevent the toxic action of chemicals (Sanchez-Hernandez, 2006; Novais et al., 2011; Mekahlia et al., 2015). Exposure of earthworms to soil contaminants leads to an immediate increase in reactive oxygen species (ROS); which include a series of anionic radicals superoxides (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$) (Zhang et al., 2014). The ROS are short-lived chemical species containing unpaired electrons, formed by the partial reduction of molecular oxygen (Moloney and Cotter, 2018). These substances are highly reactive and can attack various types of biomolecules in their vicinity, which is known as oxidative stress (Tsikas, 2017). In order to neutralize oxidative damage, a complex antioxidant defense system evolves in organisms by involving enzymatic and non-enzymatic elements of recovery and trapping of ROS (Wang et al., 2021).

Glutathione peroxidase (GPx) known as the most important peroxidase ensures the detoxification of peroxide and hydroperoxides to water and hydroxyl compounds, respectively (Pinto *et al.*, 2003) so it play a protective role against oxidative stress (Van Der Oost et al., 2003). Our results showed a significant effect in GPx activity in *A. caliginosa* earthworm adult exposed to TSP fertilizer. In contrast, no effect induced by NPK fertilizer in *A.caliginosa* adults (Halaimia, 2022). However, a marked induction in GPx activity was found in *E. vermiculata* snail exposed to Weatfert fertilizer (Attia et al., 2021), in *A. caliginosa* earthworm juvenile exposed to NPK (Halaimia et al., 2021) and in *Biomphalaria alexandrina* snails treated with high nitrogen fertilizer treatment (El-Deeb et al., 2017).

LDH is involved in the metabolism of carbohydrates in cells and plays a key role in maintaining the balance between the catabolism and anabolism of carbohydrates (Chen et al., 2001). It has also been used as an indicator of stress exposure (Diamantino et al., 2001). The results showed significant effect in LDH activity after treatment with TSP fertilizer. Such elevation of enzyme activity could be a result of the increased synthesis of particular enzymes to defend against xenobiotics stress and to increase the sources of energy production through the breakdown of energy-rich nucleotides and amino acids (Mosleh et al., 2003). A significant increase in the activity of LDH was observed in *Deroceras reticulatum* exposed to Caselio fertilizer (Abd-El Azeem and Sheir, 2018). In addition, a significant increase has been reported in the LDH activity in the juvenile earthworm, *A. calliginosa* exposed to NPK (Halaimia et al., 2021) and land snails (*Eobania vermiculata*) exposed to methomyl (Khalil, 2016)

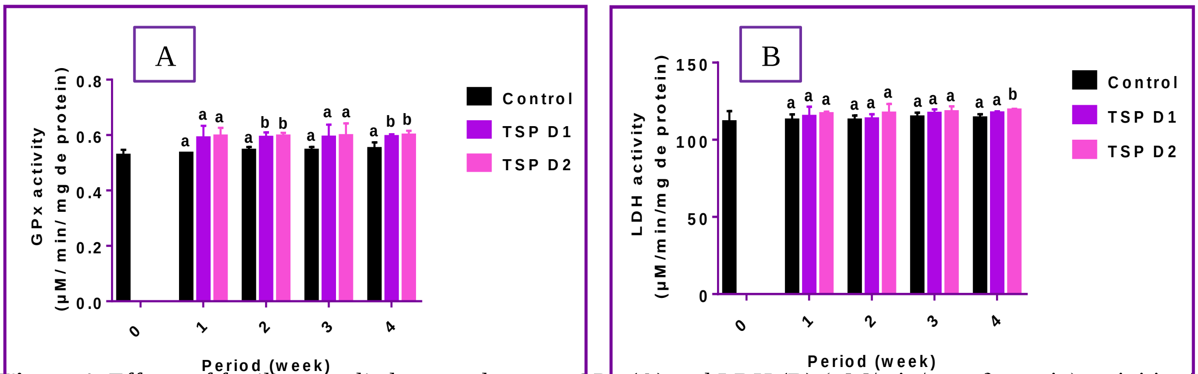

Figure 1. Effects of fertilizer applied at two doses on GPx **(A)** and LDH **(B)** (µM/min/mg of protein) activities in adult of *A. caliginosa* during the exposure period (mean ± SEM, n = 3 pools each containing 50 mg of fresh tissue). The different lowercase letters indicate significant differences at the same time based on Tukey's HSD test (p

<0.05).

Effect on histological structure

A histological section of the control and treated group (4 weeks of treatment) is illustrated in the Figures 2, 3, 4, 5 and 6. The results obtained show that TSP fertilizer affects the integuments and the intestinal wall by revealing tears in the epithelium and lysis of epithelial cells (Figs 3.B, 4.B) with destruction of the circular muscle and detachment between the longitudinal muscle fibers (Fig 2.B, 3.B, 4.B). In addition, we noticed the appearance of an irregularly shaped epidermis with destruction of the longitudinal muscles (Fig 4.B). With regard to the intestinal wall; the alterations caused fusion of the intestinal folds, narrowing and destruction of the intestinal wall with some detachment in the chloragogen tissue (Figs 5.B, 6.B) compared to controls, which shows toxicity induced by exposure to chemical fertilizer.

Histopathological analysis are short-term reactions to sublethal stresses that have been utilized as useful techniques to assess the toxicity of pollutants (Oluah et al., 2010). The histological changes observed in an organism after treatment depend on its ability to repair the lesion, the nature and duration of exposure (Haschek and Rousseaux, 1998). Previous investigations have noted earthworm histopathological reactions as useful indicators of toxicity studies (Giovanetti *et al.*, 2010; Kiliç, 2011; Lourenço *et al.*, 2011).

In the present study, extensive alterations were observed in the tissues of the body wall and intestinal tract of the earthworm exposed to TSP fertilizer. Thus, exposure to imidacloprid at different concentrations induce histological alteration in *A. caliginosa*, *L. terrestris* and *E. fetida* (Dittbrenner et al., 2011). It is also clear that the morphological, histological, and biochemical changes of *E. eugeniae* could be used as toxicity indicators (Samal et al., 2019). Earthworm (*Pheretima elongata*) intestine examined histopathologically and histochemically after exposure to the herbicide glyphosate revealed necrosis and disruption of cell membranes (Morowati, 2000). *Nsukkadrilus mbae* earthworm treated with Atrazine (herbicide) show histopathological manifestations which include a damage to the chloragogenous layer, harm to the epithelial tissues, glandular expansion of the epithelial tissues, prominent vacoulations, and pyknotic cells (Oluah et al., 2010). According to El-Deeb et al. (2015), inorganic fertilizers (high phosphorus and high nitrogen content) caused histological changes in the digestive glands of *Biomphalaria alexandrina* snails.

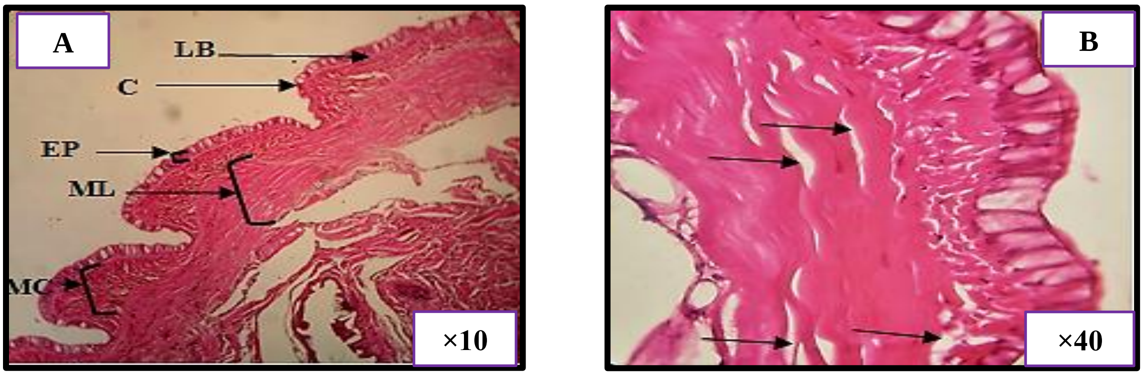

Figure 2. Longitudinal sections in the teguments of the anterior part of adult earthworm **(A),** control earthworm; **(B)**, treated earthworm (D2) (after 4weeks of exposure to TSP). Cuticule **(C)**; Epithélium **(EP)**; Circulaire muscle **(CM)**; Longitudinal muscle **(LM)**.

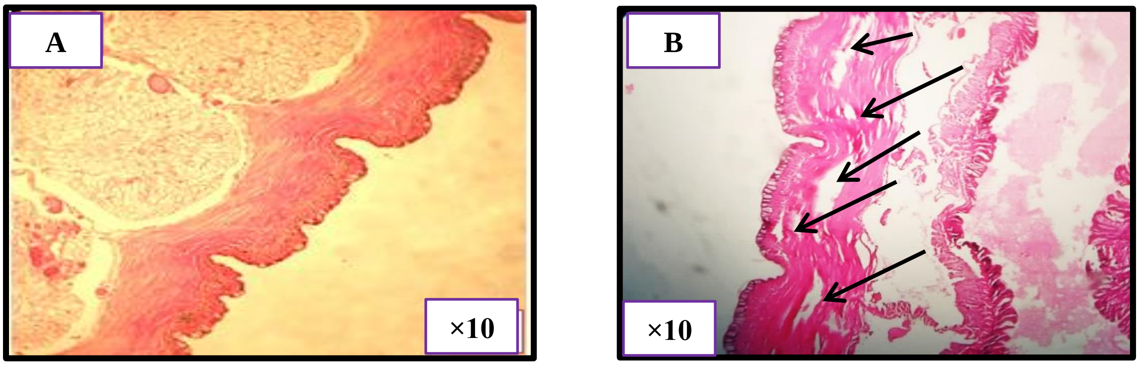

Figure 3. Longitudinal sections in the teguments of the medium part of adult earthworm **(A)**, control earthworm; **(B)**, treated earthworm (D2) (after 4weeks of exposure to TSP). Epithélium **(EP)**; Circulaire muscle **(CM)**; Longitudinal muscle **(LM).**

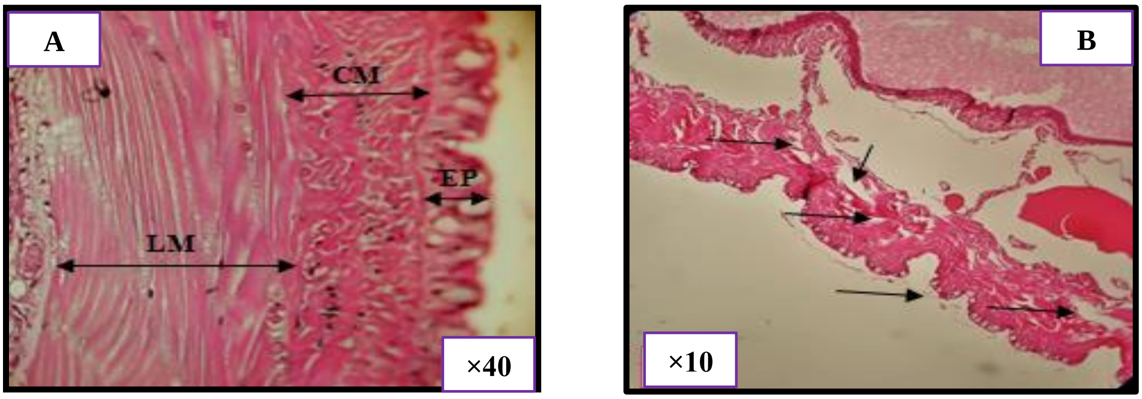

Figure 4. Longitudinal sections in the teguments of the posterior part of adult earthworm **(A),** control earthworm; **(B)**, treated earthworm (D2) (after 4weeks of exposure to TSP). Epithélium **(EP)**; Circulaire muscle **(CM)**; Longitudinal muscle **(LM)**.

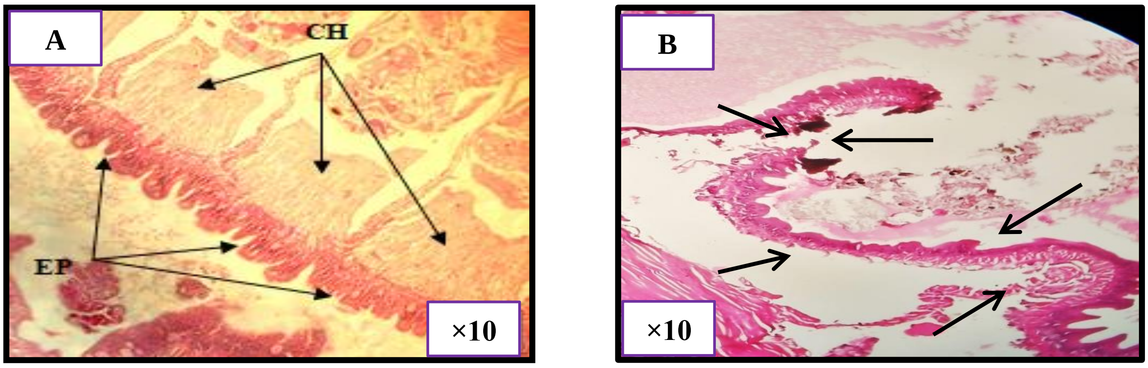

Figure 5. Longitudinal sections in the intestinal wall of the medium part of adult earthworm **(A)**, control earthworm; **(B)**, treated earthworm (D2) (after 4weeks of exposure to TSP). Intestinal epithelium **(EP)**; chloragogen tissue **(CH)**.

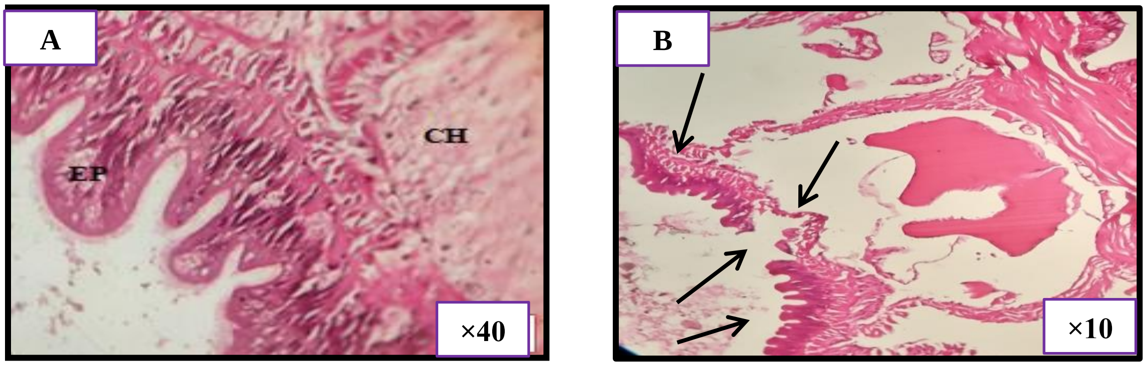

Figure 6. Longitudinal sections of the intestinal wall of the posterior part of adult earthworm **(A),** control earthworm; **(B)**, treated earthworm (D2) (after 4weeks of exposure to TSP). Intestinal epithelium **(EP);** chloragogen tissue **(CH).**

Detection of residues

The detection of residue of fertilizer by X-ray fluorescence spectrometry (XRF), showed an accumulation of TSP fertilizer in the treated earthworm tissue with the highest dose (dose 2) after 4 weeks of exposition (Table 1).

The simple fertilizer TSP consists of phosphate (P_2O_5), calcium (Ca) and some trace elements. The results obtained by the XRF technique, show the mass percentages of the elements and their oxides in the tissues of adult earthworms control and treated indicate an increase in phosphate mass percentages in treated compared to controls

Regarding calcium, the results show an increase in the mass percentages of this element and its oxide (CaO) in the treated compared to controls. Conversely, for iron, the results indicate a decrease in its percentage in treated compared to controls. The percentages of manganese (Mn), zinc (Zn) and their oxides show a slight increase in the treated compared to controls. A stability of the percentages of copper (Cu) and its oxide was noted. The results show that the elements arsenic (As) and lead (Bp) and their oxides As_2O_3 and PbO are absent in the tissues of the controls and present in those of the treated.

Our study showed an accumulation of the major elements and oxydes of TSP in the earthworm tissue. Relevant studies have reported that earthworms can absorb pollutants through the epidermal and intestinal tract (Hirano and Tamae, 2011; Huang et al., 2017; Zhu et al., 2021).

Earthworms constitute about 60-80% of soil biomass, accumulate and transfer most soil pollutants to other animals in the food chain (Chevillot et al., 2017). The tegument of earthworms is in direct contact with contaminated soil and is considered an important way of absorption of toxic substances (Reddy and Rao, 2008). Earthworms generally absorb contaminants by passive diffusion via the epidermis from interstitial soil water and intestinal absorption after oral ingestion of soil particles (Wang et al., 2014). The contribution of these two pathways depends on ecological strategies, in particular trophic behavior. Bioaccumulation of xenobiotics in Eisenia fetida earthworms increased with increasing level of exposure (Chen et al., 2017).

Table 1. The mass percentage (%) of bioaccumulation of major elements and oxides of TSP fertilizer in the earthworm tissue.

Elements	Mass Percentage (%)		Oxides	Mass Percentage (%)	
	Control	Treated (TSP)		Control	Treated (TSP)
C	24,6884	24,5762	CO_2	90,4605	90,0494
N	1,5295	1,8697	/	/	/
Na	0,2513	0,2279	Na_2O	0,3388	0,3072
Mg	0,1261	0,1486	MgO	0,2091	0,2464
Al	0,5766	0,3357	Al_2O_3	1,0894	0,6342
Si	1,0261	0,9066	SiO_2	2,195	1,9394
P	0,3851	0,4313	P_2O_5	0,8823	0,9883
S	0,3846	0,317	SO_3	0,8854	0,7915
Cl	0,2058	0,1705	/	/	/
K	0,3472	0,3768	K_2O	0,4183	0,4538
Ca	1,0686	1,6679	CaO	1,4951	2,3338
Ti	0,0193	0,0133	TiO_2	0,0321	0,0221
Cr	0,0009	0,0009	Cr_2O_3	0,0014	0,0013
Mn	0,0013	0,002	MnO	0,0017	0,0025
Fe	0,1674	0,1174	Fe_2O_3	0,2393	0,1678
Co	0,0003	0,0002	Co_2O_3	0,0004	0,0002
Ni	0,0003	0,0004	NiO	0,0004	0,0005
Cu	0,0003	0,0003	CuO	0,0004	0,0004
Zn	0,0063	0,0111	ZnO	0,0078	0,0139
As	/	0,0001	As_2O_3	/	0,0002
Se	0,0005	0,0003	SeO_2	0,0008	0,0004
Br	0,001	0,0012	/	/	/
Sr	0,0046	0,0039	SrO	0,0054	0,0046
Y	0,0001	0,0002	Y_2O_3	0,0001	0,0002
Pb	/	0,0005	PbO	/	0,0005

CONCLUSION

The data obtained show significant effects in enzymatic activity of the soft tissues of *A. calliginosa* adult earthworm's exposed to a fertilizer (TSP). The fertilizer tested led to activation of the enzymes of oxidative stress was evidenced by an increase in the activity of GPx and LDH. Lastly, structural alteration of were observed in treated earthworms. This finding could be useful as a biomarker for the evaluation of contaminated terrestrial ecosystems and the possibility of using *A. calliginosa* as a biomonitor for terrestrial pollution.

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Statement of Conflict of Interest

The authors have no conflicts of interest to declare.

Authors' Contributions

Samira HALAIMIA: Data curation, Writing, Methodology, Investigation, Formal analysis. Samir TINE, Fouzia TINE-DJEBBAR and Noureddine SOLTANI: Methodology, Investigation, Conceptualization, read and approved the article.

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Exotic plants in Ufa lemonary

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ABSTRACT: In the Educational and Experimental farm of the Ufa Forestry Technical College (Ufa Lemonary), the main cultivated crops are Citrus limon varieties Tashkent and Jubilee, which were planted in a greenhouse in 1990 with annual seedlings. Since 2009, the varieties C. limon Urman, Salavat, Leysan and Citrus medica of Uraltau and Zilya of Bashkir selection have been described and entered by the State Export Commission of the Russian Federation in the State Register of Breeding Achievements of the Russian Federation. To date, more than 600 species of plants are cultivated in lemonaria, among which there are representatives of the flora of the subtropics and tropics. Special attention is paid to fruit and medicinal species, whose representatives are in the greatest demand and are widely used in gardening and medicine. All collection plants are regularly monitored. Throughout the year, the lemonarium is a unique base for conducting excursions, theoretical, experimental and research work, practical classes on vegetative reproduction of forms and varieties of the citrus collection, rare and endangered species of woody-shrubby and herbaceous vegetation, forest-seeding and planting material cultivation for students of higher and secondary educational institutions of the city. The results of many years of research show the success of the introduction of tropical and subtropical crops in the conditions of the Southern Urals.

Keywords: Citrus fruits, Year-round greenhouse, Southern Urals, Tropical crops, Subtropical crops

INTRODUCTION

The educational and experimental farm of the Ufa Forestry Technical College (Ufa Lemonary) was established on the initiative of the Ministry of Forestry of the Bashkir ASSR in 1990, with an area of 3.9 hectares, consists of a year-round greenhouse with an area of 1 hectare and an open-ground ornamental nursery in Ufa (Fig.1-2).

Figure 1. Greenhouse inside

Figure 2. Type of greenhouse in winter

It is located on the bank of the Belaya River within the Pribelsky uvalisto-undulating plain. The climate in the area of the greenhouse intended for the laying of a lemon plantation is sharply continental, dominated by south, south-west, north and north-west winds. The average annual air temperature is +3.8 ° C, the amount of precipitation is 589 mm. Winter comes in mid-November and lasts until mid-April. The coldest month is January. During this period, the average air temperature is -12.4 ° C, often falls below -30 ° C. Snow cover is established in November and lasts an average of 155 days. The warmest month is July (+19.7°C). In some years, there are droughts. In such external climatic conditions, difficulties arise in growing any plants indoors, especially citrus fruits. Ufa Lemonary is an experimental plantation for growing lemons in closed ground conditions. The average annual yield is more than 20 tons of fruits. The main cultivated crop is Citrus limon varieties Tashkent and Jubilee, which were planted in a greenhouse in 1990 with annual seedlings. Since 2009, the varieties C. limon Urman, Salavat, Leysan and Citrus medica of Uraltau and Zilya of Bashkir selection have been described and entered by the State Export Commission of the Russian Federation in the State Register of Breeding Achievements of the Russian Federation (Sadykova,2012; Bilalova et al. 2020).

MATERIAL AND METOD

The collection of the farm is being replenished. To date, more than 600 species of plants are cultivated in lemonary, among which there are representatives of the flora of the subtropics and tropics belonging to the families *Acanthaceae, Actinidiaceae, Aizoaceae, Alstroemeriaceae, Amaranthaceae, Amaryllidaceae, Anacardiaceae, Annonaceae, Apocynaceae, Araceae, Araliaceae, Araucariaceae, Arecaceae, Asclepiadaceae, Asparagaceae, Asphodelaceae, Aspleniaceae,* Asteraceae, *Balsaminaceae, Begoniaceae, Berberidaceae, Bignoniaceae, Bromeliaceae, Cactaceae, Caprifoliaceae, Caricaceae, Caryophyllaceae, Celastraceae, Commelinaceae, Convolvulaceae, Costaceae, Crassulaceae, Cupresaceae, Cyperaceae, Davalliaceae, Droseraceae, Ericaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Gesneriaceae, Ginkgoaceae, Hydrangeaceae, lamiaceae, Lauraceae, Malvaceae, Maranthaceae, Melastomataceae, Meliaceae, Myrsinaceae, Myrtaceae, Musaceae, Nepenthaceae, Nyctaginaceae, Oleaceae, Orchidaceae, Oxalidaceae, Pandanaceae, Passifloraceae, Piperaceae, Poaceae, Podocarpaceae, Polygonaceae, Polypodiaceae, Portulacaceae, Pteridaceae, Punicaceae, Rhamnaceae, Rosaceae, Rubiaceae, Rutaceae, Sapindaceae, Solanaceae, Sterculiaceae, Strelitziaceae, Urticaceae, Verbenaceae, Vitaceae, Zamiaceae, Zingiberaceae.*

RESULTS AND DISCUSSION

In order to preserve the diversity of the collection, special attention is paid to fruit and medicinal species, whose representatives are in the greatest demand and are widely used in gardening and medicine (Table 1).

Among the diversity of cultures, plants listed in the Red Book of the Russian Federation, the Red Book of the Republic of Bashkortostan occupy a separate place.

All collection plants are regularly monitored. In order to sell seedlings to plant lovers and for educational purposes, their reproduction is regularly carried out.

Long-term observations have shown that the most important factors in growing citrus crops indoors in atypical conditions are the creation of optimal air temperature, soil and air humidity, light and proper agrotechnical measures for the care of plants all year round. The average air temperature in the greenhouse of the Ufa lemonarium during the heating season from October to May is maintained at an average of +200C, preventing a drop in temperature below +10-120C. In the summer, vents along the roof ridge of the greenhouse are used for ventilation, for the pritenka, the roof is whitewashed. Humidity is maintained in the range of 70-80%.

Table 1. Characteristics of the main fruit-bearing crops of the Ufa lemonary (2023)

Name of the species and variety	Age of plants, years	Average height of plants in a greenhouse, m	Weight of one fruit in the conditions of Lemonary , gr.
Citrus limon (L.) Osbeck Tashkent	33	3,8	110,0-170,0
Citrus limon (L.) Osbeck Jubilee	33	3,9	338,0-550,0
Citrus limon (L.) Osbeck Laysan	33	4,0	268,0-350,0
Citrus limon (L.) Osbeck Urman	33	3,5	270,0-330,0
Citrus limon (L.) Osbeck Salavat	33	4,5	550,0-890,0
Citrus unshiu Marcow.	20	2,8	60,0-85,0
Citrus paradisi Macfad.	33	4,5	106,0-125,0
Citrus sinensis (L.) Osb.	50	4,5	120,0-180,0
Citrus medica L. Pavlovsky	40	4,5	250,0-310,0
Citrus medica L. Uraltau	33	4,3	450,0-660,0
Citrus medica L. Zilya	33	3,7	230,0-300,0
Ficus carica L.	40	4,5	15,0-40,0
Eriobotriya japonica (Thunb.) Lindl.	25	4,3	10,0-30,0
Punica granatum L. nana	25	2,5	12,0-50,0
Annona squamosa L.	10	1,8	96,0-276,0
Psidium littorale Raddi	21	2,5	11,0-40,0
Coffea arabica L.	24	3,5	0,1-0,3
Feijoa sellowiana (O.Berg) Burret)	22	3,0	15,0-42,0
Monstera deliciosa Lieb.	29	3,5	367,0-734,0
Carica papaya L.	10	4,0	192,0-4500,0
Ananas comosus Mill	15	0,5	450,0-520,0
Musa × paradisiaca L.	22	4,0	12,0-22,0

Throughout the year, the lemonarium is a unique base for carrying out theoretical, experimental and research work, practical classes on vegetative reproduction of forms and varieties of the citrus collection, rare and endangered species of woody and shrubby and herbaceous vegetation, forest seed business and planting material cultivation for students of higher and secondary educational institutions of the city. Every year the number of visitors who want to see heat-loving plants increases. On average, more than 35,000 people from the Republic of Bashkortostan and beyond visit the greenhouse every year. Holding exhibitions, thematic excursions for children and adults, events on environmental education and career guidance of the younger generation is one of the activities of the Educational and Experimental farm of the Ufa Forestry Technical School.

CONCLUSIONS AND RECOMMENDATIONS

Fertility is monitored in the Republican Forest Soil and Chemical Laboratory of the Ministry of Forestry of the Republic of Bashkortostan, which is guided by methodological guidelines, GOST standards adopted in agrochemistry and soil science. Agrochemical examination of the closed ground is carried out in accordance with the "Brief methodology of soil and agrochemical examination of forest nurseries". In the samples selected for analysis, the content of humus, mineral forms of nitrogen (ammonium and nitrate), mobile forms of phosphorus, pH of acidity, exchangeable potassium and other agrochemical indicators of soils are determined to monitor fertility and develop norms for the application of basic fertilizers.

In lemonary, various methods of reproduction of all cultivated plants are used (by grafts, cuttings and layering). The method of green cuttings is widely used in the economy, which gives high results. Experiments are being conducted on inoculation and re-pollination of crops.

Currently, the farm is working on clonal micro-reproduction of *C. limon* and *C. medica* varieties of Bashkir and Uzbek breeding.

Thus, the data obtained show the success of the introduction of tropical and subtropical crops in the conditions of the Southern Urals.

Statement of Conflict of Interest

The authors have no conflict of interest.

Authors' Contributions

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Seaweed as A New Diet to Contribute to The Nature and Nutritional Deficiencies in Turkiye*

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ABSTRACT: Nearly 22% of Turkish people suffer from mineral deficiency. With meat consumption, these deficiencies have been tried to be eliminated. This study suggests that seaweed can be a suitable food source to reduce mineral and protein deficiencies in the Turkish diet while providing ecological benefits such as reducing carbon emissions, heavy metal release and transforming animal waste into nutrition in the seas. Mineral values, ecological characteristics, and distribution areas were analyzed by selecting three species naturally distributed on the Aegean and Mediterranean coasts. These analyses were compared to the species and production processes in the current seaweed farms produced in Turkiye. In addition, the religious, social, and economic reasons why Turks do not prefer to consume seaweed were examined through interviews and literature reviews. As a result, regions for ideal seaweed species and the location for the production in Turkiye were selected, and the main reason for Turk to not consume seaweed is unfamiliarity. Additionally, we could find out that the seaweed diet is not regulating any religious rule. With this study, the mineral deficiency in Turkiye can be eliminated, and the pollution rates in the Aegean and Mediterranean Seas are expected to be reduced.

Keywords: Seaweed, Nutrition diet, Water safety, Heavy metal pollution, Mineral deficiency, Sustainable food source

INTRODUCTION

A mineral is a solid chemical compound with a particular crystal structure that occurs naturally in its pure form, and people must consume minerals daily (Pettijohn, 1972). Unfortunately, 25% of the world's population suffers from a lack of minerals and is the same for the Turkiye population. According to the results of the TBSA-2010 study, the most common mineral deficiencies in Turkiye are calcium (70.2%) and zinc (44.9%). These mineral deficiencies can lead to various health problems, such as weak bones, fatigue, or a reduced immune system.

This fact shows that the traditional Turkish diet is insufficient regarding mineral intake (T.R. Ministry of Health Report, 2019). Mineral intake in Turkiye is mainly obtained from meat and dairy products. These ingredients are limited because they do not cost- and are environmentally efficient. Meat production consumes 1500 times more water than green vegetables (Mekonnen, 2010). If Turkiyre increases the amount of meat consumed as a mineral and protein source depending on the population, it will continue to consume natural resources.

Seaweeds with no root, stems, or leaves can get their minerals directly from the surrounding water (Maine seaweed council report, 2022). In 2012, humans directly consumed 40% of global seaweed production (FAO, 2022; Loureiro, *et al.* 2015). Seaweed contains seven times more calcium, twice as much iodine, six times more magnesium, three times more iron, and twice as much zinc than vegetables (USDA Food Composition Database, 2023).

While the incidence of diseases related to mineral deficiency is 10-20% in countries that consume the most seaweed in the world (Japan, South Korea, China, and Indonesia), it is 22% in Turkiye. These Asian countries consume at least five grams of seaweed daily.

It aims to define the religious, social, and economic difficulties for its adoption as a new diet in the regions where it is cultivated according to the seaweed species and farms in Turkiye.

MATERIAL AND METHOD

The naturally occurring species in Turkiye were identified using the Ocean Biodiversity Information System (OBIS) mapping. Species in Turkiye were evaluated over the overlap areas by entering the region and seaweed parameters (OBIS mapping, 2023). The densities in the regions where the species are found are colored between zero to eight, and red is the most intense region, while yellow is the least intense. The Pessarrodona (2022) mapping method shows the detected regions and their densities.

Farms in the Aegean and Mediterranean Seas producing seaweed in Turkiye were identified. The nutritional value, production methods, and environmental contributions of the seaweed product in these farms are listed. The seaweed consumer group was selected by interviewing the farms. Accordingly, the mineral deficiency of the production in the determined farms and the effect on the marine ecosystem are explained.

Challenges in society have been identified to increase seaweed consumption in Turkiye. In interviews with 20 people aged 0-18, 18-30, 30-60, and over 60, they will be asked whether they would prefer the three species in Turkiye as they first came out of the sea and the image of a recipe in Asian cuisine. Then, the benefits of the species will be explained, the images will be shown again, and their preferences will be asked. In these interviews, it was determined whether people preferred seaweed after understanding its benefits.

RESULTS AND DISCUSSION

Ecological Characteristics of Seaweed Species in Turkiye

Nearly 12,000 seaweed species have been described worldwide (over 7,000 red algae; more than 2,000 brown algae; some 1,500 greens; and perhaps 1,500 blue-greens). With an annual production of 27.3 million tons in 2014 and a growth rate of 8% year−1, seaweed aquaculture now comprises 27% of total marine aquaculture production (Duaerte, 2017). The Mediterranean Sea has the highest salinity and temperature in the Turkiye Seas. In addition to the native flora and fauna of the Mediterranean, there are also species of Atlantic and Indo-Pacific origin. The Aegean Sea contains many gulfs, coves, peninsulas, and capes on the coast and different habitats. The Aegean Sea and its islands host micro-habitats dominated by seagrass and brown algae. In Turkiye, it is the natural distribution area of different taxonomic types of seaweed from these three groups. In the Mediterranean, 1,124 seaweed species have been reported, of which at least 20% are endemic, and the most common is *Rhodophyta* (Figueroa, *et al*. 2014). The natural distribution areas of the species distributed in Turkiye were selected. *Ulva lactuca* (green seaweed), *Sargassum polycystum* (brown seaweed), and *Rhodophyta* (red seaweed) have been identified as edible seaweed species that occur naturally in Turkiye (Figure 1).

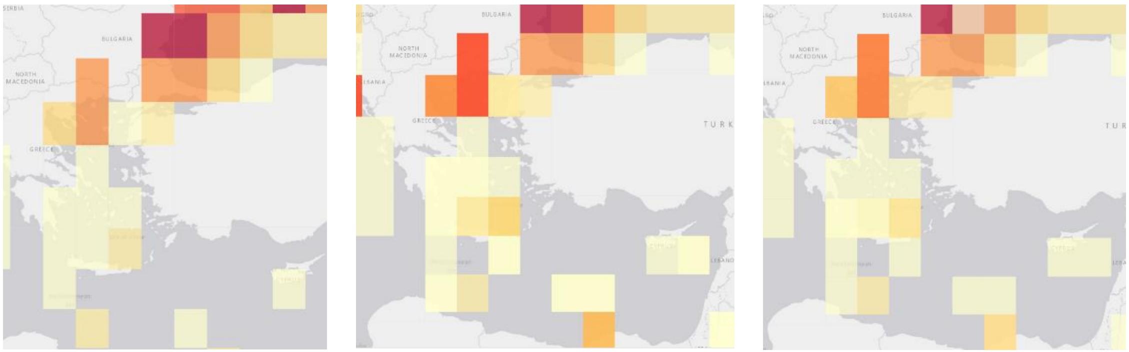

Figure 1. Green, red and brown seaweed distribution in Aegean and Meditterenean Sea in Turkiye (OBIS mapping, 2023).

Ulva lactuca is flat edible green seaweed, and its common name is sea lettuce. It is considered one of the most useful seaweeds, with applications that include its use in food, agriculture, pharmacology, and medicine. When dried in the sun, the color of seaweed can change from green to black. This makes it challenging to eat when dry. *Ulva lactuca* grows naturally worldwide, with high sun needs, and can grow at depths of up to 75 meters (Linnaeus, 1753). In Turkiye, it is found in low density (two-three) on the Aegean coasts of Izmir and Aydın. *Ulva lactuca* contains high amounts of cobalamin or Vitamin B12, which plays a powerful role in the brain and nervous system. The protein concentration of *U. lactuca* can represent up to 33% of the dry mass of the plant. Nutritionally, it contains Na (351 mg/ 100gr), K (209 mg/100gr.), Ca (180 mg /100gr), Fe (34 mg/100gr) and Zn (1,78 mg/100gr). It also has high levels of other microelements (Debbarma, 2016; Bikker, *et al.*, 2016) (Figure 2).

Figure 2.*Ulva lactuca* (Krisp, 2022)

Sargassum polycystum is edible brown seaweed, and its common name is rough-stemmed sargassum. *S. polycystum* is found at depths of 0-63 meters and in tropical climates (Prud'homme van Reine, 2016) (Figure 3). It grows on the Aegean and Mediterranean coasts of Turkiye, especially on the Izmir shores. These species can be harvested in 3-4 months in warm waters. The nutritional content of the *S. polycystum* in terms of fiber and carbohydrate makes it a nutritive supplement which makes it an important human food resource. In addition, *S. polycystum* is used as fertilizer, human food, fodder and medicine; also because it contains an auxin-like substance, to control heavy metal (Pb, Cd) pollution (Trono, 2001). Sodium (389 mg 100/g DW) was detected as the most abundant element in the *S. polycystum*, followed by K (244 mg 100/g DW), Ca (176 mg 100/g DW), Fe (32 mg/100gr DW) and Zn (5.8 mg 100/g DW) (Debbarma, 2016). The total essential amino acids content in *S. polycystum* measured 37.28%, where the highest essential amino acid was noted to be leucine and the most limiting was methionine, followed by tyrosine.

Figure 3. *Sargassum polycystum* (Low, 2020)

Rhodophyta is edible red seaweed. These inhabit a range of aquatic environments, mostly marine. Other niches include freshwater habitats and hot water springs. In the Mediterranean Sea, Rhodophyta distribution is high (Figure 4). *Rhodophyta* are also farmed and harvested for use in food and gels. These seaweeds play a major role as carbon sync along with the synthesis of many fatty acids and metabolites (Wehr, 2014). In comparison with other seaweeds, red seaweed contains a high level of calcium, sodium, and potassium, although significantly lower levels of zinc and iron. Among the three most mineral-rich red algae species (*C. crispus*, *P. palmata*, *and Gracilaria spp*.), *Gracilaria spp*. is distributed along the Aegean and Marmara coasts of Turkiye. *Gracilaria edulis* or *Gracilaria verrucosa* are the natural distributed species. *G.edulis* contains Na (423 mg/100gr), K (282 mg/100gr), Ca (180 mg/100gr), Fe (65 mg/100gr), and Zn (1.7mg/100gr)(Debbarma, 2016).

Figure 4. *Gracilaria edulis (Moody, 2007)*

While these three species are common in the deep of İzmir-Muğla in Turkiye, they also grow in warm, medium-depth (40-75 m) waters with low pollution in terms of ecological characteristics. The mineral and protein values of these three algae species differ. In terms of mineral values, *Rhodophyta* spp. first place is followed by *U. lactuca* and then *S. polycytum*. The average harvest time of these species is between 40-65 days (Sipahutar, *et al.*, 2019). They can show an average of 2-10 cm elongation per day. In recent years, the amount of phosphate has increased due to increased human activities and sea transportation in the Aegean and Mediterranean. This causes eutrophication, algae multiply rapidly, limited light transmission, organic matter accumulates at the bottom, insufficient oxygen, and fish die. Changes are seen in parameters such as odor, taste, and color in seawater. Seaweeds also help prevent this pollution (TUDAV, 2018).

Seaweed Producers and Their Effects on Consumption

Currently, seaweed producers in Turkiye have been analyzed, and production is carried out as aquaculture, which is not offshore production. Because, especially in the Mediterranean and Aegean Seas, it has been found that growing seaweed as a food is considered undesirable by companies due to the 4-10% increase in heavy metal levels. However, no legal restriction is in this regard (Akkaya, 2021). Heavy metal poisoning is caused by the accumulation of toxic amounts of metals in the body due to exposure. Heavy metals, including mercury, chromium and zinc, are fatal to humans even with exposure to just a small quantity, leading to serious illness such as cancer (Um, 2022). Most edible seaweeds have been reported to contain safe amounts of heavy metals (Leung, 2021). In Turkiye, all three seaweed species have high heavy metal absorption. Cultivation in the sea also reduces metal pollution in the water (González Fernández*, et. al.* 2023; Wael, *et. al.*, 2016; Filippini, *et. al.*, 2021) . The aquaculture farm in Muğla Seydikemer district, Turkiye's largest producer, produces 400 kg of seaweed monthly as supplementary food. The interview with the company official stated that 95% of the seaweed is sold in powder and tablet form in Turkiye, while 5% is imported. However, in the marine production of seaweed, the seaweeds take all their nutrients from the water and can clean it. Marine seaweed production farms are located close to fish farms, especially since the wastes of sea animals are a natural fertilizer for seaweed. For this reason, our research selected potential marine points by comparing the distribution areas of local seaweed species in Turkiye with marine fish farms.

One solution to reduce the quantity of heavy metals in water is adsorption, the absorbing of contaminants on the water's surface using adsorbents. To make this adsorption technique more "low-cost" and "environmentally friendly", a group of Korean scientists recently suggested the use of a type of seaweed known as kelp, or kombu, as a substance that can effectively absorb contaminants in water (Um, 2022). In addition, unused seaweed produced in seaweed farms can be reused in the sea to absorb heavy metals.

The Religious, Social, And Economic Difficulties For its Adoption As A New Diet in Turkiye

According to Kurokura (2004), seaweeds and shellfish have special religious meanings in Japanese culture. These foods were believed to provide strength and protection. Seaweed, which started to be mass-produced as food in the 17th century in East Asian countries, increased its consumption as food by 40% after the start of its trade. The seaweed diet habit in Asia is said to be directly related to the economy. This is due to the seaweed diversity, sustainable harvest and ecosystem availability of these countries. Dietary situations in Japan for the 20th century were studied in four periods. The period up to 1950 is the period of poverty, the period of reconstruction between 1950 and 60, the period of high economic growth between 1960-75, and the period of low economic growth after 1975 (Zava, 2011). Especially before 1935, diets were based on steamed rice. 21% of dishes in Japanese food culture contain seaweed. Between 1993 and 2016, calcium and iodine intakes in Japan increased 20 and 4 times (Ohta, 2016).

Dietary change in Asian cultures has often been accompanied by increased food diversity due to economic growth. In Turkiye, there have been periods of economic depression after 1960, 1980-1990, and 2020. Considering meat consumption in these periods, while it was 28 kg per person in 1980, it decreased to 16.6 kg in 1998, 14 kg in 2017, and 7 kg in 2022 (TÜİK, 2022). Vegetable consumption, on the other hand, has reduced by 10% over the years. An alternative recommendation should be made to meat consumption for adequate protein and mineral intake in Turkiye. However, this dietary change has some religious, social, and economic difficulties.

Religious difficulties

Seafood consumption in Turkiye is a religious prejudice in society. However, although seaweed grows naturally in Turkiye, the consumption of seaweed is in the form of Spirulina powder (seaweed powder). Spirulina, which is widely consumed as a powder, is preferred because it is not consumed fresh. On the other hand, there is a well-known proverb about seafood consumption in Turkiye which is "If my father comes out of the sea, I will eat" - I eat everything when I'm hungry- but in fact, with this statement, Imam Shafi (182) said, "everything from the sea is halal" (TDI, 2021). It has been observed that a proverb, which society misunderstands, affects consumption. It is not thought that religion directly affects seaweed consumption in Turkiye. People should know that they can freely eat seaweed due to their religion. People are also prejudiced against seaweed, as there is a prejudice against food exported from abroad in society. Those who do not know the Spirulina process can easily use this powder. But they do not prefer to eat fresh.

Social difficulties

Food has a great importance in social life in Turkiye. Turkish cuisine has a wide variety of meat, vegetable and legume recipes due to its geography. Sea cowpea was selected when recipes or flavors similar to seaweed were examined. Salicornia europaea (Sea cowpea) is a trendy dish in Turkish cuisine. The sea cowpea is a plant that grows on the seashore between 0-20 m in the Aegean Sea (BörülceGen, 2021). They are similar in color, texture, and taste to sea cowpea and Ulva latuca. Seaweeds produced in Turkiye are not sold fresh in the markets. However, sea cowpea is both cultivated and sold fresh in markets. The public can consume the product that has access. Seaweed is marketed as a powder in Turkiye.

Environmental difficulties

Seaweed cultivation in Asia is characterized by a series of long-line techniques with vertical droppers; similar breeding techniques have been successfully tested in Europe (Peteiro and Freire, 2009, 2011, 2012, 2013b; Sanderson et al., 2012; Handa et al., 2013; Marinho et al., 2015; Peteiro et al., 2016). Production can be carried out more effectively with the long-line technique in Turkey. In addition, seaweed cultivation, which has great market power in Asia, is carried out cheaply. However, technological modifications will be required to reduce labor costs in production in Turkey (Edwards & Watson, 2011).

The environmental conditions required to grow different types of seaweed are variable (Kerrison et al., 2015). In general, seaweed production requires areas with adequate nutrients and light for growth, salinity, and temperatures not limited by the species grown. U. lactuca, S. polycytum, and Rhodophyta species, which grow naturally in parts of the Aegean, Mediterranean, and Southern Marmara, grow in environments with different light, temperature, and salinity. For this reason, each species should be grown in seas suitable for its natural range.

Current production is largely limited to periods (mainly autumn and spring) when light and nutrients are sufficient to allow growth. High contamination levels with increased human activities during the summer can cause crop quality problems (Campbell, 2019).

The production of biologically matched hydrodynamic models should be considered a top priority in producing countries to support risk assessment, understand the carrying capacity of water bodies, and select suitable sites that minimize adverse environmental changes.

CONCLUSION

Seaweed consumption is important in Turkey for eliminating protein and mineral deficiencies, reducing heavy metal content in the seas where it is produced, increasing light transmission, and reducing the accumulation of organic matter at the bottom. However, the religious, social, and ecological reasons why Turks do not prefer seaweed are often cited. While Turks are not inherently biased against seaweed consumption, they are more familiar with consuming seaweed in the form of powder, which is a popular product in Turkey. Therefore, increasing consumption could be achieved by introducing different forms of seaweed to the domestic market. Additionally, Turkey is a multicultural country, and regional eating habits vary. While the consumption of herbs, flowers, and local vegetables is high in the Mediterranean and Aegean Seas, meat products are more commonly consumed in the Eastern Anatolia region. As such, the areas selected for seaweed production should be based on the consumer group.

If it is widely accepted that eating seaweed is not haram (forbidden) in terms of religion, and that it has numerous health benefits, it is predicted that consumption will increase. Socially, the adoption of seaweed into Turkish cuisine could be facilitated by developing recipes that are similar to existing dishes. Possible ecological benefits of seaweed production and consumption in Turkey include supporting marine biodiversity, increasing oxygen levels, reducing carbon emissions, absorbing animal waste, and trapping polluting materials by algae. Furthermore, seaweed could provide ecological support to other fish and aquaculture farms in the region. This study will focus on the farms that will be established to increase seaweed consumption in Turkiye in the future and the nutritional value levels of people in the future.

Authors' Contributions

Ceylan Sözer designed and analyzed the research, also studies arranged. Ikhwan Kim contributed the writing of the article.

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Introduction of *in silico* Toxicity Assessment Tools and Comparison of Their Capabilities for Two Common Agrochemicals

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ABSTRACT: Thousands of agrochemicals are being utilized in the global market, which poses a challenge to traditional ecotoxicity testing methodologies for in vivo tests, which are time-consuming, costly, and dependent on huge numbers of animals. Through computational modeling and algorithm prediction using toxicity data, *in silico* toxicity aims to forecast chemical toxicity. It is regarded as one of the substitutes for animal testing since it makes use of a range of computational methods to link an agrochemical's structure to its toxicity. In this study, firstly, current information on the usage areas of such software and databases, such as SwissADME, OSIRIS Property Explorer, SwissTargetPrediction, ProTox-II, T.E.S.T. and VEGA and the differences between them are presented. Since such databases collect data from many sources and have different decision algorithms, they may produce different results for the same analytes. In order to compare predicted doses from models with *in vivo* results, two compounds, glyphosate (GLY: systemic herbicide) and chlorpyrifos (CPF: organophosphate insecticide) were selected as case studies. Acute oral toxicity with prediction models for LC_{50} of *P.promelas* and *D.magna* were analyzed and compared with the data provided by ECHA. Compared to the experimental values given in the ECHA reports, for chlorpyrifos, ProTox-II (6.25%) in acute toxicity and T.E.S.T. (76.56% and 745%) in LC_{50} of fish and water flea calculated the most reliable results, while for glyphosate VEGA (2.46%, N/A and 330.68%) calculated the closest results. These findings indicate that much more computational toxicology studies are required to ensure the gradual replacement of *in vivo* testing.

Keywords: Agrochemicals, chemical toxicity, glyphosate, chlorpyrifos, bioinformatics

INTRODUCTION

Hundreds of genomes have been sequenced and kept for public research at GenBank since the sequencing of the first complete microbial genome of *Haemophilus influenzae* in 1995. Genes and genomes were followed by the collection of protein sequences and structural information. It is becoming impossible to manage the enormous amount of data produced by all those sequencing initiatives. The need for efficient data processing has been silently filled by bioinformatics. We now know more about the structure of the genome and proteome thanks to bioinformatics research. Since more than ten years ago, bioinformatics has become a crucial area of science that is facilitating biological discoveries. It is simply difficult to collect, manage, analyze, and understand the enormous volumes of data that are available, especially following whole genome sequencing studies, without the use of bioinformatics tools. For the agricultural community, sequencing the genomes and proteomes of plants and animals has huge advantages. To find the genes and proteins and to clarify their functions, bioinformatics technologies can be applied. The quality of the crops could then be improved by using this precise genetic or proteomic knowledge to generate stronger, drought, disease, and pest resistant crops. It assists with insect resistance, bettering nutritional quality, rational plant improvement, climate change studies, creating drought-resistant crop varieties, waste cleanup, and finally, the evaluation of new agrochemicals from an ecotoxicology perspective (Sharma, 2021).

While the process of creating new compounds requires years of expensive research and development, the chemical industry faces significant difficulties due to safety concerns, particularly those related to cardiac and liver toxicity (Arora, Kumar, Srivastava, Garg, & Singh, 2022). It is crucial to identify the toxicological level at which exposed organisms cannot survive. In 2007 (European Commission, 2006), the European Union's REACH legislation (1907/2006) went into effect. REACH stands for Registration, Evaluation, Authorization, and Restriction of Chemicals. The main objectives of REACH are to protect human health and the environment from potential hazards posed by chemicals and to make the EU chemicals industry more competitive. When chemicals are manufactured or imported into Europe in quantities greater than one tonne per year, REACH restricts their usage in order to accomplish these goals (“REACH Legislation - ECHA,” n.d.). Chemical producers and importers in the European Union (EU) are required to submit an application reports to the European Chemicals Agency (ECHA, www.echa.europa.eu) in order to register these substances and transmit the information required to ensure their appropriate use.

Conventional ecotoxicity testing techniques for in vivo experiments, which are laborious, costly, and reliant on numerous animal subjects (Figure 1) (“Animal Welfare - Chemical Testing on Animals | RSPCA,” n.d.), encounter difficulties due to the continuously rising number of chemicals, with more than 140,000 chemicals currently used in the global market. In order to do this, the creation of predictive toxicity tests and models has turned into a strategic issue for these businesses. As a result, it is important to take an integrated approach to knowledge-based data sources as well as *in vitro* and *in vivo* experimental data.

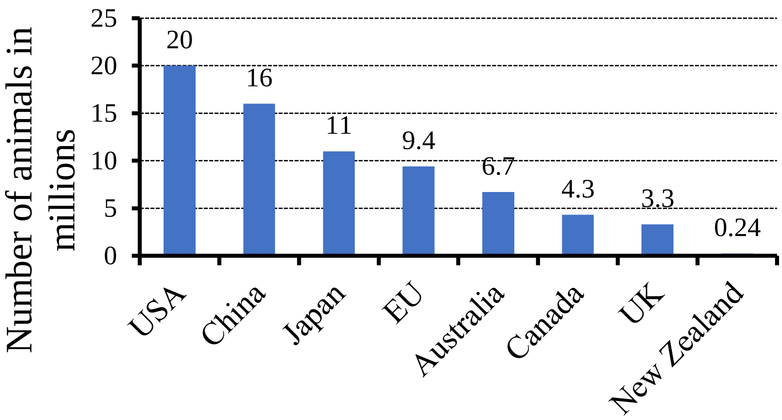

Figure 1. By of 2020, the annual global number of animals utilized for testing and research (source: The Royal Society for the Prevention of Cruelty to Animals)

Computerized toxicity predictions have advanced to new levels as the volume and variety of biological data have grown over the years (Table 1). Regulatory agencies have approved only a small number of in vitro assays, however if *in silico* models are used in accordance with OECD guidelines, they are totally permissible.

Table 1. The advantages of conventional animal testing (in vivo) and alternative predictive toxicology (in silico) approaches. (Gozalbes & Vicente de Julián-Ortiz, 2018)

	Rapidity	**Economy**	**Correlations with human adverse effects**	**Ethical/social acceptance**	**Regulatory legal acceptance**	**Industrial regulatory use**
in silico	high	high	medium	high	high	low / no
in vitro	medium	medium	medium	high	medium	medium
in vivo	low / no	low / no	high	low / no	medium	high

Through computational modeling, QSARs, and algorithmic prediction using toxicity data, *in silico* toxicity aims to forecast chemical toxicity. It is regarded as one of the alternatives to animal testing since, in general, it makes use of a range of computational approaches to link a chemical's structure to its toxicity or efficacy (Medina-Franco & Saldívar-González, 2020). To better comprehend and predict chemical activity and safety-related endpoints, biological data such as gene expression and cell shape can be employed. Quantitative and qualitative predictions of the toxicity of chemicals, mixtures, nanomaterials, and other compounds are now available thanks to computational approaches.

The adoption of one or more computational toxicology approaches depends on the complexity of the type of toxicity being examined and the baseline data that is available to us:

- the use of molecular coupling ("docking") to examine how chemicals interact with biological receptors
- the systematic examination of structure-activity connections based on prior information and experience
- structural similarity characteristics' extrapolation
- physiology-based pharmacokinetic research
- the creation and use of mathematical QSAR prediction models

The Quantitative Structure-Toxicity Relationships (QSTR) is sometimes used in place of QSAR when the goal of the study is to simulate a toxicity-related parameter. There are numerous QSTR models that can be used to estimate various toxicological parameters of chemicals, whether they are involved with the influence on human health or the environment (Silva, 2020). Overall, published models can be divided into groups based on the toxicity parameters that need to be assessed:

i. *Systemic Toxicity in Humans* (mostly forecasts of acute toxicity, reproductive toxicity, mutagenicity, and carcinogenicity)

ii. *Localized Toxicity in Humans* (prediction of phototoxicity, skin and eye irritation, respiratory sensitization, and other adverse effects)

iii. *Environmental Distribution* (the persistence, diffusion, and bioaccumulation of compounds that have hazardous effects on the environment, as well as their final fate, can all be predicted)

iv. *Ecotoxicity* (predictions of harmful effects on plants, animals, and birds, as well as aquatic and terrestrial organisms)

Numerous ecotoxicity assessment tools have been developed as a result of the cost-benefit advantages of *in silico* methodologies and regulatory backing for them:

(1) T.E.S.T.: Using QSAR techniques, the Toxicity Estimation Software Tool (TEST) assesses the toxicity, notably mutagenicity. The EPA created this program to calculate the acute toxicity based solely on the chemical compound's structure. It employs several QSAR methodologies and includes a variety of prediction models, such as the fathead minnow LC_{50} or *Daphnia magna*, oral LD_{50} in rats, BCF, and Ames test for mutagenicity and developmental toxicity. It also includes models for forecasting certain physical characteristics, such as melting point, viscosity, density, and boiling point.

(2) ToxTree: Toxtree calculates different hazardous risks using structural guidelines. It is a technology that organizes chemical compounds into categories and, using decision trees, forecasts various hazardous effects. Ames test, carcinogenicity, biodegradation and persistence, irritability of the skin, eyes, *in vivo*, and *in vitro* mutagenicity, and DNA binding protein binding are a few of the models that are accessible.

(3) VEGA: Five endpoints, including developmental toxicity, skin sensitization, mutagenicity, carcinogenicity, and the bioconcentration factor, are predicted by VEGA using either rule-based or statistically-based models. The EU supported the CAESAR project, which had the express purpose of creating QSAR models that were compliant with the REACH regulations. For five very relevant REACH properties; i.e. BCF, skin sensitization, carcinogenicity, mutagenicity, and developmental toxicity, five predictive models have been put into place. These models were created in accordance with OECD criteria and tested for predictability using a range of statistical techniques and external validation sets. Currently, the VEGA platform has the CAESAR tools installed.

There are some other free softwares available for ecotoxicological assessments. Depending on the types of calculations you want to perform, **ChemProp** has a variety of modules. The approaches used are primarily based on the 2D chemical structure. Although **CORAL-SEA** offers a straightforward user interface and can produce regression models or binary classifications, it has the drawback of being too slow for sets of compounds with an abnormally high number of chemical structures (up to 5,000). The OECD's official initiative, **QSAR-Toolbox**, makes it easier to comply with REACH regulations. It is primarily distinguished by the ability to classify molecules according to how structurally similar they are, enabling the use of previous experimental data to close knowledge gaps. **Lazar** is a web interface that uses the chemical structure to provide various toxicity predictions. It produces a logical report with these predictions, the application domain, and validation findings. Using a variety of computational techniques, such as docking and QSAR, **Virtual ToxLab** predicts probable toxicity associated with 16 proteins that are known to have negative effects.

The quantity and caliber of data available for the building of computational models is a crucial component for a strong predictive efficacy. The interlinkages between these data, which may come from *in vivo* or *in vitro* experiments, must be taken into account. The application of a bigger effort to utilise existing data should be understood as the requirement for more data rather than additional animal testing in order to expand model coverage. Chemical databases contain data on the composition, toxicity, physical characteristics, and environmental effects of chemicals.

MATERIAL AND METHOD

Numerous predictive models for aquatic toxicity are available, but more research is still needed to determine how accurate *in silico* tools are for determining priorities and assessing risks. For the purpose of contrasting the estimated doses from CompTox models with the outcomes of *in vivo*, two compounds, chlorpyrifos (CPF: organophosphate insecticide) and glyphosate (GLY: systemic herbicide), were selected as case studies.

CPF (Figure 2), an insecticide used extensively in agriculture and as a biocide to control pests that are not agricultural, is a member of the class of pesticides known as organophosphates. More than 88 nations approved the use of CPF goods in 2008. Following the risk assessment conducted by the European Food Safety Authority, the European Union denied the renewal of the approval of CPF for use as the active substance in plant protection products in 2019. Its import in Turkey was terminated on 08.04.2016 and its production was terminated on 30.04.2016. As of 21.05.2020, its use was terminated and it was included in the list of banned agrochemicals.

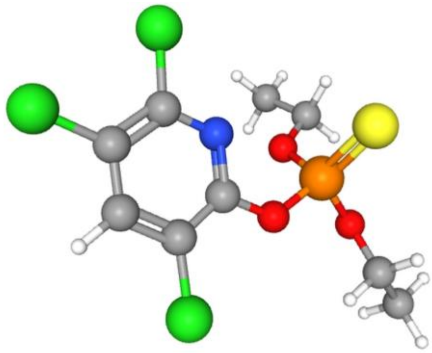

Figure 2. The 3D structure of chlorpyrifos (source: https://pubchem.ncbi.nlm.nih.gov)

In order to control broadleaf weeds and grasses, a non-selective herbicide GLY is frequently employed (Figure 3). Since 1974, it has been approved for use as a pesticide in the US. GLY was approved for use in the EU until 15 December 2022. The five-year approval was granted by the European Commission (EC) in 2017, following separate assessments by EFSA and the ECHA. The approval was extended in December 2022 for one year until 15 December 2023. This agrochemical is still in use in Turkey.

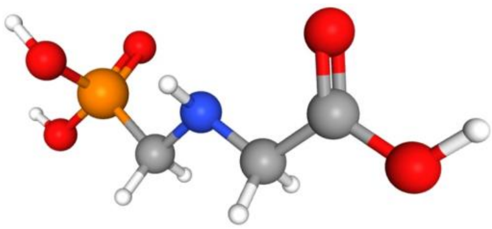

Figure 3. The 3D structure of glyphosate (source: https://pubchem.ncbi.nlm.nih.gov)

ADME/T analyses and ecotoxicological evaluations of these two agrochemicals were carried out with different software. Canonical SMILES expressions of the substances were obtained in the first stage to be used in these programmes:

Chlorpyrifos: CCOP(=S)(OCC)OC1=NC(=C(C=C1Cl)Cl)Cl

[Computed by OEChem 2.3.0 (PubChem release 2021.05.07)]

Glyphosate: C(C(=O)O)NCP(=O)(O)O

[Computed by OEChem 2.3.0 (PubChem release 2021.10.14)]

All software used either these expressions directly, or 3D .sdf exyension files derived from these expressions with the Online SMILES Translator and Structure File Generator (at https://cactus.nci.nih.gov/translate/ supported by NCI/CADD Group).

The ADME/Tox properties of these substances were firstly evaluated with software frequently used in pharmaceutical research such as SwissADME (Daina, Michielin, & Zoete, 2017) and OSIRIS Property Explorer (Sander, Freyss, Von Korff, Reich, & Rufener, 2009) and some important parameters were calculated to establish reference points and to show their toxic properties. In addition, these results were visualised with Boiled-Egg and radar plots. Although these types of software contain working logics that are similar to ChemTox software, since the aim of the study was to compare the parametric values such as EC_{50}, LC_{50}, LD_{50}, which were previously obtained experimentally for model organisms with those obtained as a result of in silico calculations, the analyses were continued with software that can perform these calculations.

ProTox-II online software and T.E.S.T, VEGA, ToxTree free software analyses were performed for GLY and CPF. In particular, 3 numerical data were tried to be obtained and compared with each other. These values are simply the lethal doses on water flea (*Daphnia magna*) and fathead minnow (*Pimephales promelas*), which are model organisms frequently used in aquatic ecotoxicology assessments, i.e. monitoring and evaluation of the toxic effects of such substances in aquatic ecosystems. The third is the acute oral toxicity value obtained and calculated from studies with the rat, which is one of the most common test animals.

The results of all these computational predictions were compared with the values stated in the latest official and valid reports of ECHA and it was tried to determine which software provides more successful results, in other words, results with highest reliability.

RESULTS AND DISCUSSION

ADME/T properties of the tested agrochemicals

CPF passed the drug compatibility test in all six parameters in SwissADME analyses and was estimated to be able to passively pass through the gastrointestinal tract. It is also reported to be unable to be removed from the central nervous system by P-glycoproteins (Figure 4). Halo-pyridine group and phosphor in its structure causes structural alerts in Brenk evaluation; and, the synthetic accessibility score was stated as 3.31 which is medium in the scale of 1 (very easy)-10 (very difficult). QSPR model created a Log K_p value of -4.92 cm/s defining the skin permeability of this substance. OSIRIS Property

Explorer analysis revealed that this compound is highly mutagenic, tumorigenic, irritant and very harmful on reproductive system. The calculated druglikeness is -9.37 and the overall drugscore is 0.03.

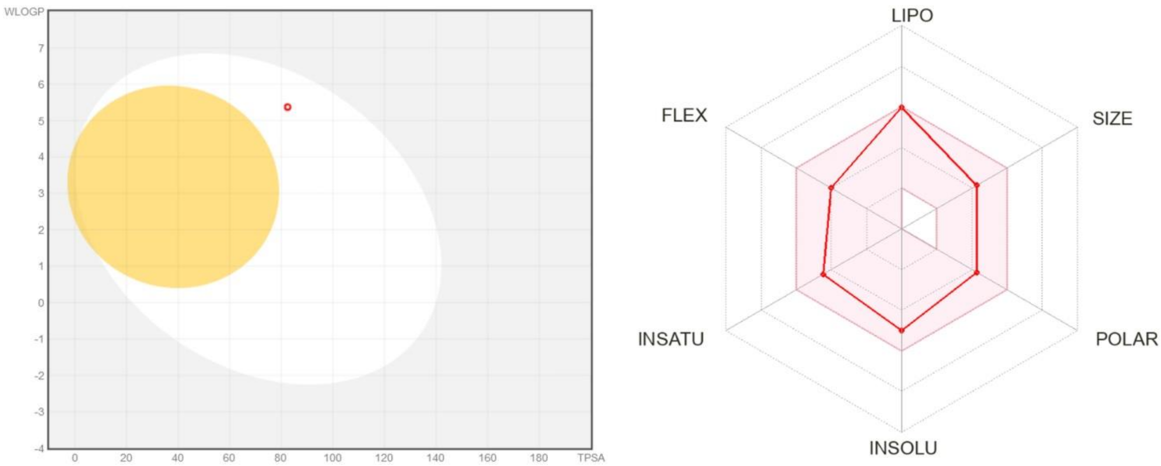

Figure 4. The boiled-EGG and radar reports of chlorpyrifos presented by SwissADME

GLY passed the drug compatibility test more successfully in SwissADME analyses in all six parameters, and was estimated to be able to passively pass through the gastrointestinal tract. However, it would be appropriate to add that this feature is weaker than that of CPF. It was also reported that, like the other one, it could not be removed from the central nervous system by P-glycoproteins (Figure 5). The phosphor group caused structural alert in Brenk evaluation and the synthetic accessibility score was stated as 3.31 pointing "easy" in the aspects of size and complexity penalties. QSPR model resulted a Log K_p value of -9.75 cm/s defining the skin permeability of GLY. Unlike CPF, GLY is predicted to be highly soluble in aquatic environment. OSIRIS Property Explorer analysis revealed that this compound is highly mutagenic, tumorigenic and very harmful on reproductive system, however, there is no indication of irritating effects found. The calculated druglikeness is -15.02 and the overall drugscore is 0.11.

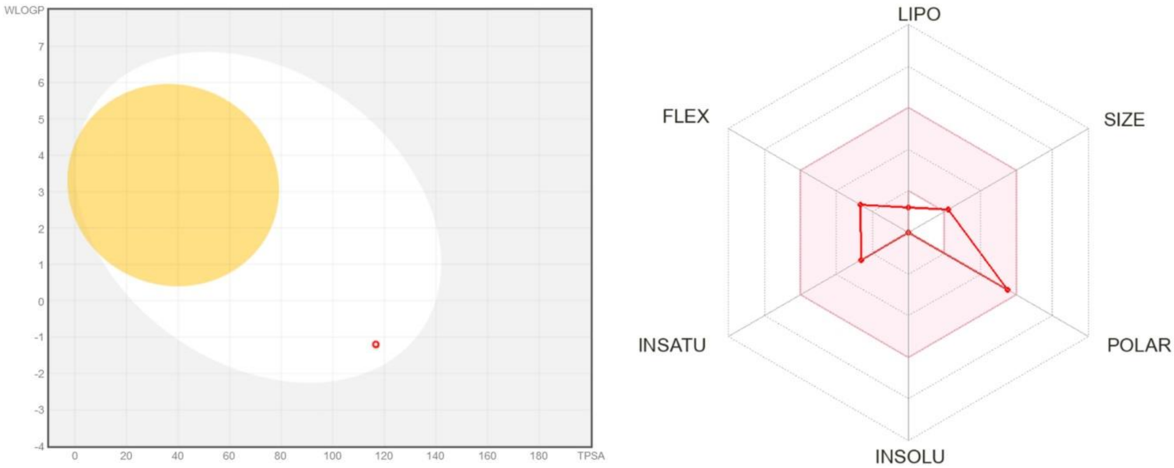

Figure 5. The boiled-EGG and radar reports of glyphosate presented by SwissADME

Analyses with SwissTargetPrediction (Daina, Michielin, & Zoete, 2019) software summarised the possible target proteins for CPF and GLY as separate piecharts (Figure 6). The report generated for the top15 protein targets can be interpreted as indicating the toxic effects of these substances as possible enzyme, lyase and protease inhibitors.

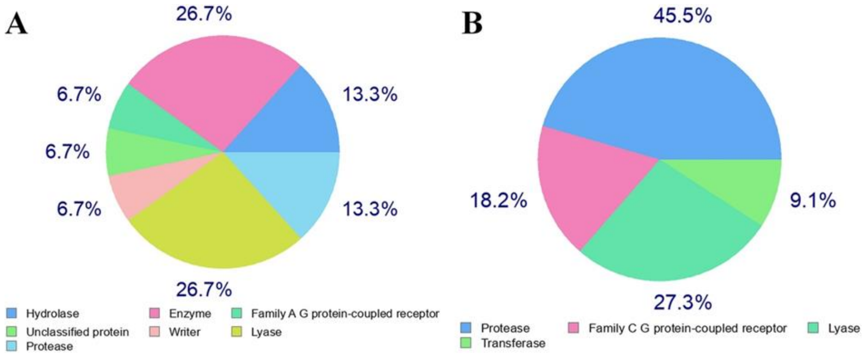

Figure 6. SwissTargetPrediction reports of (A) CPF and (B) GLY.

The comparison of CompTox software by their prediction for CPF and GLY

The predictions for the LC_{50} values for *Daphnia magna* (48 hr), *Pimephales promelas* (96 hr), and oral rat (LD_{50}) were presented as in Table 2 as the outcome of the calculations completed against the CPF and GLY canonical SMILES formulas submitted to the T.E.S.T. program.

Table 2. LC_{50} values for water flea (48 hr), fathead minnow (96 hr), and oral rat (LD_{50}) predicted by T.E.S.T. software.

	Endpoint	Experimental value	Predicted value
chlorpyrifos (2921-88-2)	*D. magna* LC_{50} (48 hr) mg/L	4.96E-04 [source: ECOTOX]	8.45E-04
	P. promelas LC_{50} (96 hr) mg/L	0.15 [source: ECOTOX]	0.75
	Oral rat LD_{50} mg/kg	82.00 [source: ChemidPlus]	99.65
glyphosate (1071-83-6)	*D. magna* LC_{50} (48 hr) mg/L	20.90 [source: ECOTOX]	N/A
	P. promelas LC_{50} (96 hr) mg/L	4.54 [source: ECOTOX]	57.08
	Oral rat LD_{50} mg/kg	4876.61 [source: ChemidPlus]	3927.52

In the analysis performed by T.E.S.T. software on CPF agrochemical for *D. magna*, when the predictions for the test chemicals in the external test set and the most similar chemicals were analysed, the mean absolute error in -Log10(mol/L) was 0.72 for the whole set and 0.56 for those with similarity coefficient ≥ 0.5. When the predictions for the test chemicals and the most similar chemicals in the training test set were analysed, the mean absolute error in -Log10(mol/L) was 0.65 for the whole set and

0.63 for those with similarity coefficient ≥ 0.5. When the same analyses were performed for the external test set of *P. promelas*, the mean absolute error in -Log10(mol/L) values were 0.65 and 0.56, and 0.70 and 0.75 for the training set, respectively. This last value indicates a negative reliability of 0.6 < SC. If we use the same parameter to assess the reliability of T.E.S.T.'s oral rat LD_{50} analyses, the values for the external test set are 0.43 and 0.50 - the latter value again indicating a negative prediction quality with a similarity coefficient < 0.6. For the training set, 0.47 and 0.63 were calculated, again indicating low reliability. Continuing with the reliability assessment of T.E.S.T.'s analyses for the agrochemical GLY, no calculations could be made for either the external test set or the training set of D. magna, again considering the same parameter. However, T.E.S.T. software analyses of the toxic effect of GLY for P. promelas reported an acceptable reliability for both the external test set (Mean absolute error in -Log10(mol/L) was 0.65 for the entire set and 0.18 for similarity coefficient ≥ 0.5) and the training set (Mean absolute error in -Log10(mol/L) was 0.70 for the entire set and 0.49 for similarity coefficient ≥ 0.5). Finally, the mean absolute error in -Log10(mol/L) for the external test in the oral rat LD50 assessment performed with the T.E.S.T. software was 0.48 for the complete set and 0.57 for those with similarity coefficient ≥ 0.5 - the latter value being an acceptable reliability. For the training set, these values have been as 0.47 and 0.41 respectively and show good reliability.

Table 3. LC_{50} values for water flea (48 hr), fathead minnow (96 hr), and oral rat (LD_{50}) predicted by VEGA software.

	Endpoint	Experimental value	Model
chlorpyrifos (2921-88-2)	*D. magna* LC_{50} (48 hr) mg/L	0.001 [EPA][α] N/A	0.2341 [EPA] 0.0005 [DEMETRA][β]
	P. promelas LC_{50} (96 hr) mg/L	0.1566 [EPA] 0.5068 [KNN-IRFMN][γ]	0.4628 [EPA] 0.5068 [KNN-IRFMN]
	Oral rat LD_{50} mg/kg	105.89 [KNN]	105.89 [KNN]
glyphosate (1071-83-6)	*D. magna* LC_{50} (48 hr) mg/L	20.08 [EPA] 125.01 [DEMETRA]	361.77 [EPA] 495.29 [DEMETRA]
	P. promelas LC_{50} (96 hr) mg/L	4.55 [EPA] N/A	16.25 [EPA] N/A
	Oral rat LD_{50} mg/kg	4877.02	4877.02

α U.S. Environmental Protection Agency
β Development of Environmental Modules for Evaluation of Toxicity of pesticide Residues in Agriculture
γ *k*-Nearest Neighbor - Istituto di Ricerche Farmacologiche Mario Negri

The results obtained from the analyses with VEGA are summarised in Table 3. VEGA software estimated the LC_{50} values for the toxic effect of CPF on *D. magna* species with low reliability. For both the DEMETRA and EPA models, in the parameters of global AD index, the concordance for similar molecules, maximum error of prediction among similar molecules, the feature had bad assessments, and the models were stated not to be reliable regarding this aspect. On the other hand, in the analyses performed by the same software for *P. promelas*, "good reliability" was reported between the LC_{50} values obtained experimentally in the EPA model and that calculated by the model; all the measured applicability domain score are fair with the model. Another model applied for this species, KNN-IRFMN, also reported "good reliability". VEGA reported "low reliability" in the assessment of GLY according to the EPA model predictions for which the feature had bad assessments, and the models were stated not to be reliable regarding the parameters of global AD index, the concordance for similar molecules, maximum error of prediction among similar molecules. In contrast, the DEMETRA model reported "good reliability" in its calculations for GLY, as in its predictions for CPF; however, the atom centred fragments similarity check had a non-optimal assessment. For *P. promelas*, the EPA model reported "good reliability" and only the atom centred fragments similarity check had a non-optimal assessment. For the same species prediction was not applicable for KNN-IRFMN model, it was not possible to perform an assessment.

For the prediction of various toxicity endpoints, such as hepatotoxicity, mutagenicity, carcinogenicity, immunotoxicity, acute toxicity, cytotoxicity, toxicity targets and adverse outcomes pathway, ProTox-II incorporates fragment propensities, molecular similarity, most frequent features, and machine learning. In the analyses performed using the ProTox-II virtual lab, LD50 values for CPF and GLY, representing oral toxicity, were found to be 60 mg/kg with predicted toxicity class of 3 and 815 mg/kg with predicted toxicity class of 4, respectively.

Table 4. The values of toxicity parameters in discussion provided by ECHA.

	Endpoint	Value reported by ECHA
chlorpyrifos (2921-88-2)	*D. magna*	EC_{50} (48 hr): 0.1 µg/L
	P. promelas	NOEL$^{\Psi}$ (weight): 1.6 µg/L NOEL (lethality): 3.2 µg/L
	Oral rat	LD_{50}: 64 mg/kg
glyphosate (1071-83-6)	*D. magna*	LC_{50} (48 hr): 74 mg/L
	P. promelas	N/A

Oral rat	LD_{50}: 2000-5000 mg/kg (*most animals tolerated even much higher doses of 5000 mg/kg*)

ψ No Observed Effect Level

The Committee for Risk Assessment (RAC) of ECHA accepts the classification of GLY as being hazardous to aquatic life and capable of inflicting serious eye damage. However, a legal procedure to ban the use of GLY will not be in place until the end of 2023. However, it was stated that the banning process for CPF was completed after its harmful effect was reported. For these two substances, the toxicity values given in the current ECHA reports that are still valid are listed in Table 4. Compared to the experimental values given in the ECHA reports, for CPF, ProTox-II (6.25%) in oral rat toxicity and T.E.S.T. (76.56% and 745%) in LC_{50} of *P. promelas* and *D. magna* calculated the most reliable results, while for glyphosate VEGA (2.46%, N/A and 330.68%) calculated the closest results.

CONCLUSION

When considering ecological risk assessments for a wide range of agrochemicals, it is important to note that CompTox software has played a crucial role in reducing experimental expenditure, making complex relationships and uncertainties between experimental data understandable, and, in particular, alleviating the financial burden and ethical concerns associated with the use of experimental animals. This study examined and compared the reliability of three *in silico* approaches (T.E.S.T., VEGA and ProTox-II) for acute aquatic toxicity to *Daphnia magna* and *Pimephales promelas* with oral rat toxicity. None of the three software programmes compared with each other was superior to the others for all parameters. When we take into account their conformity with the experimental data provided by ECHA reports and their own databases, it is clear that these software should continue to be developed in order to provide the performance and reliability levels expected from them, especially to make the use of experimental animals unnecessary.

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Statement of Conflict of Interest
The author declares that there is no conflict of interest.

Authors' Contributions
All the design and analyzes were arranged and performed by CY. The author completed the writing of the article and took part in the process of publication of the article.

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Evaluation of Hosts and Distribution of *Hyalopterus pruni* (Geoffroy, 1762) and *Sphaerolecanium prunastri* (Boyer de Fonscolombe, 1834), which are Pests of Fruit Trees in Iğdır, Turkey*

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ABSTRACT: This study was carried out in the spring, summer and autumn periods of 2013-2016 in order to determine pests in fruit trees ***Hyalopterus pruni*** (Geoffroy, 1762) and ***Sphaerolecanium prunastri*** (Boyer de Fonscolombe, 1834) in Iğdır province. These two species were economically important pests of apricot, peach, plum and cherry in the Iğdır plain. ***H. pruni*** cause important damage by covering apex of shoots. ***S. prunastri*** causes damage by covering all plant branches in plastering way. Both ***H. pruni*** and ***S. prunastri*** are seen in the nature for five months that from May to October.

Keywords: *Hyalopterus pruni*, *Sphaerolecanium prunastri*, Fruit trees, Iğdır, Turkey

INTRODUCTION

Iğdır plain is located in Eastern Anatolia Region and has a microclimate where there is a continental climate close to the Mediterranean climate. Iğdır is known which enables the cultivation of many agricultural products thanks to its microklima property and soil structure. According to 2017 data, 82163 tons of fruit production was made in Iğdir province. The production of apple, apricote and peach is more dominant than the other fruits (TÜİK, 2017). ***Hyalopterus pruni*** (Geoffroy, 1762) and ***Sphaerolecanium prunastri*** (Boyer de Fonscolombe, 1834) which are important pests for fruit trees (especially apricote and peach), spread to all regions of Turkey and on the world. *H. pruni* (Hemiptera: Aphididae) is an important pest of stone fruit trees, especially apricot, in eastern of Turkey (Toros *et al*. 1996; Atlıhan *et al*. 1999; Atlıhan and Kaydan, 2002; Özgökçe and Atlıhan, 2005; Daşcı and Güçlü, 2008; Atlıhan and Güldal, 2009). *H. pruni* causes direct damage by sucking plant sap, which induces plant deformation and indirect damage by the development of over production of honeydew. Furthermore, it also reported as a virüs that vector of the plum pox virus (Bodenheimer and Swirski 1957; Atlıhan and Kaydan, 2001; Daşcı and Güçlü, 2008). *S. prunastri* (Hemiptera: Coccidae) is a common pest on stone fruits in the Palaearctic Region (Ben-Dov, 1993). Plum scale spread over all regions in Turkey. The host plants of Plum Scale in Turkey are almond, apricot, cherry, peach and plum. This pest causes damage by sucking plant sap and by fouling the leaves with honeydew excretion, where sooty mould develops (Bodenheimer, 1953; Ülgentürk *et al*. 2001).

This study was carried out to determine distribution, host plant and short notes that the situation of pests in fruit trees in Iğdır Plain.

MATERIAL AND METHOD

H. pruni and *S. prunastri* samples were collected from pome and stone trees at different altitudes regions of Iğdır Provinces (Fig 1).

Study area: This study was conducted in Iğdır Plain, located in Eastern of Turkey Figure 1 and it has a microclimate with a continental climate close to the Mediterranean climate. Iğdır Plain altitudes range from 826 to 1545 m samples were collected from pome and stone trees orchards during the seasons of spring and summer of 2013-2016.

Figure 1. Location of study area (Anonymous, 2017).

Sampling method: Fruit trees were samples at random from the four cardinal directions. Samples were randomly collected as shown in Table 1 (Lazarov and Grigorov, 1961).

Table 1. The number of trees controlled in the sampled fields (Lazarov and Grigorov, 1961).

Number of total trees	Number of control trees samples
1-20	All trees
21-70	10-30
71-150	31-40
151-500	41-80
501-1000	15% of all trees
More than 1000	5% of all trees

The specimens were collected between May and October. The sampling process in fruit orchards involved the use of visual inspection and branch counting methods and samples were collected from contaminated plant parts such as branches, shoots, and leaves. The collected samples were transferred into tubes containing 70% ethanol for preservation.

RESULTS AND DISCUSSION

Hyalopterus pruni (Geoffroy, 1762)

Distribution in the World: Andorra, Arabian Peninsula, Austria, Azerbaijan, Balearic Islands, Belarus, Belgium, Bulgaria, Canary Islands, Caucasus, Croatia, Cyprus, Czech Republic, Denmark, Egypt, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Iraq, Ireland, Israel, Italy, Jordan, Kosovo, Latvia, Lebanon, Lithuania, Macedonia, Moldova, Netherlands, Norway, Poland, Portugal, Romania, Russia, Sardinia, Sicily, Slovakia, Slovenia, Spain, Sweden, Switzerland, Syria, Turkey, Ukraine, United Kingdom, Yugoslavia (Önder et al. 2011).

Distribution in Turkey: Ankara (Düzgüneş vd 1982); Iğdır (Özbek vd 1996; Daşcı ve Güçlü 2008); Niğde, Hatay (Uygun vd 2000); Diyarbakır (Bayhan *et al.* 2000); Malatya (Öztürk vd 2004); Van (Denizhan and Yaşar 2005); Adana, Denizli, Isparta, Kayseri, Mersin (Şahin 2007); Erzurum (Narmanlıoğlu and Güçlü, 2008); (Daşçı and Güçlü, 2008); Artvin (Görür vd 2009); Ankara, Antalya, Niğde (Önder et al. 2011); Antalya, Gaziantep, İzmir, Kahramanmaraş, Samsun (Akyürek 2013); Erzincan, Gümüşhane (Alaserhat 2015).

Host Plants: In the study, *H. pruni* was detected on plum (*Prunus domestica*), apricot (*Prunus armeniaca*) and peach (*Prunus persica*) trees. Blackman and Eastop (2006) reported that the main hosts of *H. pruni* were *P. domestica* and *P. armeniaca*, while it preferred *Phragmites communis* and sometimes *Arundo donax* as secondary hosts (Görür et al. 2009). Different researchers have identified host plants such as Apiaceae, Cyperaceae, Loganiaceae, Malvaceae, Poaceae, Rosaceae, Scrophulariaceae, and Typhaceae (Çanakçıoğlu 1975); *P. domestica, P. armeniaca, P. padus, P. dulcis, P. persicae, P. cerasus, P. insitita, P. amygdalus, P. cerasifera, P. pisardii nigra, P. spinosa, Persica vulgaris, Armeniaca vulgaris, Phragmites australis,* and *Buxus* sp. (Erkin 1983; Blackman and Eastop 2000; Bayhan et al. 2003; Kavallieratos et al. 2004; Özdemir 2004; Blackman and Eastop 2006; Kocadal 2006; Şahin 2007; Eser et al. 2009; Rakhshani 2012); *Arundinaria, Arundo, Calamagrostis, Leymus, Miscanthus, Molinia, Phalaris, Phragmites, Poa, Setaria* (Poaceae), *Brassica* (Brassicaceae), *Carex, Scirpus* (Cyperaceae), *Malus, Prunus* (Rosaceae), *Trichophorum,* and *Typha* (Typhaceae) plant species (Akyürek 2013).

Biological Observations: In this study, it was found that while aphids were more abundant on the leaves of apricot and peach trees from May to October, they were less frequently observed on the leaves of plum trees. *H. pruni* formed widespread and dense colonies, particularly on apricot and peach trees, by feeding on the undersides of the leaves. This feeding resulted in the leaves curling and turning a light green color, while also secreting a substantial amount of sweet exudates. The highest population of *H. pruni* was observed in May and July (Figure 2). The distribution of *H. pruni* in the study area is presented in Figure 3. *H. pruni* is primarily noticed on the undersides of leaves, where it forms a whitish, waxy substance. It feeds by piercing and sucking plant sap, which results in leaf discoloration. In populations with high densities of *H. pruni*, little to no leaf curling is observed in apricot leaves, whereas peach leaves exhibit more curling (Figure 4). Throughout the study period, during the high population density period from May to July, the leaves of the trees were visibly shiny and covered with a sweet substance. *H. pruni* is considered the primary pest of apricot and peach trees in the study area.

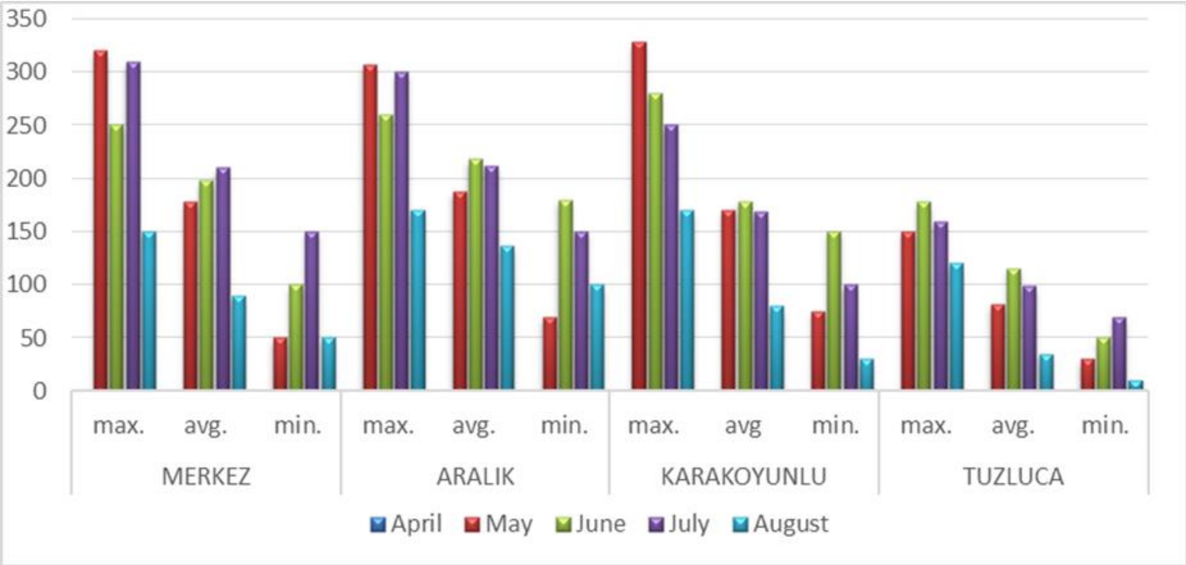

Figure 2. Population density of Hyalopterus pruni (Geoffroy 1762) by month in Iğdır province

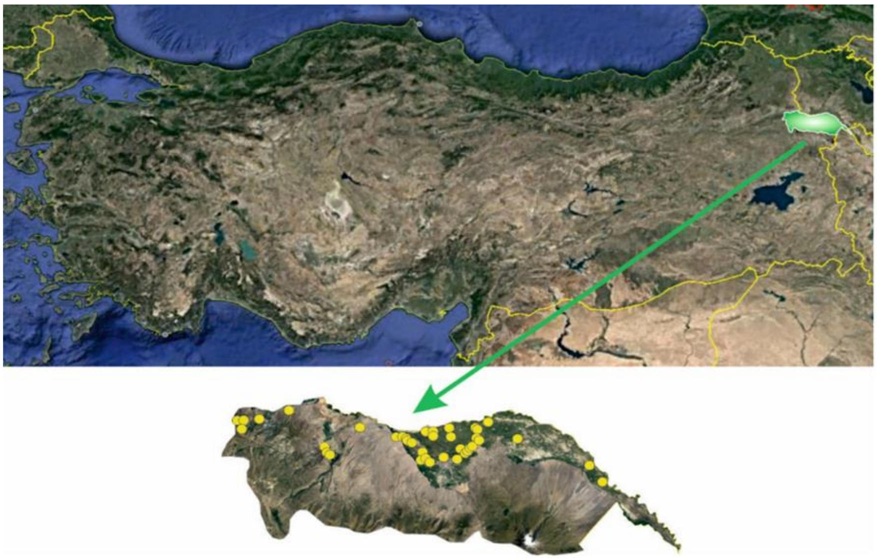

Figure 3. Distribution of Hyalopterus pruni (Geoffroy 1762) in the study area.

A similar study was conducted by Narmanlıoğlu (2013) and the researcher identified this pest in plum, apricot, and peach trees. *H. pruni* was found to form dense colonies and secrete large amounts of honeydew, and although it was more abundant on the leaves of apricot trees, it caused more curling on the leaves of plum and peach trees. Throughout the study, it was observed that during the high population density period from May to July, the leaves of the trees appeared shiny and covered with a sweet substance. In the study area, *H. pruni* is the main harmful pest for apricot and peach trees.

Figure 4. The damage and population of Hyalopterus pruni (Geoffroy, 1762) in peach and apricot

A similar study was conducted by Alaserhat (2015), who determined that the population of *H. pruni* on its host plants, including apricot, peach, plum, and almond trees, reaches its maximum level from the second week of May to the second week of July. Studies conducted in the study area have indicated that *H. pruni* is much more widespread and has a higher population compared to other aphid species on apricot and peach trees and is an economically damaging species in the region (Özbek *et al.* 1996; Daşcı and Güçlü, 2008). Various researchers have reported that this species feeds on plant sap from the undersides of leaves on stone fruit trees, causing yellowing and curling of the leaves due to this feeding, and also produces honeydew, which leads to the development of sooty mold. In addition, they act as vectors for some plant virus diseases (Öztürk *et al*. 2004; Daşcı and Güçlü, 2008; Özbek and Yıldırım, 2014).

Sphaerolecanium prunastri (Boyer de Fonscolombe, 1834)

Distribution in the World: Armenia, Austria, Azerbaijan, Bulgaria, China, Czechoslovakia, France, Georgia, Germany, Greece, Hungary, Iran, Israel, Italy, Japan, Moldova, Poland, Romania, Russia, Spain, Switzerland, Turkey, Ukraine, United States of America, and Yugoslavia (Demirözer 2004; Kaydan et al. 2009).

Distribution in Turkey: Afyon, Burdur, Isparta (Ülgentürk *et al.* 2001); Malatya (Özgen ve Bolu, 2009); Afyon, Ağrı, Ankara, Antalya, Balıkesir, Bilecik, Bitlis, Bolu, Bursa, Edirne, Hakkâri, Iğdır, İstanbul, İzmir, Kırklareli, Kocaeli, Konya Sakarya, Tekirdağ, Van (Kaydan *et al.* 2009).

Host Plants: In this study, *S. prunastri* has been detected on apricot (*Prunus armeniaca*) and peach (*Prunus persica*) trees. This species has been reported to be present in various host plants, including *Amygdalus* sp., *A. communis*, *Armeniaca* sp., *A. persica*, *Cerasus* sp., *C. arium*, *Malus sylvestris*, *Mesembryanthemum edule*, *Prunus* sp., *P. vulgaris*, *P. avium*, *P. armeniaca P. cerasifera*, *P. divaricata*, *P. domestica*, *P. dulcis*, *P. persica*, *P. salicina*, *P. spinosa*, *P. spinosa*, *P. ursina*, *Pyrus communis*, *P. malus* and *Rhamnus dahurica* (Demirözer, 2004; Kaydan *et al.* 2009).

Biological Observations: In this study, it was determined that this species is most common and abundant on apricot and peach trees, and less commonly found on plum and cherry trees. Besides, the distribution of *S. prunastri* in the study area is presented in Figure 5. In the study area, it was observed that the insect covers young branches of apricot and peach trees in a smearing manner, giving them a shiny appearance It was also found that its feeding results in the production of sugar-rich substances that cause fumigation This species is an important pest in the study area, being widespread on almost all apricot and peach trees and differing in terms of population density (Figure 6).

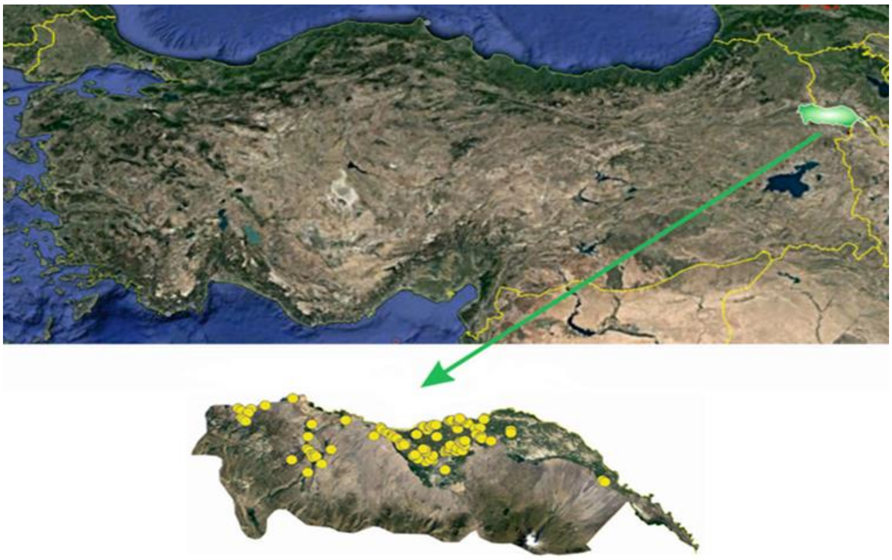

Figure 5. Distribution of *Sphaerolecanium prunastri* (Boyer de Fonscolombe, 1834) in the study area.

Figure 6. The damage of *Sphaerolecanium prunastri* (Boyer de Fonscolombe, 1834) in peach and apricot.

In a study conducted in apricot orchards in Malatya province, *S. prunastri* was determined as one of the species causing damage to apricot trees (Öztürk *et al.* 2004). In the same study area, another researcher reported that *S. prunastri* was the most prevalent and damaging species among the Coccidae family, with a prevalence rate of 23.5% and causing significant damage in orchards with high pest density (Yiğit, 2013). Another study conducted in the same region by Özgen and Bolu (2009) emphasized the necessity of continuous monitoring of *S. prunastri* populations and distributions to prevent new infestations through contaminated seedlings and grafts, as well as the need for constant control measures to keep this polyphagous pest under control in apricot orchards in Malatya province. Different studies conducted by various researchers have reported that *S. prunastri* is a species that can reach high populations, especially in areas where apricot cultivation and production of stone fruits are carried out, in the provinces of Bitlis, Iğdır, and Van (Kaydan *et al.* 2009). Different studies conducted by various researchers have reported that *S. prunastri* is a species that can reach high populations, especially in areas where apricot cultivation and production of stone fruits are carried out, in the provinces of Bitlis, Iğdır, and Van (Kaydan *et al.* 2009)

CONCLUSION

During the study, it was determined that ***H. pruni*** was more abundant on the leaves of apricot and peach trees, especially from May to October, while it was less frequently found on the leaves of plum trees. The highest population was observed in May and July, and while it did not cause leaf curling on apricot trees, it was found to cause leaf curling particularly on peach trees. It has been determined that

S. prunastri specifically covers the young branches of apricot and peach trees in the form of a scale, and it is present in all apricot trees in the study area, particularly in apricot production areas where it reaches high populations.

It is believed that the findings of this study will shed light on future research to be conducted in this field. The results obtained from this study can be used to further investigate the population dynamics, biology, ecology, and management strategies of *H. pruni* and *S. prunastri*, as well as other similar species. This can contribute to a better understanding of the pest status of these species and help develop effective pest management programs for the cultivation of stone and pome fruit crops.

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Statement of Conflict of Interest

The authors declare no conflict of interest.

Authors' Contributions

YBK designed and analyzed the research and arranged the studies. They also worked on the preparation of pictures and tables. EY provided supervision and editing support. 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.

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Estimation of Root Length Using Regression Tree Method in *Sesbania punicea* Seeds

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ABSTRACT: Regression Tree analysis, which is one of the data mining methods, does not require any assumptions about the distribution of independent variables compared to classification and clustering analysis techniques and offers more successful solutions for a large number of independent variables and data. Regression trees help to better define groups, discover relationships between them, and predict future events. The regression tree method is useful for determining the effects of several factors on the specified dependent variable. The genus *Sesbania*, which belongs to the Papilionoideae subfamily of the Leguminosae family, consists of 500 species and is distributed pantropically. Most *Sesbania* species are annuals, and some are relatively short-lived. In the present study, the effect of different nutrients and plant-friendly bacterial strains applications on stalk thickness, stalk length and number of lateral roots on root length was investigated and a prediction model was created. As a result of the application, the root length of the germinated seeds was estimated with 94% success.

Keywords: Germination, CART, *Sesbania punicea,* Stalk thickness, Stalk length

INTRODUCTION

An alternative approach to nonlinear regression is to sub-divide, or partition, the space into smaller regions, where the interactions are more manageable, and finally we get to chunks of the space which are so tame that we can fit simple models to them. The global model thus has two parts: one is just the recursive partition, the other is a simple model for each cell of the partition. Regression trees use the tree to represent the recursive partition. Each of the terminal nodes, or leaves, of the tree represents a cell of the partition, and has attached to it a simple model which applies in that cell only. A point x belongs to a leaf if x falls in the corresponding cell of the partition. To figure out which cell we are in, we start at the root node of the tree, and ask a sequence of questions about the features. The interior nodes are labeled with questions, and the edges or branches between them labeled by the answers. Which question we ask next depends on the answers to previous questions. Variables do not all have to be of the same type; some can be continuous, some can be discrete but ordered, some can be categorical, etc. You could do more-than-binary questions, but that can always be accommodated as a larger binary tree. There are several advantages to this: making predictions is fast (no complicated calculations, just looking up constants in the tree: it's easy to understand what variables are important in making the prediction: if some data is missing, we might not be able to go all the way down the tree to a leaf, but we can still make a prediction by averaging all the leaves in the sub-tree we do reach; the model gives a jagged response, so it can work when the true regression surface is not smooth; there are fast, reliable algorithms to learn these trees. Prediction trees use the tree diagram to represent the recursive partition. Each of the leaves, of the tree represents a cell of the partition, and has attached to it a simple model which applies in that cell only (Hastie & Tibshirani, 1990; Lahmann & Kottner, 2011; Loh, 2011; Mikail & Kaplan, 2021).

Sesbania punicea (Cav.) Benth. is a plant belonging to the Fabaceae family and originating from South America and is distributed in the Atlantic Forest, Pampa and Pantanal regions (Bergmann, 2014; Anonymous, 2021). It has been used as an ornamental plant in North America, Europe, South Africa and Australia and is considered as an invasive exotic species because it spreads rapidly (Woodward & Quinn, 2011; WIDEpac, 2012). In addition to being grown as an ornamental plant, this species is also used in landscape restoration, as it is successfully used in cases such as fire and storm, changes in land use, and priority forest areas (Ulibarri et al., 2002). It tolerates poor soils well and adapts easily to different climates. Therefore, it can also be used in damp or flooded areas (Kissmann & Groth, 1992).

The aim of this study is to predict factors affecting the root length of *Sesbania punicea* by means of regression tree model.

MATERIAL AND METHOD

Material

Sesbania punicea (Cav.) Benth. germinated seeds were used as study material (Çığ, 2021a; 2021b; 2022). Lead concentrations ($Pb(NO_3)_2$) were prepared as 10 and 20 ppm (Çığ, 2022). Nickel concentrations were prepared as 50 and 100 ppm (Çığ, 2021a). Zinc concentrations ($ZnSO_4.7H_2O$) were prepared as 100 and 200 ppm (Çığ, 2021b). The bacteria used in the experiment were obtained from Siirt University, Faculty of Agriculture, and Department of Field Crops. Bacterial isolates were isolated from the Siirt ecological condition and their PGPB activity was detected. *Paenarthrobacter nitroguajacolicus* (KF3B), *Bacillus zhangzhouensis* (KF3A), *Microbacterium oxydans* (KF58C), *Brevibacterium frigoritolerans* (KF58B) and *Paenibacillus xylanilyticus* (KF63C) bacteria used were diagnosed with the microbial identification system (MIS) and identified as Plant Growth Promoting Bacteria (PGPB) activity under laboratory conditions. All traits were measured manually for high precision. The parameters used in this study are root length (cm), lateral root number, stalk thickness (mm), stalk length (cm).

Method

The space X is partitioned by a sequence of binary splits into terminal nodes (Fig. 1).

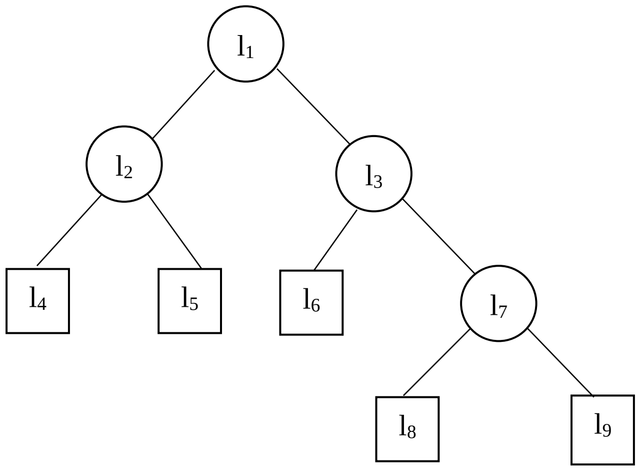

Figure 1. Scheme of regression trees

In each terminal node l, the predicted response value y(l) is constant.

Starting with a learning sample, three elements are necessary to determine a tree predictor:

1. A way to select a split at every intermediate node
2. A rule for determining when a node is terminal
3. A rule for assigning a value y(l) to every terminal node l

It turns out, as in classification, that the issue of the node assignment rule is easiest to resolve. We start with the resubstitution estimate for R*(d), that is,

$$R(d) = \frac{1}{N}\sum_n \left(y_n - d(x_n)\right)^2 \qquad \text{(Eq. 1)}$$

The value of y(l) that minimizes R(d) is the average of y_n for all cases (x_n, y_n) falling into l; that is, the minimizing y(l) is

$$\bar{y}(l) = \frac{1}{N(l)}\sum_{x_n \in l} y_n, \qquad \text{(Eq. 2)}$$

where, the sum is over all yn such that $x_n \in l$ and N(l) is the total number of cases in l. The proof is based on seeing that the number a, which minimizes $\sum(y_n - a)^2$ is n

$$a = \frac{1}{N}\sum_n y_n \qquad \text{(Eq. 3)}$$

Similarly, for any subset y_n, the number which minimizes $\sum(y_n - a)^2$ is the average of the y_n.

From now on, the predicted value in any node t will be $\bar{y}(l)$. Then, using the notation R(L) instead of R(d),

$$R(L) = \frac{1}{N}\sum_{l \in \tilde{L}} \sum_{x_n \in l} (y_n - \bar{y}(l))^2 \qquad \text{(Eq. 4)}$$

Set

$$R(l) = \frac{1}{N}\sum_{x_n \in l} (y_n - \bar{y}(l))^2 \qquad \text{(Eq. 5)}$$

so (Eq. 4) can be written as

$$R(L) = \sum_{l \in \tilde{L}} R(l) \qquad \text{(Eq. 6)}$$

These expressions have simple interpretations. For every node l, $\sum_{x_n \in l}(y_n - \bar{y}(l))^2$ is the within node sum of squares. That is, it is the total squared deviations of the y_n in l from their average. Summing over $l \in \tilde{L}$ gives the total within node sum of squares, and dividing by N gives the average.

Given any set of splits S of a current terminal node l in $\tilde{L}$, the best split s* of l is that split in S which most decreases R(L).

More precisely, for any split s of l into l_L and l_R, let

$$\Delta R(s,l) = R(l) - R(l_L) - R(l_R) \quad \text{(Eq. 7)}$$

Take the best split s* to be a split such that

$$\Delta R(s^*,l) = \max_{s \in S} \Delta R(s,l) \quad \text{(Eq. 8)}$$

Thus, a regression tree is formed by iteratively splitting nodes so as to maximize the decrease in R(L). In classification trees, choosing the best splits to be the ones that minimized the resubstitution misclassification rate had undesirable properties. The best split at a node is that split on the x variables which most successfully separates the high response values from the low ones. At each intermediate node l, one of $\bar{y}(l_L), \bar{y}(l_R)$ is considerably lower than $\bar{y}(l)$ and the other higher (Breiman et al, 1993; Mikail & Kaplan, 2021).

RESULTS AND DISCUSSION

There are 4 continuous (stalk length, stalk thickness, root length and the number of lateral roots) and 2 categorical (minerals and bacteria) variables used to predict root length. Table 1 shows descriptive statistics of continuous variables. Table 2 represents the frequency and percentage of categorical variables.

Table 1. Descriptive statistics for the continuous variables

	N	Mean	Minimum	Maximum	Std.Dev.	Coef.Var.
Stalk length	135	8,813615	0,00	15,56000	3,234428	36,69809
Root length	135	5,537333	0,00	13,72000	3,185835	57,53373
Stalk thickness	135	0,166074	0,00	0,42700	0,072380	43,58282
Number of lateral roots	135	3,644444	0,00	12,00000	2,432573	66,74744

Table 1 shows that the most variation is in the number of lateral roots, followed by root length.

Table 2. Frequency and percentage values for several categorical variables

	N	Percent
Control	45	33,33
Ni 1	15	11,11
Ni 2	15	11,11
Pb 1	15	11,11
Pb 2	15	11,11
Zn1	15	11,11
Zn2	15	11,11
KF3A	27	20,00
KF63C	27	20,00
KF3B	27	20,00
KF58C	27	20,00
KF58B	27	20,00

The regression tree diagram is depicted in Fig. 2.

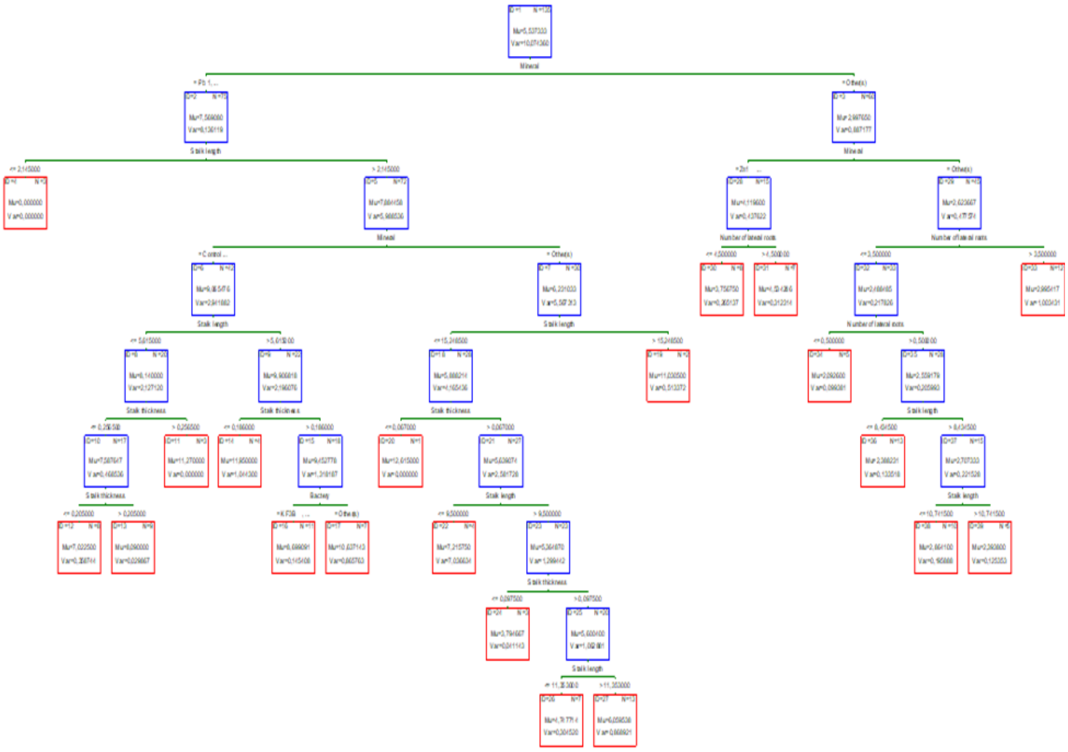

Figure 2. Regression tree diagram for root length

20 terminal nodes (red rectangles) and 19 non-terminal nodes (blue rectangles) were generated by the regression tree. The node definitions and splitting values for root length were given in Table 3.

At the top of regression tree diagram, Node 1, which gave general descriptive statistics of root length, was divided into new two child nodes, with respect to mineral addition (Pb1 and other contents). The root length averages of these two child nodes, Nodes 2 was estimated as 7.569 cm and Node 3 was estimated as 2.998 cm. With an average of 12.615 cm, Node 20 gave the highest root length. Numbers (proportions) of records were 75 (56%) for Node 2 and 60 (44%) for Node 3. Node 2 was newly divided into Nodes 4 and 5, depending on stalk length factor. Corresponding average values for these two new child nodes were 0 and 7.88 cm, respectively. Node 4 has 3 records and is the terminal node. Right side nodes always show high level mean. Node 3 was also re-branched into two child nodes as Nodes 28 and

29, in association with mineral content factor, respectively. The root length averages for these two nodes were found as: 4.12 and 2.62 cm, respectively. Numbers (proportions) of records were established as 15 (11%) and 45 (33%), respectively. Mineral content factor remarkably influenced the root lengths of germinated seeds of *Sesbania punicea* whose records were consisted of Zn1 in the Node 3.

Table 3. Node definitions and splitting values for root length predictions

	Left - branch	Right - branch	Size of - node	Node - mean	Node - variance	Split - variable	Split - constant	Split - category	Split - category	Split - category
1	2	3	135	5,53733	10,07436	Mineral		Pb 1	Pb 2	Control
2	4	5	75	7,56908	8,13612	Stalk length	2,14500			
4			3	0,00000	0,00000					
5	6	7	72	7,88446	5,98854	Mineral		Control		
6	8	9	42	9,06548	2,94188	Stalk length	5,61500			
8	10	11	20	8,14000	2,12712	Stalk thickness	0,25650			
10	12	13	17	7,58765	0,46854	Stalk thickness	0,20500			
12			8	7,02250	0,35874					
13			9	8,09000	0,02987					
11			3	11,2700	0,00000					
9	14	15	22	9,90682	2,19608	Stalk thickness	0,18600			
14			4	11,9500	1,04430					
15	16	17	18	9,45278	1,31819	Bacteria		KF3B	KF58C	
16			11	8,69909	0,14541					
17			7	10,6371	0,86576					
7	18	19	30	6,23103	5,56731	Stalk length	15,2485			
18	20	21	28	5,88821	4,16544	Stalk thickness	0,06700			
20			1	12,6150	0,00000					
21	22	23	27	5,63907	2,58173	Stalk length	9,50000			
22			4	7,21575	7,03663					
23	24	25	23	5,36487	1,29944	Stalk thickness	0,09750			
24			3	3,79467	0,04114					
25	26	27	20	5,60040	1,06288	Stalk length	11,3530			
26			7	4,74771	0,30452					
27			13	6,05954	0,86892					
19			2	11,0305	0,51337					
3	28	29	60	2,99765	0,88718	Mineral		Zn1		
28	30	31	15	4,11960	0,43762	Number of lateral roots	4,50000			
30			8	3,75675	0,26514					
31			7	4,53429	0,31231					
29	32	33	45	2,62367	0,47757	Number of lateral roots	3,50000			
32	34	35	33	2,48848	0,21783	Number of lateral roots	0,50000			
34			5	2,09260	0,09938					
35	36	37	28	2,55918	0,20599	Stalk length	8,43450			
36			13	2,38823	0,13352					
37	38	39	15	2,70733	0,22153	Stalk length	10,7415			
38			10	2,86410	0,19589					
39			5	2,39380	0,12535					
33			12	2,99542	1,00343					

On account of the fact that Nodes 4, 11, 12, 13, 14, 16, 17, 19, 20, 22, 24, 26, 27, 30, 31, 33, 34, 36, 38 and 39 were terminal nodes, there was not any separation operation in further processes for providing satisfactory homogeneity in those nodes.

In the current study, an average of the root length was 5.54 cm, which was an average of all the records. According to Fig. 3, stalk thickness was significantly identified as the most important factor influencing root length. Fig. 3 shows importance level of the independent variables and their percent were given in Table 4.

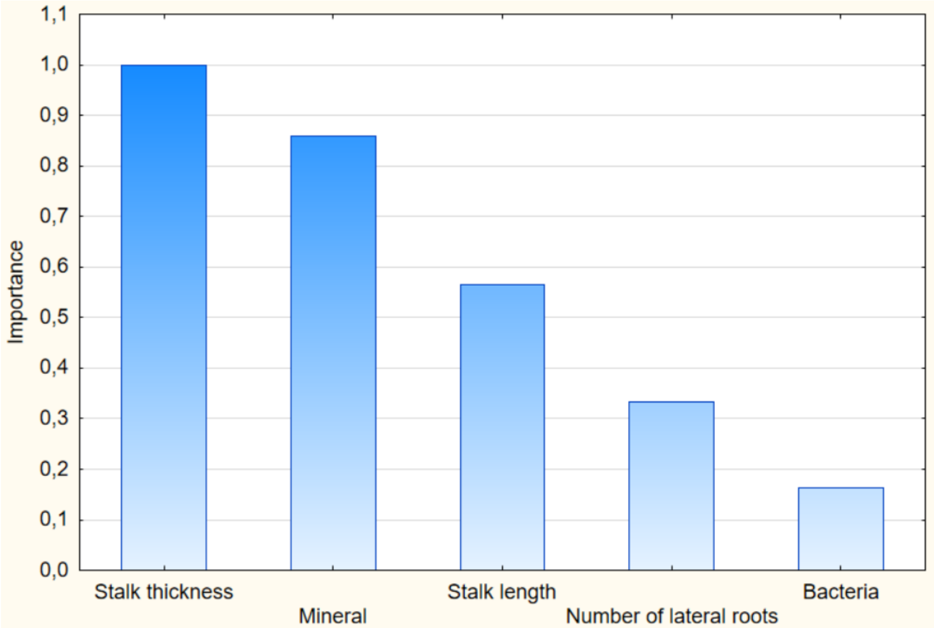

Figure 3. Importance level of independent variables affecting root length

Table 4. Importance level of independent variables affecting root length

	Importance (%)
Stalk thickness	100
Mineral	86
Stalk length	57
Number of lateral roots	33
Bacteria	16

As shown from the Table 4 bacteria application was not found statistically significant in root length prediction. The correlation relationship between the observed and estimated root lengths is given in Fig. 4.

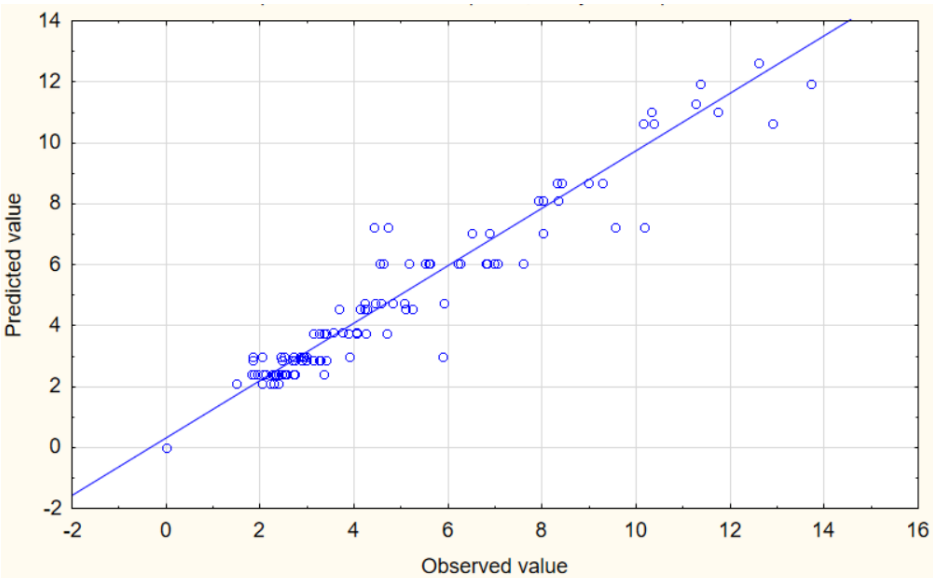

Figure 4. Scatterplot between observed and predicted root length

The R^2 of the prediction model is 94%, which is significantly high.

CONCLUSION

Due to the fact that CART is not a parametric method, the data used here is not required be belonged to a particular type of distribution. Also, this method can easily determine effects of both continuous and categorical variables on dependent continuous variable.

In regression tree diagram, the stalk thickness trait was determined to be the most affective factor for root length prediction, followed by mineral content of nutrition, stalk length and the number of lateral roots.

As a result, the routing of the algorithms leading to the terminal node will help to determine the criteria for the classification in the regression tree and the application of the test data to this algorithm.

Statement of Conflict of Interest

The authors declare that they are no conflict of interest.

Authors' Contributions

NM and AÇ designed and analyzed the research, NM and AÇ studies arranged. NM 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.

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Prediction of Lateral Root Numbers in *Sesbania punicea* with Different Minerals and Bacteria Applications by Using Sugeno Fuzzy Logic Model

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ABSTRACT: Seen as a technique that can express linguistic uncertainty mathematically, fuzzy set theory is a mathematical criterion for a wide variety of uncertain events, including the concept of probability. Here, the uncertainty is modeled with the logical system. Due to the different structures of fuzzy systems, fuzzy inference methods are also different. Frequently used fuzzy inference techniques are Mamdani and Sugeno methods. In Takagi-Sugeno fuzzy system, linear functions are formed instead of fuzzy sets in the results of fuzzy rules. The output of this system is a "fuzzy" combination of a set of linear functions. The Sugeno method works well with optimization and adaptive techniques, making it easy to model especially for dynamic nonlinear systems. The aim of this study is to estimate the lateral root numbers of the germinated seeds of *Sesbania punicea* by applying the Sugeno fuzzy inference method. Different plant-friendly bacterial strains applications and nutrient elements were used as input. In this application, the results obtained by the multiple regression method will be compared with the results obtained by the Sugeno inference method.

Keywords: Fuzzy rules, Germination, Prediction, *Sesbania punicea,* Takagi-Sugeno

INTRODUCTION

In 1985, Takagi and Sugeno (Mallows, 1973) modified the consequence of implication from fuzzy sets to linear functions and developed the so-called "Takagi-Sugeno fuzzy systems" which were applied to parking control of a model car (Sugeno, 1985; Zhang et al, 1994; Chak et al, 1998). The format of their fuzzy rules is

If x_1 is A_1 and x_2 is A_2 and … x_n is A_n, then $y = a_0 + a_1x_1 + \dots + a_nx_n$.

The structure of these systems varies significantly from that of the conventional one. As a consequence of implication, they contain a linear function by which the output can be computed. The aim of the linear function in Takagi-Sugeno fuzzy systems is to describe the local linear behavior of the system. Fuzziness, which appears only in the premise part of the fuzzy rule, indicates the uncertainty about which the output range of the linear function varies. Takagi-Sugeno fuzzy systems have a number of advantages by their nature. The systems can be easily understood and the local system equations can be directly related to the local behavior of the system. Takagi-Sugeno fuzzy systems include two kinds of knowledge: one is the qualitative knowledge represented by the if-then rules, and the other is the quantitative knowledge represented by the local functions. The systems allow us to formulate these two kinds of knowledge into a unified mathematical framework (Chak et al, 1998). Today fuzzy logic has taken place widespread not only in designing and manufacturing but also in practice with what we call smart robots as integral parts of our life within the area of technology. Recently in Türkiye fuzzy logic has an important place in learning and application of system and control principles, at least in scientific and research areas. Research and Development units of many international businesses have been needed fuzzy system and control mechanisms. This necessity has emerged in Turkey as well (Şen, 2001).

The aim of this study is to estimate the number of lateral roots in *Sesbania punicea* by applying the Sugeno fuzzy inference method.

MATERIAL AND METHOD

Material

Sesbania punicea (Cav.) Benth. germinated seeds were used as study material (Çığ, 2021a; 2021b; 2022). Lead concentrations ($Pb(NO_3)_2$) were prepared as 10 and 20 ppm (Çığ, 2022). Nickel concentrations were prepared as 50 and 100 ppm (Çığ, 2021a). Zinc concentrations ($ZnSO_4.7H_2O$) were prepared as 100 and 200 ppm (Çığ, 2021b). The bacteria used in the experiment were obtained from Siirt University, Faculty of Agriculture, and Department of Field Crops. Bacterial isolates were isolated from the Siirt ecological condition and their PGPB activity was detected. *Paenarthrobacter nitroguajacolicus* (KF3B), *Bacillus zhangzhouensis* (KF3A), *Microbacterium oxydans* (KF58C), *Brevibacterium frigoritolerans* (KF58B) and *Paenibacillus xylanilyticus* (KF63C) bacteria used were diagnosed with the microbial identification system (MIS) and identified as Plant Growth Promoting Bacteria (PGPB) activity under laboratory conditions. All traits were measured manually for high precision. The parameter used in this study is the number of lateral roots.

Method

Sugeno-style fuzzy inference is very similar to the Mamdani method. Sugeno changed only a rule consequent. Instead of a fuzzy set, he used a mathematical function of the input variable. The format of the Sugeno-style fuzzy rule is

IF x is A
AND y is B
THEN z is $f(x, y)$

where x, y and z are linguistic variables; A and B are fuzzy sets on universe of discourses X and Y, respectively; and $f(x,y)$ is a mathematical function.

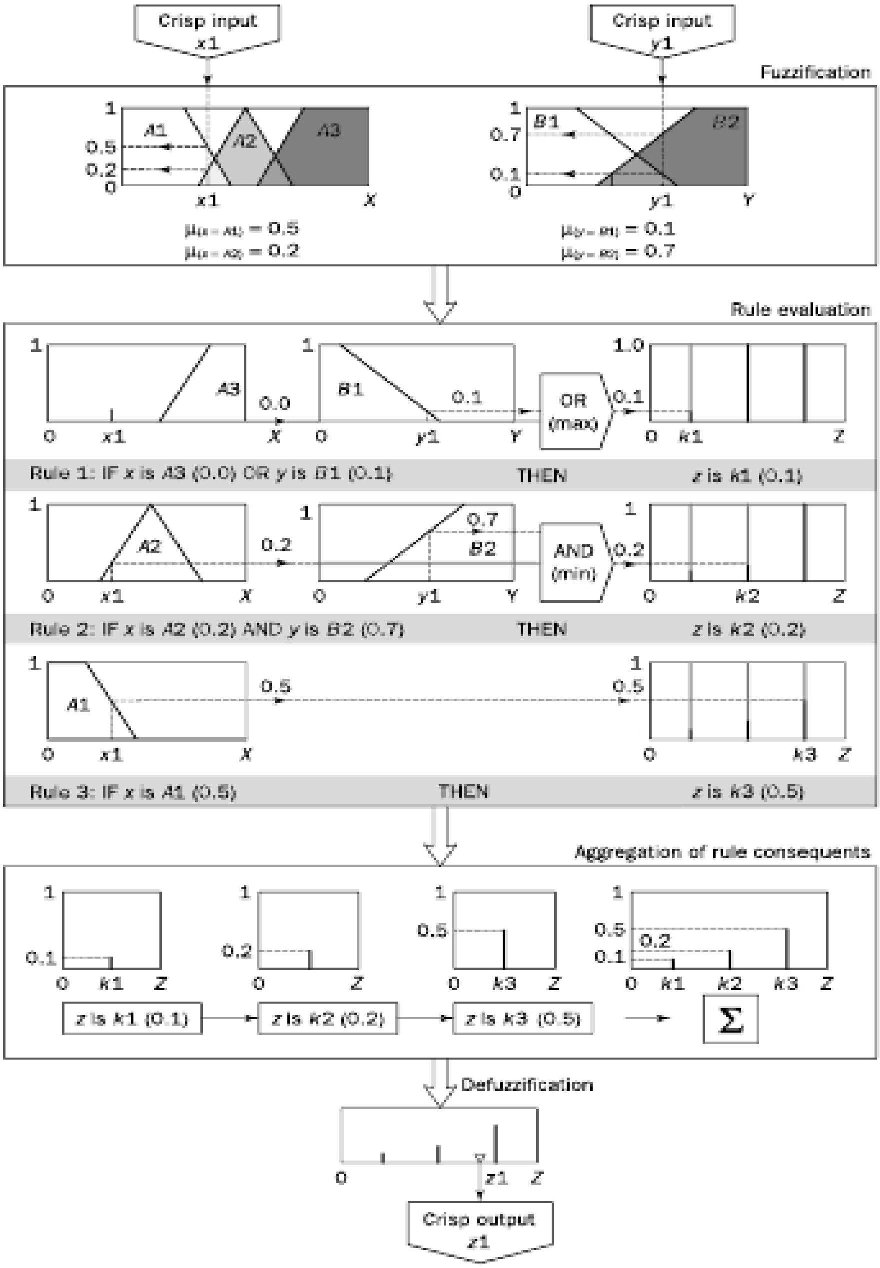

Figure 1. The basic structure of Sugeno-style fuzzy inference (Negnevitsky, 2005)

The most commonly used zero-order Sugeno fuzzy model applies fuzzy rules in the following form:

IF $\quad x$ is A
AND $\quad y$ is B
THEN z is k

where k is a constant.

In this case, the output of each fuzzy rule is constant. In other words, all consequent membership functions are represented by singleton spikes. Fig. 1 shows the fuzzy inference process for a zero-order Sugeno model.

The Sugeno method is computationally effective and works well with optimization and adaptive techniques, which makes it very attractive in control problems, particularly for dynamic nonlinear systems (Negnevitsky, 2005). MATLAB Fuzzy Logic Toolbox (Mathwork, 2009) was used for modeling.

Performance Criteria

The estimated performance was calculated with coefficient of determination (R^2).

$$R^2 = \frac{\sum_{i=1}^{n}\left(Y_i - \bar{Y}\right)^2}{\sum_{i=1}^{n}\left(Y_i - \bar{Y}\right)^2}$$

Where,
Y_i – observed value,
$\hat{Y}_i$ – predicted value,
$\bar{Y}$ – Arithmetic mean,
n – the total number of observations (Ameen and Mikail, 2018).

RESULTS AND DISCUSSION

For the fuzzy logic model realized via the MATLAB program, 4 inputs (Pb, Zn, Ni and Bacteria) and 1 output (The number of lateral roots) were available. Fuzzy sets for nutrients were developed as illustrated in Fig. 1-3.

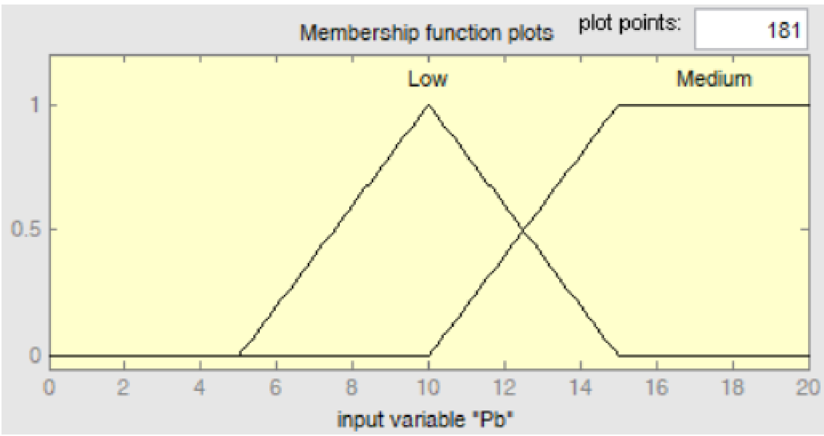

Figure 1. Fuzzy sets for Pb

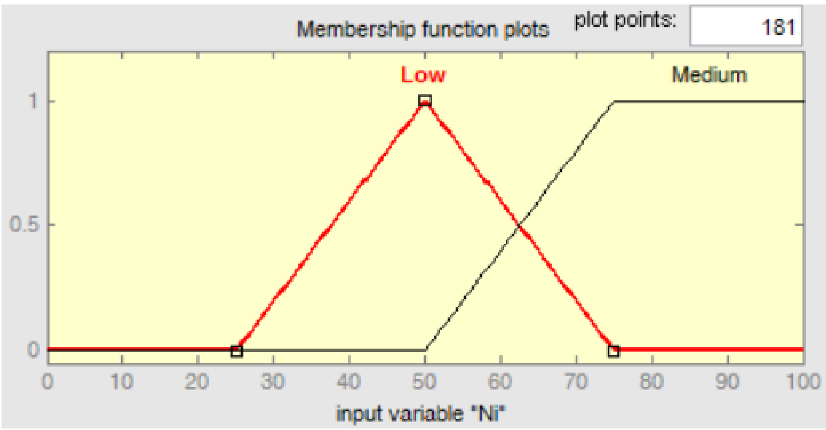

Figure 2. Fuzzy sets for Ni

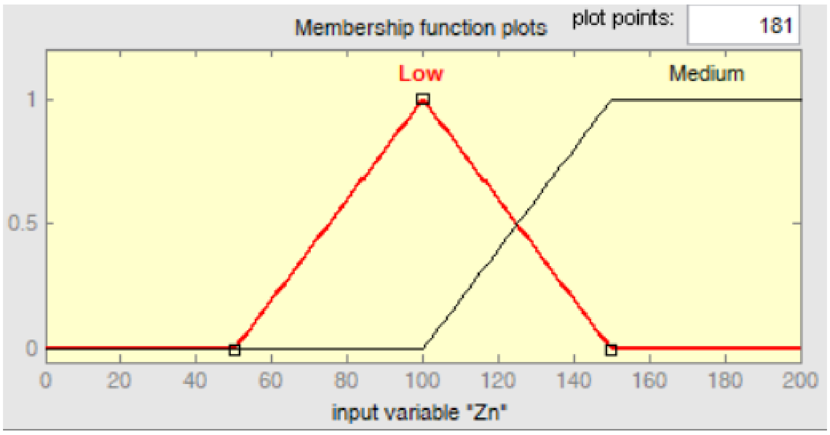

Figure 3. Fuzzy sets for Zn

The Bacteria graph formed in MATLAB is illustrated in Fig. 4. In the fuzzy logic model (FLM), however, this input is entered as discrete.

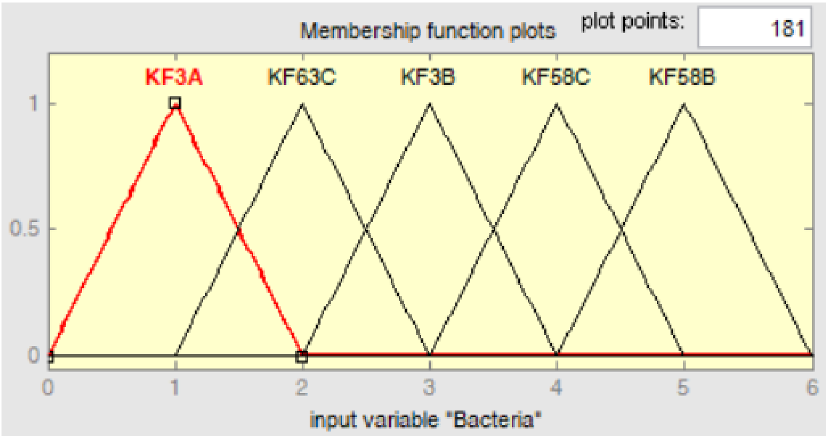

Figure 4. Fuzzy sets for bacteria variable

Rule base of the FLM was contained of 75 rules. Below are some rules:

1. If (Bacteria is KF3A) and (Ni is Low) then (Lateral_roots is 1) (1)
2. If (Bacteria is KF3A) and (Ni is Low) then (Lateral_roots is 2) (1)
3. If (Bacteria is KF3A) and (Ni is Low) then (Lateral_roots is 3) (1)
4. If (Bacteria is KF3A) and (Ni is Medium) then (Lateral_roots is 1) (1)
5. If (Bacteria is KF3A) and (Ni is Medium) then (Lateral_roots is 2) (1)
6. If (Bacteria is KF63C) and (Ni is Low) then (Lateral_roots is 2) (1)
7. If (Bacteria is KF63C) and (Ni is Low) then (Lateral_roots is 3) (1)
8. If (Bacteria is KF63C) and (Ni is Low) then (Lateral_roots is 4) (1)
9. If (Bacteria is KF63C) and (Ni is Medium) then (Lateral_roots is 1) (1)
10. If (Bacteria is KF63C) and (Ni is Medium) then (Lateral_roots is 3) (1)

The relationships between the number of lateral roots and nutrients and bacteria were shown in Fig. 5 (a,b,c,d), respectively.

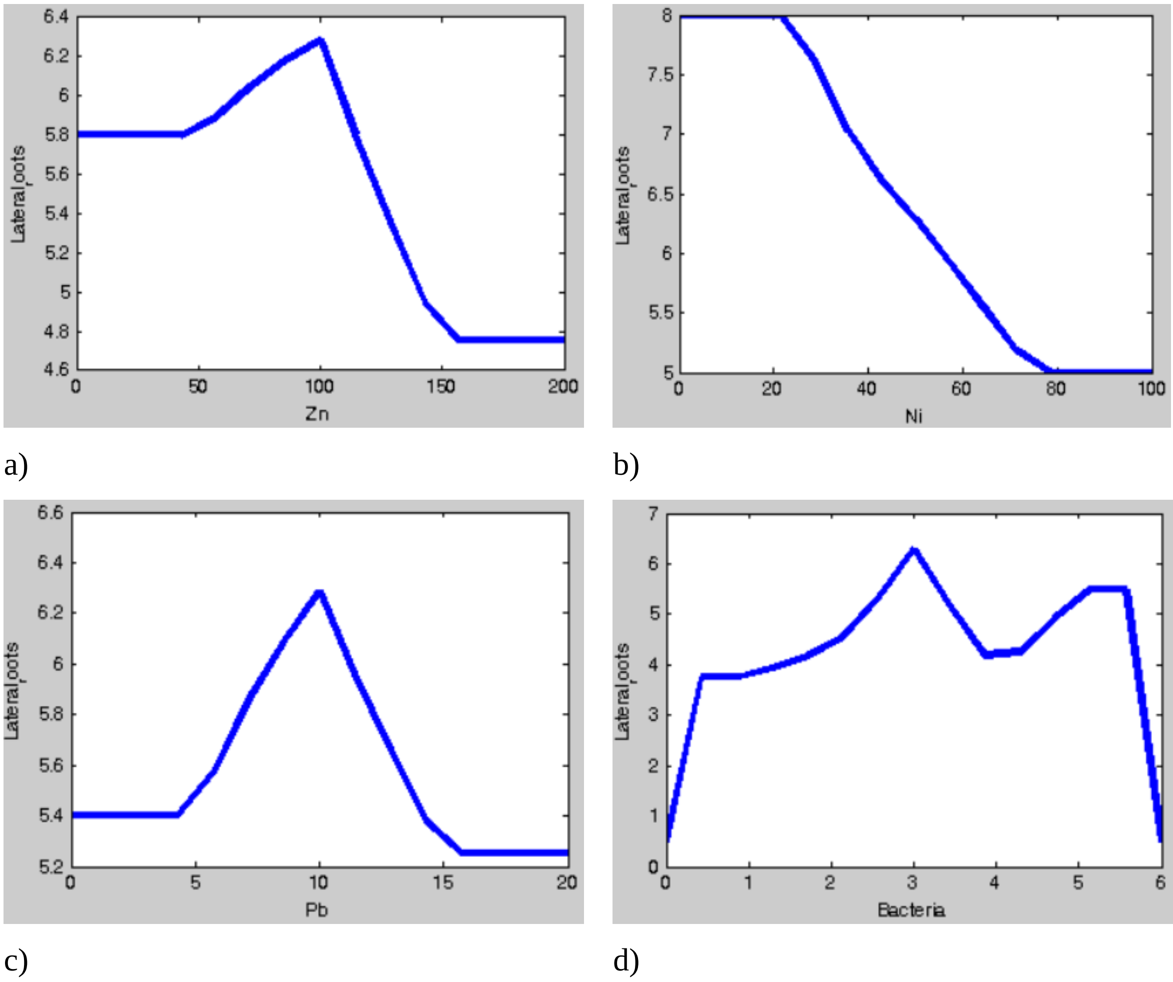

Figure 5. Relations between Zn and the number of lateral roots (a), and between Ni and the number of lateral roots (b), between Pb and the number of lateral roots (c), between bacteria and the number of lateral roots (d)

Fig. 6 shows the 3-dimensional relationships between the inputs and the output.

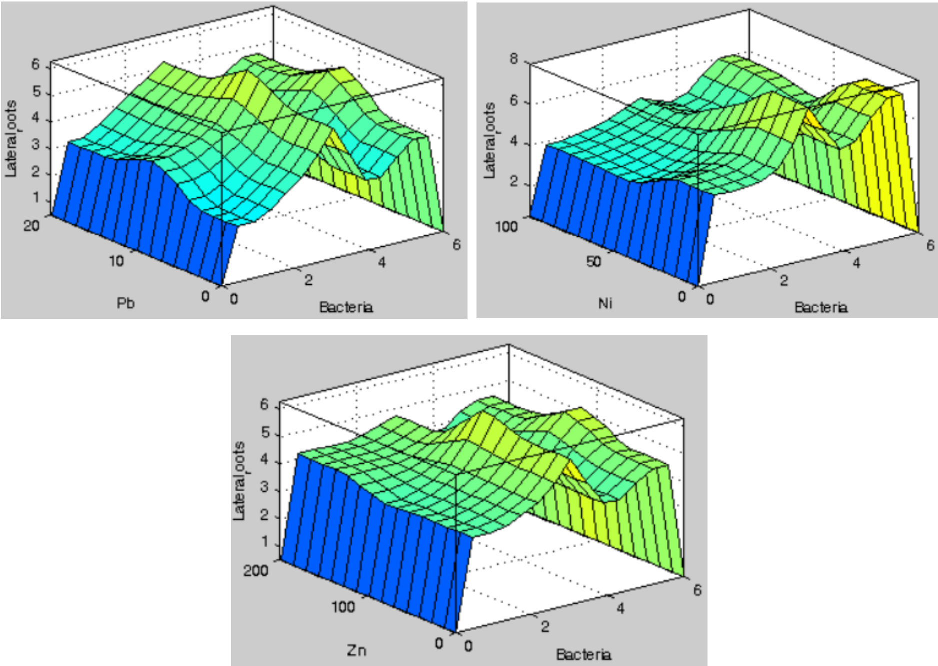

Figure 6. Relations between nutrients, bacteria and the number of lateral roots

For example, with given 10 ppm $Pb(NO_3)_2$ and KF3B bacteria, the observed output value appears next to the number of lateral roots (8.5). Other results can be found by supplying different input data. Thereafter, all data were run through the FL model, with the results of the number of lateral roots (Fig. 7).

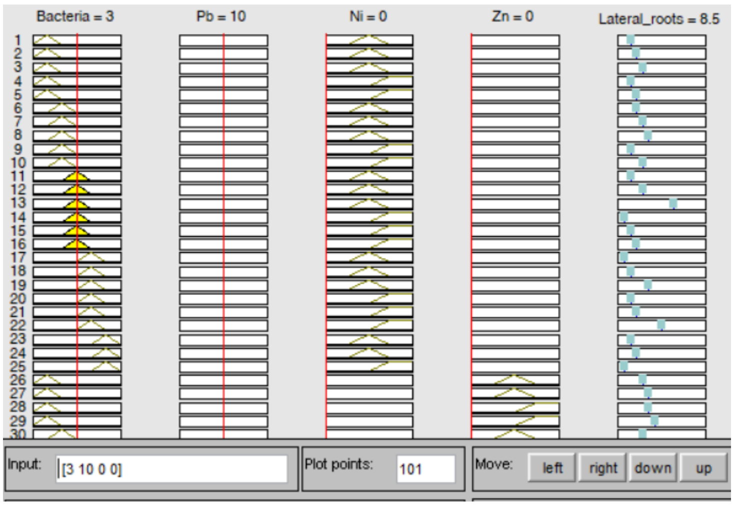

Figure 7. Results window of FLM

The coefficient of correlation between real estimated and predicted by means of FL was r=0.69.

CONCLUSION

This paper was aimed to estimate the number of lateral roots by means of FL. Using the input data of different doses of minerals like Pb, Zn, Ni and bacteria model was able to predict the number of lateral roots of *Sesbania punicea* at a level of success. In the end, the study's FL model revealed coefficient of determination rate of r=0.69. It can be said that a fuzzy logic model can be applied in areas where conventional methods is not productive. Considering some different factors, a more punctual and efficient system can be developed in the future.

Statement of Conflict of Interest

The authors declare that they are no conflict of interest.

Authors' Contributions

AÇ and NM designed and analyzed the research, AÇ and NM studies arranged. NM 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.

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The Effect of Bacteria Applications Showing ACCD Activity on the Seedling of *Citrullus lanatus (Tunb)* Seeds under Salt Stress

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ABSTRACT: Watermelon seeds were germinated in a salty condition and coding was done to encourage plant growth. In the study, the results of bacterial application were investigated against the germination inhibitory effect of salt environments. Germination percentages and germination rate of *Citrullus lanatus* (Thunb) seeds used in the study were recorded. The effects of TV14B (*Stenotrophpmonas maltophilia*) bacteria used in the study on the germination rate and germination rate of watermelon seeds germinated in salt medium were determined. Salt stress was induced by using two doses of NaCl (control, 150 and 300 mM NaCl). As a result of the research, the effect of TV14B (*Stenotrophpmonas maltophilia*) bacteria on salt stress was determined in terms of germination rate and germination rate of *Citrullus lanatus* (Thunb) seeds. Observations were made daily to determine the rooting and germination percentage of watermelon seeds.

Keywords: Bacteria, Germination, NaCl, *Citrullus lanatus* (Thunb), Salt stress

INTRODUCTION

While only 10% of agricultural production areas are not damaged by any stress, the remaining 90% area is under the negative effects of some stresses. While salinity affects 20% of agricultural production areas, drought affects 26% negatively and severely (Blum, 1985; Ashraf, 1994). It is predicted that 50% of agricultural land will be lost due to salinity in the middle of the 21st century (Alcázar et al., 2020). Salinity is one of the most important environmental stresses limiting production (Majeed et al., 2010). According to FAO (2021b) determinations, salinity is one of the leading abiotic stresses that negatively affect the production potential at a rate of 46 million ha/year. Salinity, which lowers the plant biomass, negatively affects the physiological activities of plants and also causes damage to their biochemical activities. Salinity, which is effective in every stage of vegetative activities from seed germination to product production, in short, shows its effect in all stages of seedling development, green parts formation and seed formation.

Cultivation of the watermelon vegetable dates back to ancient times; It has a history of 4000 years in Egypt, and its cultivation in China and Russia dates back to the 10th century. It was thanks to the Spaniards that the American continent met the watermelon vegetable (Robinson and Deckers-Walters, 1997). The continuity of the development of new varieties resistant to biotic (fusarium, etc.) and abiotic (drought, salinity, etc.) conditions shows the importance of this vegetable in agriculture. The watermelon vegetable, whose roots reach up to about 2 m (1.8 m), is drought tolerant with this feature. *Citrullus* species including watermelon, which is one of the important species of *Cucurbitaceae* family, have diploid (2n=22) chromosome number. It can be grown especially in the hot regions of Africa, Asia and the Mediterranean with high evaporation rates (Jeffrey, 1975; Whitaker and Davis, 1962; Robinson and Decker-Walters, 1997). Africa is the gene center of the *Citrullus* species.

Most members of cucurbits (*Cucurbitaceae*) (pumpkin, watermelon, melon, cucumber, conomon) are grown in our country, and our country is one of the diversity centers of this family. For example, Türkiye (Van) is the secondary gene center of the melon vegetable. For this reason, the necessity of developing varieties resistant to abiotic stresses such as salinity and drought is also important in terms of product range. Because abiotic stress factors are among the most important factors limiting food production (worldwide). Among abiotic stresses, drought and salinity were found to cause more severe damage than other abiotic stresses. This species is known to be resistant to abiotic stresses. For example, melon, one of the important species of the *cucurbit* family, is one of the first vegetables that can grow in arid and semi-arid areas under drought and salinity conditions (Kusvuran et al., 2010). Rhodes et al. (2002) states that vegetables that are resistant to salinity and drought conditions, which are abiotic stresses, accumulate a few osmolites with organic compatible soluble properties under osmotic stress conditions. Osmolytes increase the water uptake of plants from the soil and ensure that the turgor (cell) capacity is not affected (Wang et al., 2003). For example, citrulline was found to accumulate more in salinity and drought conditions. The largest producer of watermelon vegetables, which is approximately 100 million tons, is the country of China (FAO, 2021a). In terms of watermelon production, Asia is followed by America, Africa, Europe and Oceania in the order of continents. Produced in hot and warm climates, the watermelon vegetable is produced in large areas in the world due to the climate demand. Due to its nutritional value and being a popular vegetable, the amount of production is high.

MATERIALS AND METHODS

The study was conducted in Siirt University Faculty of Agriculture, Department of Field Crops Tissue Culture Laboratory under sterile conditions with 3 replications according to "Randomized Lots Test Pattern". In each recurrence, the seeds were sown in glass petri dishes, 10 seeds each, on coarse filter paper, and watered with sterile distilled water as needed. All steps of the treatments were carried out under sterile conditions and the petri dishes with the seeds were kept in darkness in a climate chamber set at 24±2 °C. Daily observations were made and recorded in order to calculate germination speed and germination percentage ratios of the seeds. The obtained data were analyzed by Excel software and the average % rooting and germination values were calculated.

The effects of the bacteria on the initial development period of *Citrullus lanatus* plant in salt conditions were investigated by coding the seeds of *Citrullus lanatus* plant to encourage germination percentage ratios of the seeds and germination speed. Bacteria have been isolated from Van lake ecological conditions. 1 bacterial strain (TV14B), which was found to have ACCD (1-aminocyclopropane-1-carboxylate deaminase) activity, which were proven to be superior in laboratory tests and tests that determined their effects on plant growth and which are effective in terms of resistance to stress conditions, were used. Abiotic stress in the study was created by using salt (control, 150 mM and 300 mM NaCl). NaCl concentrations were applied at the seed sowing stage and once at 3 ml. As a result of the research, the effect of bacteria showing ACCD activity on NaCl stress in *Citrullus lanatus* plant was determined.

Preparation of Bacterial Suspensions and Inoculation Techniques:

Nutrient Broth (NB) medium was used to prepare the inoculum. A loopful of 24-hour bacterial cultures previously developed in Nutrient Agar (NA) broth was inoculated into 100 ml of NB and incubated overnight at 30 ºC at 150 rpm/min on a horizontal shaker. Then the mixture was diluted with sterile distilled water and the bacterial concentration was adjusted to 108 CFU/ml with a spectrophotometer.

Seed Disinfection and Bacterial Inoculation:

Citrullus lanatus seeds were soaked in 20 % sodium hypochlorite for 30 minutes and disinfected, then washed with sterile distilled water and dried (Inan, 2007). The seeds were then inoculated with the bacteria to be tested before sowing. The inoculation process was carried out by immersing these seeds in a bacterial suspension at a concentration of 108 CFU/ml for 60 minutes. Seeds that were not treated with bacteria for control were kept in NB diluted with sterile distilled water (Heinonsalo et al., 2004).

RESULT AND DISCUSSION

The differences between the germination rate and rate of watermelon seeds tried to germinate under salt stress conditions were found to be statistically significant (Table 1). The fact that the bacteria application alone is lower than the control application, and the fact that the salt applications alone have a higher germination rate and speed than the other interaction groups and control, is also a subject that needs to be studied. It was determined that the application with the highest germination rate among the applications had the Salt (EC) application value (93.3%), while the lowest value (46.7%) was obtained from the bacteria (TV14B) application. When examined in terms of the effects of different applications on the germination rate of watermelon seeds, the applications were statistically divided into two groups;

It was determined that EC groups, EC*TV14B interaction groups and control group were in the first group statistically, while the bacteria (TV14B) group took the last place.

Table 1. Germination rate and speed in different applying

Aplications	Germination Rate (%)	Germination Speed
EC 1	93,3 a	31,1 a
p	< 0,05	< 0,05
EC 2	90,0 a	30 a
p	< 0,05	< 0,05
Control	86,7 a	28,9 a
p	< 0,05	< 0,05
TV14B	46,7 b	15,6 b
p	< 0,05	< 0,05
TV14B*EC1	83,3 a	27,8 a
p	< 0,05	< 0,05
TV14B*EC2	90,0 a	30,0 a
p	< 0,05	< 0,05

When the germination rate values were examined, it was determined that there were differences between the applications in parallel with the germination percentage values, and they were in two different groups statistically. It was recorded that the highest germination rate value (31.1) was obtained from the EC1 application, and the lowest value (15.6) was obtained from the bacterial application with the TV14B code. It has been determined as the research output that should be emphasized that the control application is in the first statistical group and its value is higher than both the bacteria group alone and the bacteria*salt interaction groups. It is stated in the literature that the *Cucurbitaceae* family is tolerant and even resistant to drought and salt stress with the various enzymes it contains. Kusvuran et al. (2010) pointed out that the melon vegetable is one of the first vegetables that can be grown in arid and semi-arid areas. Especially in areas where the summer season is dry, waterless melon and watermelon cultivation is common in our country. Because vegetables that are resistant to abiotic stresses such as salinity accumulate osmolite in their bodies (Rhodes et al., 2002). Wang et al. (2003) also determined that osmolytes increase the water uptake of plants from the soil and ensure that the cell turgor capacity is not affected.

Statement of Conflict of Interest

The authors declare that they are no conflict of interest.

Authors' Contributions

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.

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The Effect of Bacteria Applications Showing ACCD Activity on the Seedling of *Solanum melongena* Seeds under Lead Stress

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ABSTRACT: *Solanum melongena* seeds were tried to germinate under lead stress conditions and coded with plant growth promoting bacteria (PGPB). In the research, the results of the bacterial application against the negative effects of lead applications on germination were examined. In addition to lead and bacteria applications, a control group was also formed. The effects of *Stenotrophomonas maltophilia* (TV14B) bacteria used in the study on the germination speed and germination rate of eggplant seeds in lead stress conditions were determined. Abiotic stress in the study was created by using Pb [Pb(NO3)2] (control, 50 and 100 ppm). The experiment was set up in a randomized block design with 3 replications. 10 *Solanum melongena* seeds were used in each replication. Compared with the control plots, it was determined that bacterial application had a positive effect on germination rate and germination rate in both lead stresses.

Keywords: PGPB, Lead, Abiotic stress, Seedling, *Solanum melongena*

INTRODUCTION

Eggplant (*Solanum melongena* L.), which is frequently included in the daily nutrition menu, can be grown in temperate and tropical climates. The most eggplant production in the world takes place in China. While India ranks second with 12 million tons of eggplant production, our country ranks 4th in the world with approximately 830 thousand tons (FAO, 2021). Eggplant, which contains phenolics that are especially effective in type 2 diabetes, is a nutritious vegetable that can regulate blood sugar and normalize the glucose ratio, as well as being a source of fiber. Eggplant, which contains various vitamins, is rich in minerals such as magnesium, iron, calcium, as well as various compounds (polyphenolic), fatty acids and amino acids Kwon et al., 2008; Sharma and Kaushşk, 2021).

Eggplant, which is defined as brinjal in its homeland India, has a diploid chromosome (2n=24) structure. It is grown abundantly in European countries such as Türkiye, Italy and France, especially in Far East countries (Kalloo, 1993).

In addition to the name Brinjal, the name of the eggplant, known by names such as Guinea squash, melongena, eggplant, aubergine, derives from their appearance or the meaning of local peoples or transfer from various languages. For example, small eggplants with white fruit color are called eggplant in the USA and Australia, or the scientific definition of the name melongena. It is widely grown in Asian countries, especially in China, India, and in the Middle East and Mediterranean regions (Daunay, 2008; Cericola et al., 2013). Eggplant, which is from the *Solanaceae* family, is in the genus Solanum (Sukprasansap et al., 2019). Niño-Medina et al. (2017), while eggplant is divided into three types (egg, long, thin and dwarf) according to fruit shape, Ullah et al. (2014), on the other hand, classified the eggplant vegetable with a more detailed grouping, with different evaluations from cooking quality to the color of the midrib, even taking into account local biodiversity.

Heavy metal accumulations not only reduce the quality of life of people but also create negative effects on other living things. Heavy metals, which affect the nutrition quality of people directly (vegetables-fruits) or indirectly (forage plants, etc.) and create important health problems, become more and more important with the rapid spread of the industry. Because heavy metals reduce the quality of the healthy environment of people, as well as deteriorate the content quality of plants. As a result of heavy metal contamination of soil and water, it means that nutritional arguments are also negatively affected. Heavy metal accumulation, especially lead, is in question in production areas intertwined with areas where industrialization is intense. Industry is the primary source of heavy metal accumulation, especially in cases where industrial residues are likely to contaminate water resources or mix with groundwater. Heavy metals disrupt the normal functioning of the life cycle, primarily by disrupting plant photosynthesis activities. As a result of the accumulation in various organs of the plant (leaf, petioles, stem, etc.), it causes both damage to the content of the plant and the metabolism of the living things (human, animal) that consume the plant in question, causing various and important health problems (Dere, 2019). Lead accumulation does not show itself immediately, damage to health may appear in the future. Heavy metals such as lead cause anion cation exchange in the soil and the roots cannot develop in the plant. The accumulation of lead also causes the leaves to remain small and therefore the photosynthesis function is not fulfilled sufficiently. Lead accumulation, which affects the negative changes of internal chemistry from the plant leaf area to the cell wall, can also cause negative effects in the genetic structure of the plants (Sharma and Dubey, 2005; Bradley et al., 2018). As in all stress situations, changes in the self-defense mechanism against various changes occur in plants exposed to heavy metal accumulation. These changes, which are beneficial to a certain extent, cause damage both to themselves and to those who benefit from them in the event of continuity and overdoses. Heavy metals accumulated in the plant are eliminated with phytochelatin in plant roots (Salt et al., 1995). In areas with high lead concentration, the intake of some macro and micro nutrients is also adversely affected. For example, zinc, manganese phosphorus intake decreases in high lead environment. Although lead accumulation differs between cultivars, it has been found to reduce dry matter accumulation in tomato vegetables (Aksu and Yıldız, 2007) and eggplant rootstocks (with variations between tolerance and sensitive genotypes) (Topal et al., 2017). According to the findings of Akıncı and Çalışkan (2010), it was determined that the eggplant vegetable was more sensitive to lead stress than the other types used in the experiment. It is among the other research findings that morphological developments are affected in plants under lead stress (Özkay et al., 2014). Lead content at a reference range of 2-300 mg/kg in the soil, which is the basis of plant growth, is accepted as acceptable. However, when it comes to plants, this range is; It is predicted to be between 0.2-20 mg/kg (Kabata-Pendias and Pendias, 1984).

While organic acids, phenolics, sugars etc. root secretions support the life of bacteria, these secretions also increase their bioavailability. Bacteria both contribute positively to biomass increase and create various positive changes in the tolerance of plants to abiotic stresses. According to the determinations of Gulzar and Mazumder (2022), PGPRs direct phytostabilization and regulate phytoextraction activities. Thanks to bacteria, plants provide resistance to abiotic stresses (Jan et al., 2021; Nadeem et al., 2014; Rashid et al., 2021). Among the most important features of plant growth promoting bacteria (PGPR), it promotes colonization on root surfaces and provides more nutrient uptake from the soil. Since PGPRs are mobile in the soil, they can adhere to all parts of the root surfaces (Jan et al., 2021). PGPRs, which activate systemic resilience in plants under abiotic stress conditions, are a group of microorganisms that accelerate plant growth (Dutta and Khurana, 2015; Etesami and Beattie, 2018).

MATERIALS AND METHODS

The study was conducted in Siirt University Faculty of Agriculture, Department of Field Crops Tissue Culture Laboratory under sterile conditions with 3 replications according to "Randomized Lots Test Pattern". In each recurrence, the seeds were sown in glass petri dishes, 10 seeds each, on coarse filter paper, and watered with sterile distilled water as needed. All steps of the treatments were carried out under sterile conditions and the petri dishes with the seeds were kept in darkness in a climate chamber set at 24±2 °C. Daily observations were made and recorded in order to calculate germination speed and germination percentage ratios of the seeds. The obtained data were analyzed by Excel software and the average % rooting and germination values were calculated.

The effects of the bacteria on the initial development period of *Solanum melongena* L. plant in Pb [Pb(NO3)2] conditions were investigated by coding the seeds of *Solanum melongena* L. plant to encourage germination percentage ratios of the seeds and germination speed. Bacteria have been isolated from Van lake ecological conditions. 1 bacterial strain (TV14B), which was found to have ACCD (1-aminocyclopropane-1-carboxylate deaminase) activity, which were proven to be superior in laboratory tests and tests that determined their effects on plant growth and which are effective in terms of resistance to stress conditions, were used. Abiotic stress in the study was created by using Pb [Pb(NO3)2] (control, 50 and 100 ppm). Pb concentrations were applied at the seed sowing stage and once at 3 ml. As a result of the research, the effect of bacteria showing ACCD activity on Pb stress in *Solanum melongena* L. plant was determined.

Preparation of Bacterial Suspensions and Inoculation Techniques:

Nutrient Broth (NB) medium was used to prepare the inoculum. A loopful of 24-hour bacterial cultures previously developed in Nutrient Agar (NA) broth was inoculated into 100 ml of NB and incubated overnight at 30 ºC at 150 rpm/min on a horizontal shaker. Then the mixture was diluted with sterile distilled water and the bacterial concentration was adjusted to 108 CFU/ml with a spectrophotometer.

Seed Disinfection and Bacterial Inoculation:

Solanum melongena L. seeds were soaked in 20% sodium hypochlorite for 20 minutes and disinfected, then washed with sterile distilled water and dried (Yaşar et al., 2011). The seeds were then inoculated with the bacteria to be tested before sowing. The inoculation process was carried out by immersing these seeds in a bacterial suspension at a concentration of 108 CFU/ml for 60 minutes. Seeds that were not treated with bacteria for control were kept in NB diluted with sterile distilled water (Heinonsalo et al., 2004).

RESULT AND DISCUSSION

Despite the statistical difference between the applications, contrary to expectations, high germination rate and speed were not found in bacteria (Table 1). Interestingly, the highest germination percentage (93.3% and 31.1%) and germination rate were recorded from the highest lead application. Pb1 application was followed by Pb2 (90.0%) and TV14B*Pb2 (90.0%), respectively. Another interesting result is that bacterial application alone had the lowest germination percentage (46.7%) and the lowest germination rate (15.6). It was determined that the control group had lower germination percentage (86.7%) and germination rate (28.9) than both Pb applications. TV14B*Pb1 application (83.3%) provided a lower germination percentage than Pb1 (93.3%) application alone. Similar results are seen in germination rate; It was determined that TV14B*Pb2 germination rate (30.0) was lower than Pb1 application (31.1) and Pb2 application had the same values as germination rate (30.0).

Table 1. Germination rate and speed in different applying

Applications	Germination Rate (%)	Germination Speed
Pb 1	93,3 a	31,1 a
p	< 0,05	< 0,05
Pb 2	90,0 a	30 a
p	< 0,05	< 0,05
Kontrol	86,7 a	28,9 a
p	< 0,05	< 0,05
TV14B	46,7 b	15,6 b
p	< 0,05	< 0,05
TV14B*Pb1	83,3 a	27,8 a
p	< 0,05	< 0,05

TV14B*Pb2	90,0 a	30,0 a
p	< 0,05	< 0,05

According to the research of Özay (2008), it was determined that there was a decrease in the germination rate, underground organ and stem lengths in feed turnips together with lead concentration. It has been determined that plants under lead stress are affected from germination success to nucleic acid structures (Munzuroğlu and Geçkil, 2002). The reason for the difference with our findings may be due to the use of different species.

It is understood from the literature that the high lead concentration causes the accumulation of dry matter and the change (deterioration) of the internal chemical structure of the plants rather than the first stages;

As the lead concentration increases in plant cultivation areas, decreases are observed in the phosphorus, zinc and manganese intake of the plant in question. Lead accumulation the dry matter accumulation of tomato vegetable (with differences between cultivars) was affected. In the study examining the effects of lead stress on cultivar performance among tomato cultivars, it was noted that although dry matter accumulation varied among cultivars, dry matter accumulation decreased in all cultivars (Aksu and Yıldız, 2007). In the analyzes made on plants in a high-density (300 ppm) lead environment, an increase in SOD (superoxide dismutase) and GR (gutation reductase) activities was observed, as well as low dry matter accumulation of plants, short stature in various organs (stem, root), and decrease in leaf areas that directly affect photosynthesis (Kıran et al., 2015).

It is understood that the real results of our research will be understood in a long time, and the negative effects of lead concentration will appear in the advanced stages of plants.

Statement of Conflict of Interest

The authors declare that they are no conflict of interest.

Authors' Contributions

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.

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Investigation of the Chemical Profiles, Antioxidant and Enzyme Inhibition Activities of the Polar and Apolar Extracts of *Elaeagnus umbellata*

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ABSTRACT: The utilization of plants as sources of both active compounds and precursors of them has been increasing since the discovery and identification of phytochemicals, and the exploration of novel candidates for plant-derived drugs or supplements is still a prominent topic. Herein, a comprehensive evaluation was performed on the potential for pharmaceutical and nutritional usage of *Elaeagnus umbellata*, a promising source of bioactive compounds. The qualitative and quantitative phytochemical composition (a total of 60 phenolic and triterpene compounds) of solvent extracts (water and ethanol) *of Elaeagnus umbellata* fruit, prepared by ultrasound-assisted extraction, was analyzed using LC-MS/MS and GC-MS techniques. The antioxidant activity of the extracts was determined using ABTS radical scavenging activity and CUPRAC assays. The inhibition activities of the extracts against the enzymes that are the main targets of the treatment of neurodegenerative disorders (tyrosinase, acetylcholinesterase, and butyrylcholinesterase), ulcers (urease), hyperpigmentation (elastase, collagenase), and hypertension (angiotensin-converting enzyme) were investigated. The maximum ACE inhibitory activity (77.86%) was obtained from the polar extract of *Elaeagnus umbellata* fruit, while the apolar extract inhibited only 55.86% of the activity. The results revealed the biological properties of *Elaeagnus umbellata* extracts and the effects of solvent polarity on these properties.

Keywords: *Elaeagnus umbellata*, Phenolic profiles, Triterpene content, Enzyme inhibition, Antioxidant activity

INTRODUCTION

Plants have been essential components of traditional medicines used to treat a variety of human and animal diseases throughout civilizations since prehistoric times. They have become one of the main components of modern medicine with the revelation of the phytochemical content of plants and their bioactivity, along with technological developments. The extracts of different parts of several plants have been used as plant-derived pharmaceutical products due to a variety of therapeutic characteristics. Since less than 30% of the secondary metabolites of plants have been isolated and their biological activities have been revealed (Wink, 2010), *it is crucial to enhance our knowledge of the functional roles of plants in order to identify novel potential therapeutics.*

Elaeagnus umbellata, also called cardinal olive, autumn olive, or autumn elaeagnus, is one of the 70–80 species belonging to the Elaeagnaceae family, which shows distribution throughout Asia, North

America, Europe, and Australia. *E. umbellata* is a deciduous 3–5-m shrub that grows in temperate and subtropical climates at altitudes ranging from 1200 to 2100 m and produces a bright red, tiny, tasty, juicy drupe (Nazir et al., 2020). The edible berries of this plant have been used in traditional medicine to treat inflammation, asthma, pulmonary infections, coughs, and high blood pressure (Gamba et al., 2020). The diversity of their folkloric uses makes it important to investigate the pharmacological potential of *Elaeagnus* species by revealing their chemical composition. A limited number of scientific studies have revealed that the autumn olive berry is an excellent source of vitamins (particularly vitamins A, C, and E), minerals, and fatty acids (Ahmad et al., 2006). Moreover, this species has been shown to contain secondary metabolites such as β-carboline alkaloids, coumarins, anthraquinones, glycosides, sterols, carotenoid, and flavonoids (Paudel et al., 2019). Bioactivity analysis indicated that different parts of *E. umbellata* have antiplasmodial, antioxidant, antibacterial, anti-proliferative, and antihypertensive activities (Minhas et al., 2013; Ozen et al., 2017; Paudel et al., 2020).

The aim of the present work is to advance our knowledge of *E. umbellata* by assessing its fruits as possible sources of bioactive substances. For this purpose, polar and apolar extracts (water and ethanol) of *E. umbellata*'s fruits were prepared by ultrasound-assisted extraction. The presence of 53 phenolic compounds in the extracts was comprehensively investigated qualitatively and quantitatively by LC-MS/MS techniques. The presence of seven triterpene species in the extracts was examined using GC-MS techniques. The antioxidant activity of the extracts was determined using ABTS radical-scavenging activity and CUPRAC assays. The inhibition activities of the extracts against the enzymes that are the main targets of the treatment of neurodegenerative disorders (tyrosinase, acetylcholinesterase (AChE), and butyrylcholinesterase (BChE)), ulcers (urease), hyperpigmentation (elastase, collagenase), and hypertension (angiotensin-converting enzyme (ACE)) were investigated.

MATERIAL AND METHODS

1. Plant collection

The dried fruits of *E. umbellata* were purchased from a local market in Giresun, Turkey.

2. Preparation of polar and apolar extracts of the *E. umbellata*'s fruits

10 g of dried fruits crushed in a laboratory blender (Waring Commercial Blender, USA) were transferred to a centrifuge tube, and 100 mL of either deionized water or a mixture of ethanol and water (50:50, v:v) was added to the berries, which were then sonicated in an ultrasonic water bath (SK06GT Kudos ultrasonic water bath, Korea) for 30 minutes. During sonication, the temperature of the ultrasonic bath was kept in the range of 30–40 °C with the addition of ice. The extract was centrifuged at 8,000xg (Hanil Science Industrial Combi 514R, Korea) for 15 minutes. The supernatant was taken into a different tube. 30 mL of ethanol-water (50:50, v:v) or deionized water mixture was added to the pellet and

sonicated again. After repeating this process three times, the three supernatants were combined. The extracts were stored at -80 °C until analysis (Meng et al., 2011).

3. Qualitative and quantitative analyses of phytochemical content of the extracts

3.1. Determination of the phenolic components using LC-MS/MS

The phenolic composition of the extracts was analyzed according to the method reported by Yener et al. (2018) and Yilmaz et al. (2020) using a Shimadzu-Neexera model UHPLC (ultra-high performance liquid chromatograph) combined with a Shimadzu-LCMS 8040 model triple quadrupole mass spectrometer. The liquid chromatography system included an analytical column (Inertsil ODS-4 model C18, 100 mm×2,1 mm, 2µm), an autosampler (SIL-30AC model), binary pumps (LC-30 AD model), a degasser (DGU- 20A3R model) and a column oven (CTO-10ASvp model). The final concentrations of the extracts were 250 mg/L and all samples were filtered before the injection. The solvent flow rate was 0.5 mL/min and the injection volume was 5 mL. The eluent A consisted of water, 5 mM ammonium formate, and 0.1% formic acid; and the eluent B consisted of methanol, 5 mM ammonium formate, and 0.1% formic acid. The gradient elution profile was as follows: 20–100% B (0–25 min), 100% B (25–35 min), 20% B (35–45 min) (Yener et al., 2018; Yilmaz, 2020)

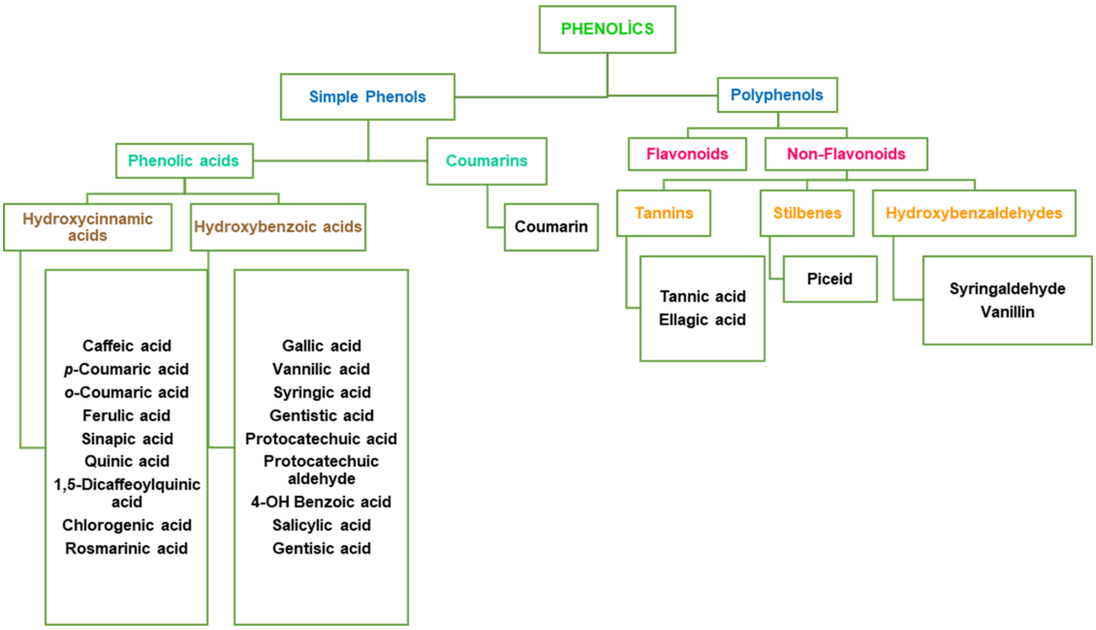

Figure 1. Phenolic standards used for LC-MS/MS analysis

3.2. Determination of the triterpenoid contents using GC–MS

The presence of seven triterpenoids in the extracts was assessed using an Agilent 7890A model gas chromatography and an Agilent 5977B model mass spectroscopy system. A HP-5MS column (30 m × 0.25 mm × 0.25 µm film) was used for chromatographic separation. Fixed helium gas (1 mL/min, 20 psi) was installed as the carrier gas. The GC oven was preheated to 80 °C for 2 minutes, then raised to 300 °C at a rate of 5 °C per minute, where it remained for 14 minutes. The volume of injection was set to 1.0 μL and the split ratio was 1:10. The ionization energy of the electron ionization/mass spectrometer (EI/MS) was adjusted to 70 eV. MS data were collected by setting the complete scan mode and scan m/z to a density of 50-800 atomic mass units (amu). N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane was used for derivatization of samples. The concentration of the samples was 1000 mg/L (Bakir et al., 2020).

4. Determination of the enzyme inhibition activity

4.1. Tyrosinase inhibition activity

Tyrosinase inhibition activity was performed with slight modifications based on the method suggested by Hearing and Jiménez (1987)(Hearing & Jiménez, 1987). Briefly, 150 μL of phosphate buffer (pH=8), 10 μL of the extract (4 μg/L), and 20 μL enzyme solutions (28 nM) were mixed in a microplate and the mixture was incubated at 37 °C for 10 minutes. Then, 20 μL of L-DOPA (0.5 mM) was added as substrate, and the mixture was incubated at 37 °C for 10 minutes. The absorbance of the samples was measured at 475 nm. α-kojic acid was used as the standard.

4.2. Elastase inhibition activity

Elastase inhibition activity was carried out with some modifications based on the method suggested by Kraunsoe et al. (1996) (Kraunsoe et al., 1996). Briefly, 40 μL of Tris-Cl buffer (0.1 M, pH=8), 10 μL of extract (4 μg/L), and 20 μL of enzyme solutions were mixed and preincubated for 10 minutes at 37 °C. 30 μL of 1.015 mM N-succinyl-(Ala)3-nitroanilide solution in 0.1 M Tris-Cl (pH:8) was added to the mixture and further incubated at 37 °C for 20 minutes. The absorbance of the samples was measured at 410 nm. Oleanolic acid was used as the standard.

4.3. Collagenase inhibition activity

Collagenase inhibitory activity was carried out with slight modifications based on the method suggested by Thring et al. (2009). 50 μL of collagenase solution (0.8 U/mL), 50 μL of extract, and 0.9 mL of Tris buffer (pH:7.5) were mixed and incubated at 25°C for 30 minutes. Then, 0.05 mL of 1 mM N-(3-[2-Furyl] acryloyl)-Leu-Gly-Pro-Ala was added to the mixture. The mixture was further incubated at 25 °C for 15 minutes. The absorbance of the samples was measured at 340 nm. Epicatechin gallate was used as the standard (Thring et al., 2009).

4.4. Acetylcholinesterase and butyrylcholinestase inhibition activity

The AChE and BChE inhibition activities were carried out according to Ellman's method with minor modifications (Ellman et al., 1961). 130 μL of phosphate buffer (0.1 mM, pH 8) and 20 μL of AChE (Type-VI-S, EC 3.1.1.7, 0.22 U/mL) or BChE (EC 3.1.1.8, 0.1 U/mL) were added to all wells of a 96-well microplate. 10 µL of extract solution in ethanol (0.5-0.125 mM), galanthamine (a positive control), or etanol (a negative control) were added to the wells. The plate was incubated at 25 °C for 10 minutes. Subsequently, 10 µL of DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)) was added to each well. The reaction was started by the addition of 20 µL of acetylthiocholine (0.71 mM) or 10 µL of butyrylthiocholine iodide (0.2 M) for AChE or BChE, respectively. The formation of the 5-thio-2-nitrobenzoic acid anion was monitored using a microplate reader (Multiscan Sky, USA) at 412 nm.

4.5. Urease inhibition activity

The urease inhibition activity was assessed using the method described by Hina et al. (2015)(Hina et al., 2015). 25 µl of urease solution was mixed with the 10 µl each extract (4000 ppm) and incubated at 30 °C for 15 minutes in a 96-well plate. Then, 50 µL urea (100 mM) was added, and the plate was incubated at 30 °C for 15 minutes. 45 µl each of phenol reagent (1%, w/v, phenol and 0.005%, w/v, sodium nitroprusside) and 70 µL of alkali reagent (0.5%, w/v, NaOH and 0.1% NaOCl) were added to the wells, and the plate was reincubated at 30 °C for 15 minutes. The absorbance of the samples was measured at 630 nm. Thiourea was used as a standard. The percentage of inhibition was calculated from the following equation:

$$\text{Inhibition\%} = (A_{control} - A_{sample}) / A_{control} \times 100$$

4.6. ACE inhibition activity

The ACE inhibition assay was carried out by adapting the method reported by Kwon et al. (2006). 50 µL of the extracts were added to 200 µl of NaCl-borate buffer (0.3 M, pH 8.3) containing 2.0 mU ACE-I solution and pre-incubated at 25 ºC for 10 minutes. 100 µl of hippuryl-histidyl-leucine (5.0 mM) solution was added to the reaction mixture and further incubated at 37 ºC for 1 hour. 150 µl of 0.5N HCl was used to stop the reaction. Lisinopril was used as the standard. The formation of hippuric acid was monitored using HPLC with an UV detector at 228 nm (Kwon et al., 2006). The inhibition percentage was calculated with the following equation.

$$\text{Inhibition\%} = [\text{Area}_{control} - (\text{Area}_{sample} - \text{Area}_{blank})]/(\text{Area}_{control} - \text{Area}_{blank}) \times 100$$

5. Determination of the antioxidant activity of the fruit extracts

Both the ABTS assay and the CUPRAC assay were used to examine the total antioxidant activity of the extracts. Each experiment was performed in triplicate.

5.1. ABTS radical cation decolorization assay

ABTS radical scavenging activity was assessed using the method described by Re et al., 1999. 10 mg of the sample extracts were dissolved in 10 mL of ethanol to create stock solutions. 2, 5, 10, and 20 µL of the stock solutions were taken, and their final volumes were completed to 40 µL with ethanol. The solutions were then mixed with 160 µL of a 7 mM ABTS cation radical solution and was incubated at 25 ºC for 6 min in the dark. The absorbance of each sample was measured at 734 nm. BHT and α-tocopherol were used as the standards. The ABTS radical scavenging activity (% inhibition) was calculated using the following equation.

Inhibition % = ($A_{control}$ – A_{sample}) / $A_{control}$ x 100

5.2. Cupric reducing antioxidant capacity (CUPRAC) assay

The CUPRAC method was applied according to the method suggested by Apak (2004), with some modifications (Apak et al., 2004). In the CUPRAC method, copper (II)-neocuproine (2,9-dimethyl-1,10-phenanthroline) is reduced to a colorful product, which gives absorbance at 450 nm in the presence of antioxidant compounds. 10 mM copper (II) chloride, 7.5 mM neocuproine, and 1 M ammonium acetate buffer at pH 7.0 were prepared and mixed to create the CUPRAC reagent. The CUPRAC reagent was mixed with sample extracts and standards at various concentrations, and the sample absorbances were measured at 450 nm after 1 hour of incubation. The absorbance of the samples was compared to the standards.

RESULTS AND DISCUSSION

Herein, a comprehensive evaluation of the phytochemical composition and bioactivity of the fruit of *E. umbellate* was conducted, and the results provided a comprehensive understanding for assessing the potential of the fruit for pharmaceutical application, also showing the effects of the polarity of the extraction solvent on phytochemical composition and bioactivity.

1. Contents of Phenolic Compounds in the polar and apolar extracts of the fruit of *E. umbellata*

The presence of 53 phenolic compounds in the extracts was investigated by LC-MS/MS, and 19 of the species were found at different levels in at least one of the extracts. Phenolic compounds in the extracts were listed in Table 1.

Table 1. Identification and quantification of phenolic compounds in the extracts of the fruit of *E. umbellate* by LC-MS/MS

No	Analytes	Parent İon (m/z)[a]	MS^2 (Collision Energy)[b]	Quantification (mg analyte/g extract)	
				Polar extract	Apolar extract
1	Quinic acid	190.8	93.0	50.762	8.884
2	Quercetin	301.0	272.9	2.782	3.313
3	Naringenin	270.9	119.0	1.978	2.629
4	Fumaric acid	115.2	40.9	1.263	0.718
5	Chlorogenic acid	353.0	85.0	0.889	0.074
6	Acacetin	283.0	239.0	0.648	1.197
7	Hesperidin	611.2	449.0	0.445	0.213
8	Protocatechuic acid	152.8	108.0	0.305	0.090
9	Hesperetin	301.0	136.0/286.0	0.225	0.237
10	Nicotiflorin	592.9	255.0/284.0	0.224	0.049

11	Isoquercitrin	463.0	271.0	0.145	0.053
12	Kaempferol	285.0	239.0	0.103	0.198
13	Apigenin	268.8	151.0/149.0	0.013	0.020
14	Astragalin	447.0	255.0	0.026	ND
15	Gallic acid	168.8	79.0	0.021	ND
16	Caffeic acid	179.0	134.0	0.018	ND
17	Protocatechuic	137.2	92.0	0.014	ND
18	Salicylic acid	137.2	65.0	0.010	0.005
19	Luteolin	284.8	151.0/175.0	0.003	0.005
20	Ferulic acid-D3	196.2	152.1	IS	IS
21	Rutin-D3	612.2	304.1	IS	IS
22	Quercetin-D3	304.0	275.9	IS	IS

a: Parent ion (m/z): Molecular ions of the standard compounds (mass to charge ratio),b: MS2(CE): MRM fragments for the related molecular ions (CE refers to related collision energies of the fragment ions), IS: Internal standard, N.D: not detected

The content of the respective phenolic compounds differed depending on the use of solvents that had different polarities. The diversity of phenolic compounds in apolar extract was found to be weaker than that of polar extract. Gallic acid, astragalin, caffeic acid and protocatechuic aldehyde were found only in polar extract. The order of the ten most predominant phenolic compounds detected in the polar extract, from the most abundant to the least abundant, was as follows: quinic acid (50.762 mg/g extract), quercetin (2.782 mg/g extract), naringenin (1.978 mg/g extract), fumaric acid (1.263 mg/g extract), chlorogenic acid (0.889 mg/g extract), acacetin (0.648 mg/g extract), hesperidin (0.445 mg/g extract), protocatechuic acid (0.305 mg/g extract), hesperetin (0.225 mg/g extract), and nicotiflorin (0.224 mg/g extract). The ranking in apolar extract was as follows: quinic acid (8.884 mg/g extract), quercetin (3.313 mg/g extract), naringenin (2.629 mg/g extract), acacetin (1.197 mg/g extract), fumaric acid (0.718 mg/g extract), hesperetin (0.237 mg/g extract), hesperidin (0.213 mg/g extract), kaempferol (0.198 mg/g extract), chlorogenic acid (0.074 mg/g extract), and isoquercitrin (0.053 mg/g extract). According to the quantitative examination of the phenolics in the extracts, the polar extract had significantly larger concentrations of the total examined phenolic components than the apolar extracts did. Quinic acid was found to be the most abundant phenolic compound in all extracts. Especially the polar extract was rich with quinic acid, and there was almost a 6-fold difference in the level of quinic acid compared to the apolar extract (Fig. 2). The 34 phenolic compounds were not detected in any of the extracts. Apparently, these species are unusual markers in the fruit of *E. umbellate*.

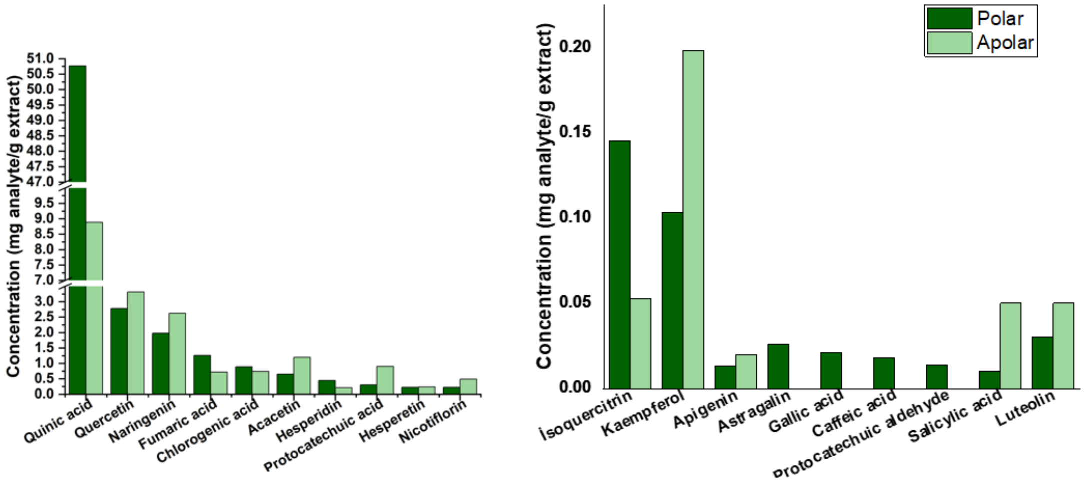

Figure 2. Quantitative analysis of phenolic compounds in the extracts of the fruit of *E. umbellate*

A limited number of studies have been found in the literature investigating the phytochemical content of the fruit of *E. umbellate*. The study conducted by Spinola et al. (2019) showed that the methanol extract of the fruit contained quercetin, kaempferol, diosmetin, gallo(epi)catechin, and ellagic acid derivatives, and in this study, moderate α-glucosidase, α-amylase and lipase inhibition activity was reported (Spínola et al., 2019). The content of total polyphenols, proanthocyanidins, α- and γ-tocopherols of the the fruit of *E. umbellate* was reported in a study conducted by Pei et al., 2015 (Pei et al., 2015). Ozen et al. (2017) also reported the presence of fumaric acid, 4- hydroxybenzoic acid, rutin and quercetin-3-β-D-glucoside, neohesperidin, hesperidin in the fruit extracts (Ozen et al., 2017).

The results revealed that the fruit of *E. umbellate* is an excellent source for phenolic substances with essential biological functions. Quinic acid, chlorogenic acid, and caffeic acid are the simple phenols belonging to the hydroxycinnamic acid classes, and their bioactivities have been proven in the literature. They were found to have anti-inflammatory (Maalik et al., 2016)(Zeng et al., 2009), antiviral (Wang et al., 2009)(Cheminat et al., 1988), anti-diabetic, and anticancer activities (Ooi et al., 2011).

Gallic acid, protocatechuic acid, protocatechuic aldehyde, and salicylic acid are also phenolic acids belonging to the hydroxybenzoic acids. Gallic acid has been used as antioxidants, anticancer, antimicrobial, antiviral, and antidiabetic reagents for pharmacological applications (AL-Zahrani et al., 2020). Antiproliferative, antioxidant, and antiadipogenesis activities of protocatechuic acid and protocatechuic aldehyde have been reported (Byun et al., 2016). Salicylic acid was reported to act as an anti-inflammatory, anti-proliferative, anti-hyperlipidemic, and anti-hyperglycemic reagent (Sidhu & Capalash, 2021).

Apigenin, luteolin, acacetin, quercetin, isoquercitrin, kaempferol, nicotiflorin, astragalin, naringenin, hesperetin, and hesperidin are the flavonoids that were detected in the extracts, and some of them have proven bioactivities as follows: apigenin; anti-inflammatory, anti-proliferation, antibacterial, anti-oxidation, and anti-cancer activity (Bi et al., 2023), luteolin; antioxidant, antibacterial, anti-inflammatory, anti-diabetic, and anti-cancer activities (Punia Bangar et al., 2023), acacetin; neuroprotective, cardioprotective, anticancer, anti-inflammatory, antidiabetic, and antimicrobial activities (Semwal et al., 2019), quercetin; anti-obesity, anti-cancer, anti-oxidant, anti-cardiovascular, anti-aging, and neuroprotective activities (Zou et al., 2021), isoquercitrin; antioxidant, anti-inflammatory, anticancer, antiviral, antihypertensive, and anti-diabetic activities (Valentová et al., 2014), kaempferol; anti-inflammatory, antioxidant, antibacterial, anticancer, and neuroprotective activities (Dong et al., 2023), nicotiflorin; analgesic, antioxidant, anti-hypertensive, and anti-anaphylactic effects (R. Li et al., 2006), astragalin; neuroprotective, anti-inflammatory, antiobesity, antiosteoporotic, antiulcer, antioxidant, anticancer, and antidiabetic activities (Riaz et al., 2018), naringenin; anti-inflammatory, antioxidant, neuroprotective, hepatoprotective, and anti-cancer activities (Motallebi et al., 2022), hesperetin; antioxidant and anticancer (Sohel et al., 2022), hesperidin; antioxidant, anti-inflammatory, and neuroprotective effects (X. Li et al., 2023).

2. Contents of triterpeneoids in the polar and apolar extracts of the fruit of *E. umbellate*

The extracts of the fruit of *E. umbellate* were analyzed for their terpenoid contents using GC/MS. The presence of seven triterpenoid species (Fig. 3.b) was screened in the extracts. The results are given in Fig. 3. Among the seven screened compounds, α-amyrin, oleanolic acid, and ursolic acid were detected in the apolar (ethanolic) extract in remarkable amounts. The results exhibited that the apolar extract was very rich in ursolic acid (3733.45 μg analyte/g extract), whose anti-aging potential was proven in the literature (Bahrami & Bakhtiari, 2016). None of the species were detected in the polar extract. No study has been found in the literature on the triterpenoid content of the fruit of *E. umbellate*.

(a)

	Triterpenoids (μg analyte/g extract)						
	Alpha Amyrin	Moronic Acid	Oleanonic Acid	Oleanolic Acid	Betulinic Acid	Ursolic Acid	Ursonic Acid
Polar extract	ND	ND	ND	ND	ND	ND	ND
Apolar extract	1157.026	ND	ND	828.552	ND	3733.452	ND

(b) (c)

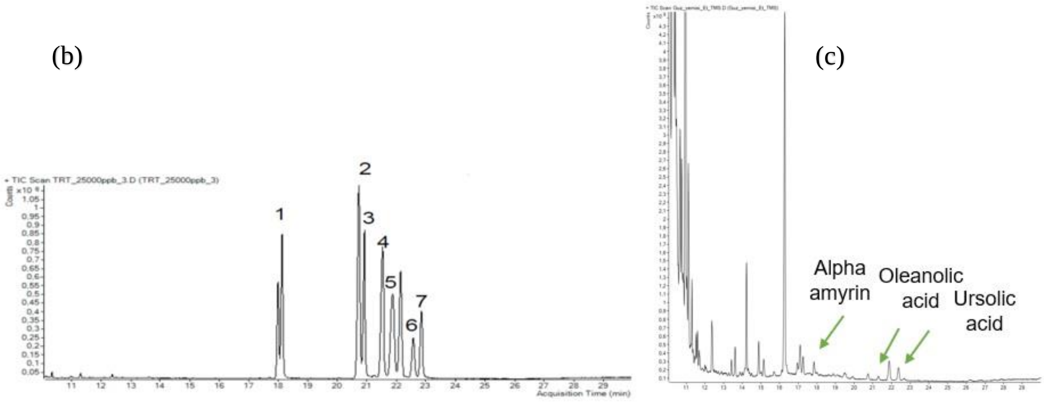

Figure 3. (a) The triterpeneoids results of the fruit extracts of *Elaeagnus umbellate,* (b) TIC chromatogram of standards analysed by GC-MS method. 1: Alphaamyrin, 2: Moronic acid 3: Oleanonic acid, 4: Oleanolic acid, 5: Betulinic acid, 6: Ursolic acid l, 7: Ursonic acid, (c) GC-MS chromatogram of the apolar extract of the fruit of *Elaeagnus umbellate*

3. Enzyme inhibition activities of the polar and apolar extracts of the fruit of *E. umbellate*

To assess the enzyme inhibition activity of the fruit extracts of *E. umbellate,* seven enzymes that are the main targets of the treatment of neurodegenerative disorders (tyrosinase, AChE, and BChE), ulcers (urease), hyperpigmentation (elastase, collagenase), and hypertension (ACE) were evaluated (Fig. 4). While the inhibitory activity of the extracts against elastase, collagenase, and ACE was detected, no activity was observed against tyrosinase, acetylcholinesterase, butyrylcholinesterase, or urease enzymes. The highest inhibition activity was obtained against the ACE in both polar (inhibition: 77.86±1.04 %) and apolar (inhibition: 55.86±1.03%) extracts. The inhibition activity was as high as the standard, lisinopril, which is a commercial ACE inhibitor. ACE, a Zn-dependent peptidase, regulates blood pressure. Therefore, ACE inhibition is a crucial therapeutic strategy used in the treatment of hypertension, which is a serious disease that causes cardiovascular, brain, and kidney damage (Wu et al., 2022). The results of ACE inhibition activity showed that the fruit extracts of *E. umbellate* are a promising source to use as either a supplement or a pharmaceutical reagent for the treatment of hypertension. This is the first study showing the inhibitory activity of the fruit of *E. umbellate* against ACE.

Collagen and elastin are the main components of connective tissue, and their excessive reduction by elastase and collagenase in the tissues causes wrinkle formation, sagging, loss of skin elasticity, and joint pain (Figueiredo et al., 2023). Due to their benefits in preventing connective tissue disorders and skin aging, inhibition of these enzymes has gained attention recently. The extracts showed low inhibitory activity against elastase and collagenase. While the polar extract showed 15.70±0.09% inhibition activity against elastase, the apolar extract inhibited only 12.80±0.18% of the elastase activity. Collagenase activity was only 7.3±0.02% inhibited by the apolar extract. No activity was detected for the polar extract against collagenase. All displayed low activity in comparison to the controls. No study on the inhibition of these enzymes has been found in the literature.

The extracts investigated in this study did not exhibit inhibitory activity against tyrosinase, AChE, BChE, or urease enzymes. However, Ozen et al. (2017) reported the inhibitory activity of the ethanol extract of the fruit against AChE (IC_{50}: 424 µg/mL), BChE (IC_{50}: 941 µg/mL). No activity was observed against urease. The water extract also showed inhibition activity against three enyzmes as follows: AChE, IC_{50}: 260 µg/mL; BChE, IC_{50}: 424 µg/mL; urease, IC_{50}:182.46 µg/mL. The activity of positive controls was as follows: galantamin for AchE: IC_{50}: 410.52 µg/mL; for BChE: IC_{50}: 350.34 µg/mL and thiourea for urease: 95.80 ± 9.65 (Ozen et al., 2017).

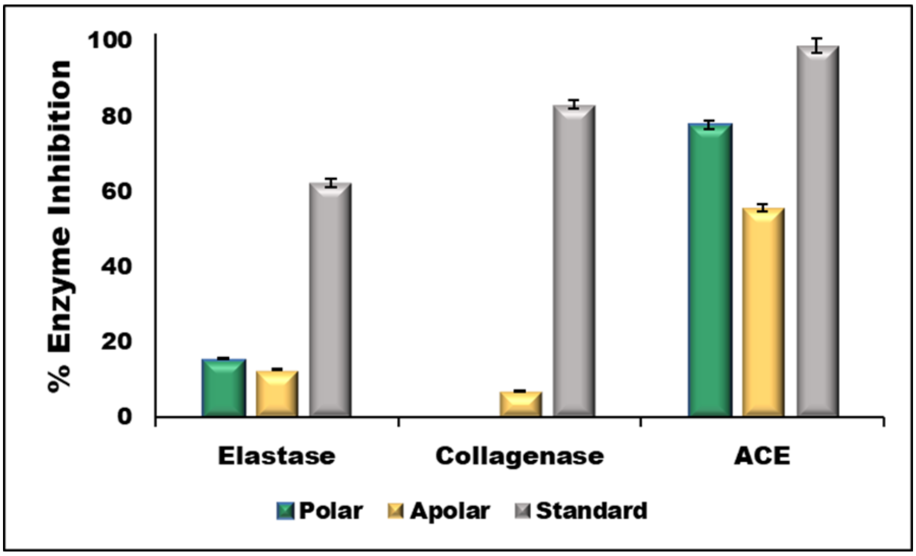

Figure 4. The enzyme inhibition activity of the fruit extracts of *E. umbellate*

4. Antioxidant activity of the fruit extracts of *E. umbellate*

The antioxidant activity of the polar and apolar extracts was investigated using ABTS and CUPRAC methods, and the activity of the extracts was compared with standards (Tables 3 and 4). Both extracts showed moderate antioxidant activity. Two standards were used for the antioxidant assay: BHT and α-tocopherol. The concentration of the extracts had been increased at least 50-fold to reach the same activity as standards. The major phenolic acid found in the extracts was quinic acid, and quinic acid is not classified as a phenolic antioxidant. Studies showed that it does not have antioxidant activity (Ercan & Dogru, 2022). Therefore, it could be the reason why the extracts exhibited lower antioxidant activity compared to standards.

Table 2. $ABTS^{\cdot+}$ radical scavenging capacity of the extracts and standard antioxidants

	% inhibition of standards and extracts					
Concentrations (µg/mL)	10	25	50	100	500	5000
BHT	20.12±0.8	36.2±1.5	54.18±2.1	87.26±2.8	-	-
α-TOC	34.55±1.2	46.34±1.6	65.12±1.8	90.50±3.2	-	-
Polar extract	-	-	-	-	32.04±1.3	82.92±1.7
Apolar extract	-	-	-	-	11.84±0.3	25.14±0.8

Different antioxidant activity results were reported in the literature. In a study using extracts at the same concentration (500 µg/mL), the $ABTS^{\cdot+}$ radical scavenging capacity of the extracts was found to be 11.81% for ethanol extract and 41.83% for water extract, which are very similar to our findings (Ozen et al., 2017).

Table 3. Cupric-reducing antioxidant capacity (CUPRAC) of the extracts and standard antioxidants

	The absorbance in 450 nm of standards and extracts					
Concentrations (µg/mL)	10	25	50	100	500	5000
BHT	0.218±0.01	0.567±0.02	1.438±0.06	2.845±0.13	-	-
α-TOC	0.564±0.02	0.691±0.03	1.274±0.05	2.273±0.12	-	-
Polar extract	-	-	-	-	0.105±0.01	0.378±0.01
Apolar extract	-	-	-	-	0.135±0.01	0.432±0.01

Khattak (2012) reported that the methanol extract of the fruit of *E. umbellate* represented 90.2% scavenging activity at a concentration of 120 µg/mL using the DPPH assay. However, no standard was used to compare in the study (Khattak, 2012). The antioxidant activity of the extracts obtained from the mixture of berries, leaves, and twigs of the plant was evaluated using the DPPH assay (Uddin & Rauf, 2012). The antioxidant activities, which vary depending on the solvent, are as follows: the methanol extract, EC_{50}: 5.5 µg/mL; the ethyl acetate extract, EC_{50}: 42.5 µg/mL; the hexane extract, EC_{50}: 65.4 µg/mL; the chloroform extract, EC_{50}: 250.6 µg/mL and querceitin, EC_{50}: 4.12 µg/mL. The results showed that antioxidant activity varied depending on the solvent used.

CONCLUSION

In this study, the phytochemical content of polar and apolar extracts obtained from the fruit of *E. umbellata* was investigated. The results showed that the fruit of *E. umbellata* is an excellent source for quinic acid, quercetin, naringenin, fumaric acid, chlorogenic acid, acacetin, and hesperidin. The apolar extract contains α-amyrin, oleanolic acid, and ursolic acid. The ACE, elastase, and collagenase inhibitory potential of fruit has been revealed for the first time in the literature. The polar extract has high inhibitory activity against ACE, which is the main target in the treatment of hypertension. The fruit of *E. umbellata* could be a promising supplement for pharmaceutical and nutritional applications.

Statement of Conflict of Interest

The authors declare that they are no conflict of interest.

Authors' Contributions

Bahar Tuba Findik and Hilal Yildiz designed and analyzed the research. 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.

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An important pest in Erzurum: White peach scale (WPS) (*Pseudaulacaspis pentagona* (Targ.-Tozz.) (Hemiptera: Diaspididae)

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ABSTRACT: *Pseudaulacaspis pentagona* (Targioni-Tozzetti) (Hemiptera: Diaspididae), is an important mulberry pest in Turkey, is known to cause economic damage to stone fruits as well. Since it is a polyphagous pest, it is also seen in ornamental and forest plants. In 2015, some observations were made on the bioecology of the species in Ayvali village, Oltu District of Erzurum. In the last week of March (24 March 2015), it was determined that *P. pentagona* spent the winter as a fertilized female. Samples were taken from the field every 4-7 days and brought to the laboratory. The first eggs were found in the second week of April (April 14) in the samples received on April 10, 2015, and a very dense egg laying was observed on April 19 and 20. The first nymphs hatched from these eggs on April 20-21, and on April 27-28, nymphal emergence intensified. In field conditions, the first eggs were found on 20 April and the first nymphs on 3 May. In field conditions, the first eggs were found on 20 April and the first nymphs on 3 May. The presence of *P. pentagona* was recorded for the first time with this study in the Eastern Anatolia Region (Erzurum-Oltu). In addition, the distribution and hosts of the species in Turkey are given. It is of great importance to know the existence of such an important species and to carry out studies to control it accordingly.

Keywords: White peach scale, *Pseudaulacaspis pentagona*, Brief biology, Erzurum, Türkiye

INTRODUCTION

Turkey is known for its wide geographic distribution of little-known plants, including its rich flora and fruits (Özkan, 2019; Cengiz and Korkut, 2020; Maras-Vanlioğlu et al., 2020).

Mulberry belongs to the *Morus* genus in the Moraceae family of the Urticales order, with 10-12 species found worldwide (De Candolle, 1967). *Morus alba* L. (white mulberry), *Morus nigra* L. (black mulberry), and *Morus rubra* L. (red mulberry) are the most common species globally (Bellini et al., 2000; Datta, 2002; Choudhary et al., 2013; 2015; 2018a,b). In Turkey, which experiences temperate, subtropical, and even small tropical climatic regions, numerous fruit species exhibiting rich biological diversity and valuable genetic resources have been cultivated in the wild for centuries (Can et al., 2021).

Mulberries have high adaptability to different climates and soil conditions. The species is resistant to abiotic stress conditions such as low soil fertility or harsh climates. Due to this characteristic, this little-known fruit is naturally grown in fruit valleys, high-altitude plateaus, and even at elevations above 2500 m (Engin and Mert, 2020; Özkan et al., 2020).

Mulberry is one of Turkey's most important traditional fruits and is consumed either fresh or transformed into various traditional products. Mulberry trees (genotypes) belonging to *M. alba*, *M. nigra* and *Morus rubra* L. exhibit high phenotypic diversity (Orhan et al., 2020).

Mulberries contain significant amounts of biologically active compounds that may be associated with certain potential pharmacological activities. Therefore, they are beneficial for health and categorized as superfoods. In most countries where mulberries are grown, the fruit is typically consumed fresh, dried, or processed. Due to its delicious taste, pleasant color, low-calorie content, and high nutritional value, it is used to make wine, fruit juice, and jam (Güngör and Şengül, 2008; Yuan and Zhao, 2017; Zhang et al., 2018).

Like all cultivated plants, harmful insect species exist that can reduce the quality and yield of the mulberry plant. One of these is the white peach scale (=mulberry scale) (*Pseudaulacaspis pentagona* (Targioni-Tozzetti) (Hemiptera: Diaspididae)). This pest feeds on the plant's sap by forming colonies on its thin and thick branches, causing the branches to dry out and the leaves to fall off. If the pest's population continues to be dense for several years, the trees can completely dry up and die (Anonymous, 2008).

Although WPS was first recorded in Italy by Targioni in 1886, it is believed that this pest originated from China or Japan (Brancsome, 2016). White peach scale, which is distributed worldwide, threatened the silk industry in Italy by killing mulberry trees and also caused damage to the peach industry in the United States in the early 1900s (Gossard, 1902; Van Duyn and Murphey, 1971) due to its ability to kill trees. According to Hanks and Denno (1993), *P. pentagona* destroyed numerous peach orchards in Florida and southern Georgia at the beginning of this century.

WPS has caused epidemics in many regions of the world areas (EPPO Global Database, 2022) (Figure 1) in recent years, particularly in peach, mulberry, kiwi, and ornamental plant (Whitmore and Medina Gaud, 1974; Kozarzhevskaya and Vlainic, 1981; Kozarzhevskaya et al., 1986; Ram and Pathak, 1987; Hickel et al., 1996; Ben-Doy *et al.,* 2015). It has been reported that the epidemic has also spread to regions with different climatic conditions in Europe, and its severity has increased (Kozár and Dávid, 1986; Kosztarab and Kozár, 1988; Kozár et al., 1994; Jansen, 1995; Kozár et al., 1995; Kozár, 1997; Mani et al., 1997). Various researchers have reported that the severity of epidemics is increasing with global warming (Kozár and Dávid, 1986; Kozár, 1992; Yamaguchi et al., 2001; Kozár and Szentkirályi, 2005).

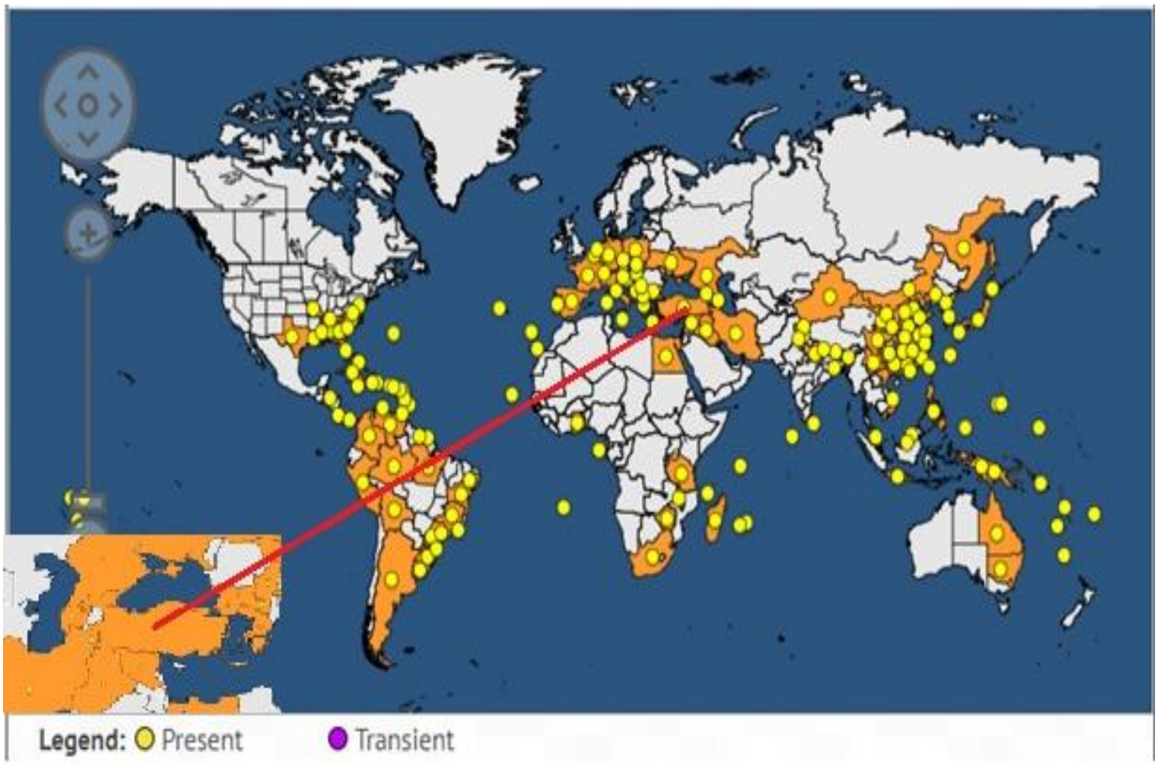


Figure 1. Distribution of *Pseudaulacaspis pentagona* (Targioni-Tozzetti) in the world (EPPO Global Database, 2022)

P. pentagona was first identified in Turkey in 1912 by Süreyya ÖZEK around Istanbul (Bodenheimer 1949). The first study on this species was conducted by Keyder (1952). Although it is called "Dut Kabuklubiti" in Turkey, it is known as "White Peach Scale (WPS)" around the world. Mulberry Scale was named "Oleander Scale" in Bermuda in 1920, where it caused significant damage to oleander trees (Collins and Whitcomb, 1975). It is the primary pest of not only peach trees but also mulberry trees in Turkey (Kıroğlu 1981, Zeki et al., 2004; Güncan, 2009; Mohammed, 2017; Yaşar, 2020). Its locations in Turkey are reported as Adana, Ankara, Antalya, Aydın, Balıkesir, Bartın, Bilecik, Burdur, Bursa, Giresun, Hatay, Istanbul, Izmir, Kocaeli, Kastamonu, Kayseri, Manisa, Mersin, Muğla, Ordu, Rize, Sakarya, Samsun, Tekirdağ, and Trabzon (Yaşar, 2020) (Figure 2).

Coccoidea (Hemiptera), known as scale insects and mealybugs, have waxy coverings or shield-like shells on their bodies. These covers provide protection against adverse weather conditions, natural enemies and control measures. They are typically immobile or have limited mobility, anchor themselves to plants and feed by sucking plant sap with their stinging-sucking mouthparts. As a result of their feeding, color changes in the feeding places, slowing of plant growth, yellowing, early leaf fall, fruit and flower drop can be seen. If the damage continues to increase, the ends of the branches begin to dry out and eventually lead to the complete drying of the plant. In addition, they play a role in the transmission of some plant diseases (Kosztarab and Kozár, 1988).

Figure 2. Distribution of *Pseudaulacaspis pentagona* (Targioni-Tozzetti) in Turkey (Source: Yaşar, 2020)

Detailed studies have been conducted on WPS in the Black Sea Region (Kıroğlu, 1981), Marmara Region (Keyder, 1952; Gürkan, 1982), Mediterranean Region (Erkılıç and Uygun, 1995), Aegean Region (Güncan, 2009), and Central Anatolian Region (Mohammed, 2017) regarding its harmful effects. Additionally, various studies on WPS and other shellfish in the Aegean region (Bodenheimer, 1949, 1953; Aysu, 1950; Göker, 1973; Yaşar, 1990, 1995a, 1995b; Öncüer et al., 1999, 2001; Karsavuran et al., 2001; Ülgentürk and Çanakçıoğlu, 2004) have been conducted, all of which focus on its host and distribution.

It is reported that WPS causes damage to 108 plant species belonging to 58 families (Miller and Davidson, 2005) and is a polyphagous pest. It is the main pest of mulberry, peach, and nectarine trees. It is also present in many other plants such as apricot, almond, walnut, poplar, willow, rose, apple, plum, kiwi, cherry laurel, lilac, geranium, blackberry, raspberry, grape, silk tree, and horse chestnut (Anonymous, 2008)

Nowadays, species of the Diaspididae family, which has the highest number of species in the Coccoidea (Hemiptera) superfamily with about 2400 species belonging to 380 genera, cause significant damage by feeding on the phloem or parenchyma of plants (Miller and Davidson, 2005). Adult female individuals of this family are called "scale insects" because they are hidden under a hard, waxy and scaly structure that they create by secreting from their specialized pygidia (Foldi, 1990). Scale insects have been recorded to live on host plants of more than 1380 genera belonging to 182 families (Borchsenius, 1966). Thanks to their adaptations and biotic relationships in morphology, reproduction, and life cycle, scale insects have developed better survival skills than other insect groups (Gullan and Kosztarab, 1997).

Approximately 199 species are known to cause damage worldwide (Miller and Davidson, 1990), and 96 species have been identified in Turkey (Kaydan et al., 2007). One of most important of these species is the White Peach Scale, *P. pentagona*.

The studies conducted on *P. pentagona* in Turkey over the past 15 years are listed below.

Ülgentürk et al. (2008) investigated the species of the Coccoidea superfamily in Istanbul, providing information on the host plants of a total of 42 species belonging to 7 families, and reported that WPS was heavily present on mulberry trees. Güncan (2009) conducted a study on the status of WPS in peach trees in important districts producing peaches in İzmir province between 2006 and 2009, and determined the distribution areas, hosts, and natural enemies of WPS in İzmir province. In addition, Güncan (2009) determined that WPS gives 3 generations per year in İzmir province. Ülgentürk et al. (2009) reported WPS in kiwi gardens in Rize as the first record of the species and also noted that it is widespread and potentially harmful for kiwi. Kaydan et al. (2013) investigated the Coccoidea superfamily in Turkey, updating it to include a total of 359 species belonging to 134 genera in 18 different families. They reported that the family Diaspididae, with 134 species belonging to 42 genera, had the most species, followed by the families Pseudococcidae with 101 species belonging to 32 genera, and Coccidae with 67 species belonging to 28 genera.

Ülgentürk and Ayhan (2014) detected 11 scale insect species on the fruits sold in markets and bazaars in Ankara and determined that WPS from these species is most commonly found in kiwi fruits. Ülgentürk and Mohammed (2016) also identified eight coccoid species on mulberry trees in various cities, including Ankara, with WPS being the most common and significant pest. Mohammed et al. (2016) recorded WPS as a widespread and damaging pest on 23 ornamental and park plants in Ankara, with *M. alba* being particularly economically important in the Ayaş district.

In March 2015, an application was made to the Dean of Atatürk University Faculty of Agriculture by mulberry growers in Ayvali village of Oltu district of Erzurum. Producers reported that a pest creates a dense population on mulberry trees and asked for help in its control. The members of the Department of Plant Protection and Horticulture and the officials of the Oltu Agriculture District Directorate conducted fieldwork in the region. According to our research in the village of Ayvali, *P. pentagona* has become a serious threat to mulberry production, and some growers have even cut their trees. It caught our attention that this species was seen for the first time in the region and caused an important epidemic. The aim of this study is to reveal biological data about the species so that mulberry producers can apply effective control methods at the right time.

MATERIAL AND METHOD

Material

Egg, nymph and adult stages of WPS obtained from mulberry plants in Erzurum Province Oltu District Ayvalı village (Figure 3) and brought to Atatürk University Faculty of Agriculture Plant

Protection Department Insect Systematics Laboratory formed the main material of the study. The obtained specimens were kept in 30 × 45 × 30 cm plastic containers at 25 ± 2 °C temperature, 65 ± 5% relative humidity, and 16-hour light: 8-hour dark conditions, and some information was attempted to be obtained on the bioecology of the species.

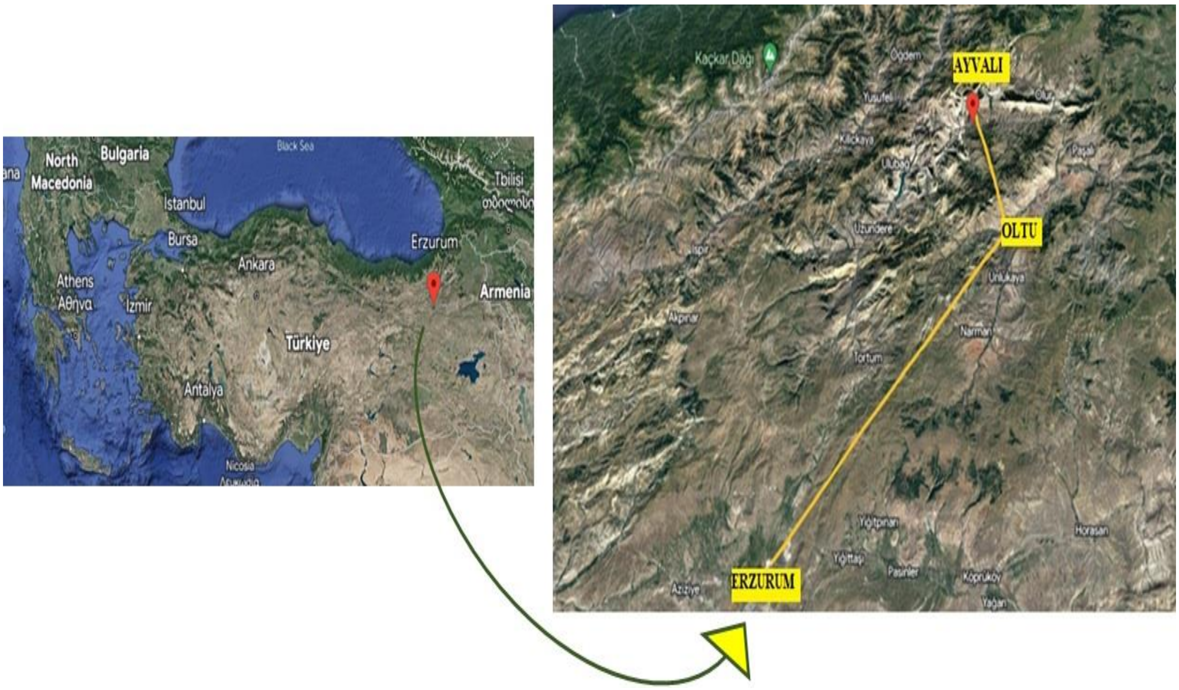


Figure 3. Study area where *Pseudaulacaspis pentagona* (Targioni-Tozzetti) was detected

Method

From the beginning of March to the end of May, samples were taken from the field every 4-7 days and related observations were made in the laboratory.

RESULTS AND DISCUSSION

Pseudaulacaspis pentagona (White Peach Scale)'s place in the animal kingdom (Burckhardt and Watson, 2004).

Domain: Eukaryota

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Uniramia

Class: Insecta

Order: Hemiptera

Suborder: Sternorrhyncha

Superfamily: Coccoidea

Family: Diaspididae

Tribus: Diaspidini

Genus: *Pseudaulacaspis* Macgillivray, 1921

Species: *pentagona* (Targioni-Tozzetti, 1886)

The adult females of *P. pentagona* have a circular, convex, dirty-white colored shell with a diameter of 2.0-2.5 mm (Figure 4a). The bodies of the adult females located beneath the shell are orange-yellow, have a broad pear shape, and have a body length of 0.9-1.5 mm (Figure 4b). The eggs seen beneath the female shell are uniformly oval (Figure 4c), with female individuals being pinkish-orange and male individuals being yellowish-white (dichroism). After hatching, the nymphs are mobile and wander around on the host for a certain period before anchoring themselves. The adult males have a body length of approximately 0.7 mm and a wingspan of 1.4 mm (Figure 4d). They are found in groups and have a pair of wings when they have completed their development. Their movements are quite slow, and they only live for about 24 hours.

As a result of the applications made by the farmers, the first samples were taken from the Ayvali Village of the Oltu district on March 4, 2015 by the officers of the Oltu District Directorate of Agriculture and sent to the Plant Protection Department. As a result of the examination of the samples, it was determined that the species was *Pseudaulacaspis pentagona*. These samples were then cultured in the Insect Systematics Laboratory. It was also determined that the species spent the winter as a fertilized female.

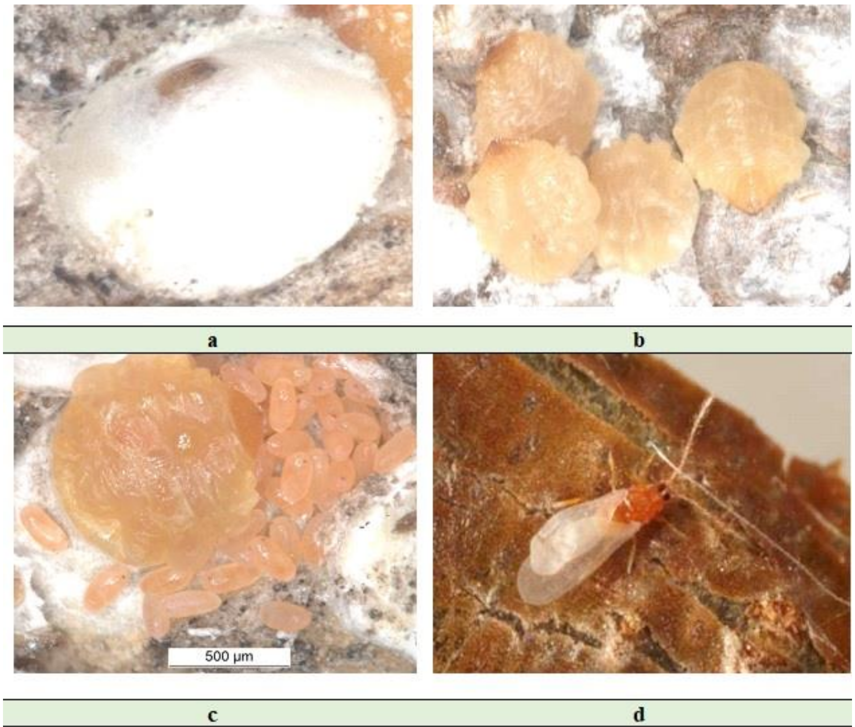


Figure 4. Some biological stages of *Pseudaulacaspis pentagona* (Targioni-Tozzetti), a. adult female shell (Original), b. adult female (Original), c. egg (Original), d. adult male (Source: Anonymous, 2023)

In samples taken from the field on March 18, 2015, the first eggs were observed on March 28 and 29. Similarly, the first nymph emergence from these eggs was observed on April 5th and 6th, and the nymphs were seen moving on the branches.

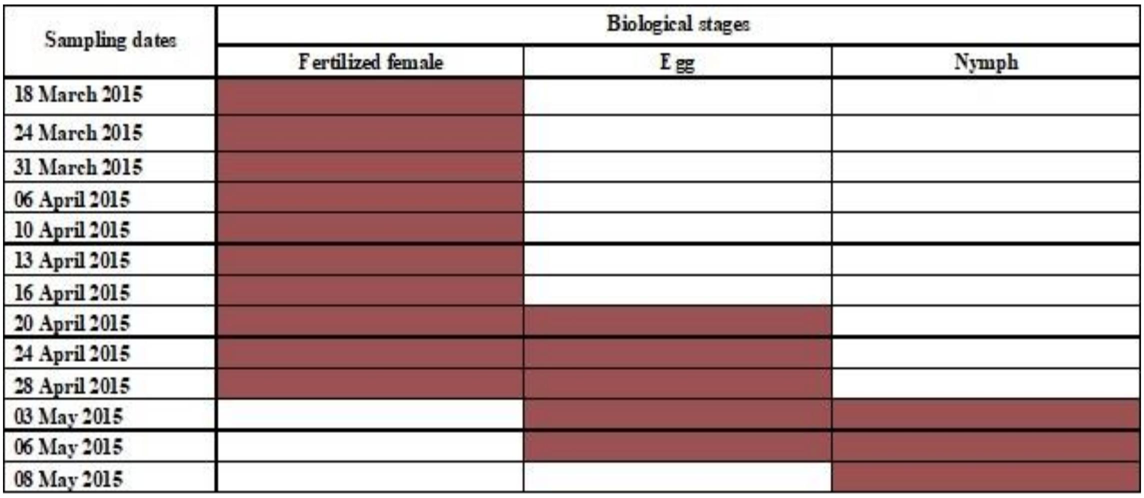
Field inspections and sample collections were conducted on March 24th, 2015, and it was observed that the females under the shells had not yet laid eggs. The same situation continued in the samples collected on March 31st, April 6th, April 10th, April 13th, and April 16th.

In samples brought from the field on April 10th, the first eggs were found in the laboratory on April 14th. The egg density increased on April 19th and 20th. In the egg counts conducted on April 14th and 15th, 2015, the number of eggs laid by 10 females was 11, 21, 51, 13, 88, 24, 22, 94, 32, and 74 (average of 43). The first eggs were observed in samples brought on April 6th on April 18th.

In samples taken from the field on April 20th, 2015, the first eggs were found. Again, it was noted that the females laid a significant number of eggs on April 19th to 21st, in the samples brought on April 13th and April 16th. In the samples brought on April 10th, the first nymph emergence occurred on April 20th and 21st.

Eggs were observed in samples from the field on April 24th and 28th. Intense nymph emergence was observed in the samples brought on April 6th, April 13th, and April 16th during the laboratory checks on April 27th and 28th. The nymphs were very active. During the laboratory checks on May 3rd, eggs were seen in samples from the field on April 24th and 28th, and eggs as well as nymphs were observed in samples taken on April 26th. Eggs and first nymphs were observed in samples taken from the field on May 3rd and 6th. Intense nymph emergence was detected in the laboratory on May 5th and 6th. On May 8th, in samples taken from the field, nymphs were seen scattered on the branches, and unopened eggs (transparent white and orange-colored) were found under the shells. During the laboratory checks on May 18th, only a small number of nymphs and eggs were found in samples taken on May 6th. On May 8th, intensive spraying was carried out by the producers in the field under the supervision of the Agricultural District Directorate staff. Therefore, sample collection from the field was discontinued as the results would not be reliable. Only samples taken on May 26th were examined, and it was determined that the spraying significantly affected the population. It was also learned that the producers sprayed once again in the first week of June. The biological stages of *P. pentagona* observed under field conditions are given in Table 1.

Table 1. Biological stages of *Pseudaulacaspis pentagona* (Targioni-Tozzetti) determined in field conditions



Sampling dates	Biological stages		
	Fertilized female	Egg	Nymph
18 March 2015			
24 March 2015			
31 March 2015			
06 April 2015			
10 April 2015			
13 April 2015			
16 April 2015			
20 April 2015			
24 April 2015			
28 April 2015			
03 May 2015			
06 May 2015			
08 May 2015			

This study is of great importance as it is the first record of WPS detected in mulberry trees in the Eastern Anatolia Region (Erzurum-Oltu-Ayvali Village). It is seen that the species can give two generations in the region and has the potential to negatively affect the quality and quantity of mulberry production in a very short time. The climate data of 2015, when the study was conducted, are given in Table 2.

It is reported that WPS gives 2 generations per year in Marmara, Black Sea and Central Anatolia regions, and 3 generations in Aegean and Southern Anatolia regions (Anonymous, 2017). It is also recorded that the first generation started in the last week of March, the second generation started at the end of June and the third generation started in mid-July in İzmir (Güncan, 2009).

Table 2. Monthly average temperature, humidity and precipitation at Oltu (Erzurum, Turkey), in 2015 (Taken from the records of Republic of Türkiye Ministry of Environment, Urbanization and Climate, General Directorate of Meterology)

Monthly Average Temperature (°C)												
Year/Months	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
2015	-1.5	1.4	5.4	8.7	13.8	19.5	23.6	24.3	21.4	12.1	4.6	-2.8
Monthly Average Humidity (%)												
Year/Months	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
2015	65.2	60.6	53.2	54.2	57.1	50.0	38.7	39.6	33.8	62.0	59.8	62.1
Monthly Average Precipition (mm)												
Year/Months	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
2015	9.40	27.6	22.80	60.80	70.40	0.20	0.00	30.60	5.60	47.20	43.60	4.80

Figure 5. Damage of *Pseudaulacaspis pentagona* (Targioni-Tozzetti) (Original)

CONCLUSION

This study is the first conducted on *P. pentagona* (WPS), which is a harmful pest for mulberry trees in the Eastern Anatolia Region (Erzurum-Oltu-Ayvalı).

The study revealed that *P. pentagona* has the potential to negatively impact both the quality and quantity of mulberry production. Given the risk of the pest quickly spreading to large areas, measures, especially internal quarantine, need to be taken urgently. This is especially important since fruit cultivation is carried out in the neighboring Yusufeli, İspir, Oltu, Uzundere, and Tortum districts. Obtaining detailed bio-ecological data that can be used in the fight against this new pest in the region, especially identifying the emergence times of nymphs from eggs, is of great importance. Furthermore, it is believed that the pollution (especially the dusting of leaves) caused by the dam construction process in the region negatively affects the natural enemy populations.

It is of great importance for Agricultural and Forestry District Directorates to conduct surveys and inform farmers considering the natural enemy status and climatic conditions (such as temperature, humidity and precipitation) of the pest. Although a mite species was obtained as a natural enemy during the observations, its species description has not yet been confirmed.

It is thought that this cosmopolitan species with a polyphagous feeding habit can reproduce and limit mulberry production in the region when suitable conditions are present.

The fact that mulberry growers cannot choose the appropriate time for chemical control leads to excessive use of chemicals and thus often insufficient results In addition, the wide host range (polyphagous) of the mulberry scale species plays an important role in its survival and re-emergence in production areas.

As a result, producers should be informed about *P. pentagona*, and control programs that take into account the environment and human health should be implemented within the scope of integrated pest management.

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Essential Oils: The Next Frontier in Antifungal Mastery and Mycotoxin Safeguarding-A review

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ABSTRACT: The application of plant essential oils (EOs) in mitigating fungal plant pathogens and mycotoxins is a burgeoning area of research. EOs, extracted through diverse methods like steam distillation and supercritical CO_2 extraction, are renowned for their broad-spectrum antimicrobial effects, low toxicity, and high utilization rates. Recent reports on plant families utilized for EO extraction against fungal plant pathogens spotlight the dominance of Lamiaceae, particularly Garden thyme, in inhibiting various pathogens. With 27 plant families explored, such as Myrtaceae and Apiaceae, the efficacy of specific EOs against diverse plant pathogens becomes evident. The antifungal mechanisms include membrane inhibition, mitochondrial dysfunction, discharge pump suppression, ROS induction, respiratory metabolism disruption, and genetic material influence. Additionally, promising findings reveal the potential detoxification of mycotoxins by certain EOs, offering novel avenues for mycotoxin reduction. This review explores the multifaceted applications and mechanisms, underscoring the transformative potential of plant EOs in plant pathology and mycotoxin management.

Keywords: Plant EOs, Distillation, Microwave-assisted extraction, Mycotoxins, Antifungal properties.

INTRODUCTION

Plant essential oils, also known as aromatic or volatile oils, are secondary metabolites extracted from various plant tissues. These oils, often differing in color and extracted from different plant parts, are typically oily and named based on their plant source (Perczak et al., 2016). Most EOs are colorless or light yellow, possess hydrophobic properties, are easily soluble in organic solvents, and exhibit volatility at room temperature, characterized by a distinct, enduring aroma (Cai et al., 2022). Mycotoxins, on the other hand, are toxic metabolites produced by fungi in food or feed, posing serious health risks (Chen et al., 2020). Recognizing the limitations and risks associated with traditional antifungal methods, the study emphasizes the potential of natural plant EOs, with their broad-spectrum antifungal effects due to low toxicity, and high utilization rates (Miguel et al., 2018; Oliveira et al., 2019). The combination of these oils may offer an alternative to synthetic pesticides, benefiting gastrointestinal health (Xue et al., 2020). This review explores the inhibitory effects of plant EOs on pathogenic fungi and mycotoxins, aiming to mitigate pollution and safeguard human and animal, and improving crop production for sustainable agriculture.

Essential Oil Extraction Methods

Various extraction techniques are employed for obtaining plant EOs, including distillation, organic solvent extraction, pressing, adsorption extraction, lipolysis, enzyme fermentation, and supercritical

CO_2 extraction (Moumni et al., 2020). Distillation and pressing methods were found to yield lower extraction efficiency compared to microwave-assisted extraction (Marković et al., 2018). Steam distillation employs steam to release aromatic compounds from plants, relying on the density difference between oil and water for separation, although it suffers from slow processing and high temperatures causing component degradation. Cold pressing disrupts plant cell structure, allowing oils to flow out without heat damage; however, it has low yields and complex purification steps, limiting its suitability for large-scale production (Cai et al., 2022). Methods using non-polar solvents like ethanol leverage the hydrophobic nature of EOs but are mainly suitable for lab research due to challenges in separating non-volatile macromolecules (Dzah et al., 2020; Wen et al., 2018). Supercritical CO_2 extraction, which alters CO_2 density through pressure and temperature changes, selectively extracts components based on their boiling points and molecular weights, demonstrating feasibility for extracting EOs from natural sources. Ultrasonic-assisted extraction utilizes ultrasonic waves to break plant cell walls, enhancing solvent permeability and extraction efficiency, particularly useful for oils with poor thermal stability (Tavakolpour et al., 2017). Microwave-assisted extraction selectively heats substances to separate them efficiently, offering advantages of time efficiency, resource utilization, and high aromatic content in the final EOs (Razzaghi et al., 2019).

Recently Report of Plant Families used for the extraction of EOs against Fungal plant Pathogens

EOs are highly concentrated plant extracts that contain the aromatic compounds responsible for the characteristic scent of the plant. EOs obtained from various plant sources have been found to exhibit antifungal properties (Caprari et al., 2023). They are extracted using methods such as steam distillation, cold-pressing, or solvent extraction. These natural compounds have been used for centuries as a traditional remedy for fungal infections (Davari and Ezazi, 2022). During the present study, a total of 100 recent studies from the last 5 years that used various EOs to control the fungal plant pathogens were collected, and it was concluded that in the last 5 years, a total of 27 plant families (*Lamiaceae, Myrtaceae, Apiaceae, Asteraceae, Poaceae, Lauraceae, Cupressaceae, Zingiberaceae, Fabaceae, Verbenaceae, Piperaceae, Pinaceae, Primulaceae, Anacardiaceae, Myristicaceae, Adoxaceae, Burseraceae, Cannabinaceae, Amaranthaceae, Aristolochiaceae, Boraginaceae, Acoraceae, Meliaceae, Ericaceae, Arecaceae, Rutaceae*, and *Amaryllidaceae*) were used to extract the EOs and control the fungal plant pathogens (Table 1). Of all 27 plant families, *Lamiaceae* is the most prevalent and effective plant family to controlling fungal plant pathogens, and a maximum of 23 plant species of this family was recorded (Tagnaout et al., 2022; Garzoli, 2023). Out of 23 plant species 'Garden thyme' is found in maximum studies followed by Oregano, Summer savory, Peppermint, Spearmint, Europe sage, English lavender, Zaatar, Sweet basil, Chinese mint, Rosemary, Pudding grass, Pot marjoram, Winter savory, and Mexican chia are the most common plant species of the family *Lamiaceae* were used to control the fungal plant pathogens (Maurya et al., 2021; Cao et al., 2022). After the *Lamiaceae*, the *Myrtaceae* and *Apiaceae* families were found with the maximum number of 8, 8 plant species, and in the Myrtaceae family the EOs of Clove was used in the maximum number of studies to control the plant pathogen followed by Blue gum, Allspice, Tea tree, and Lemon-scented ironbark, In *Apiaceae* family, the EOs of Common fennel was used in the maximum number of studies to control the plant pathogen followed by Moshkoorak, Cumin, Anise, Ferulago, and Dhania. After the *Myrtaceae* and *Apiaceae*, the *Asteraceae* family with plant species African marigold, Pot marigold, Indian chrysanthemum, Rhanterium, Curry plant, Green propolis, and Bachelor's button, the *Poaceae* family with plant species Lemongrass, Palm rose, and Citronella grass, the family *Lauraceae* with plant species Camphor, Bay laurel, Dalchini, and Chinese cinnamon, the family *Cupressaceae* (Rguez et al., 2018; Meenu et al., 2023) with plant species Common juniper, China fir, Araar tree, Italian cypress, and Monterey cypress, the family *Zingiberaceae* (Abdullahi et al., 2020) with plant species Common ginger, Greater galangal, and Cardamom, the family *Fabaceae* (Lala, 2021; Samara, 2021) with plant species Indian rosewood, Clover, and Methi, the family *Verbenaceae* (Tomazoni et al., 2018; Ju et al., 2022) with plant species Common verbena, and lemon verbena, the family *Piperaceae* (Valadares et al., 2018; Muñoz and Olivas, 2020) with the plant species Black pepper, and spiked pepper and the family *Pinaceae* (Kumar et al., 2020; Grzanka et al., 2021) with plant species Pine, and Deodar cedar were found with a maximum number to extract the EOs to control the fungal plant pathogens. The remaining families such as *Primulaceae* (Mississippi loosestrife), *Anacardiaceae* (Brazilian pepper tree), *Myristicaceae* (Nutmeg or mace), *Adoxaceae* (American elder), *Burseraceae* (African myrrh), *Cannabinaceae* (Common hop), *Amaranthaceae* (Mexican tea), *Aristolochiaceae* (Wild ginger), *Boraginaceae* (Black sage), *Acoraceae* (Sweet flag), *Meliaceae* (Neem tree), *Ericaceae* (Bilberry), *Arecaceae* (Oil palm), *Rutaceae* (Lemon), and *Amaryllidaceae* (Garlic) are found with a maximum of one plant species that are given in bracket with family (Bocquet et al., 2018; Ammad et al., 2018; Zefzoufi et al., 2020; Mangalagiri et al., 2021). This study highlighted that *Lamiaceae* is the most powerful and effective plant family for the extraction of EOs against fungal plant pathogens. This family has been reported in various previous studies (Rus et al., 2015; Hanana et al., 2017; Nieto et al., 2017; Potente et al., 2020; Valerio et al., 2021), due to their effective active ingredient and production of highly effective aromatic volatile compounds against destructive plant pathogens but the present study highlighted the most recent and new plant families that the less used and new in the extraction of EOs against the fungal plant pathogens such as *Primulaceae*, *Adoxaceae*, *Ericaceae*, *Acoraceae*, *Amaranthaceae*, *Burseraceae*, and *Boraginaceae*. The EOs extraction from Primulaceae is effective against the *Alternaria alternata*, *Fusarium solani*, and *Fusarium fujikuro* (Mouni, 2023; Aguele et al., 2023), EOs extraction from *Adoxaceae* is effective against the *A. alternate*, *A. infectoria*, and *A. Flavus* (Anžlovar et al, 2020), EOs extraction from *Ericaceae* is effective against the *Sclerotinia sclerotiorum* (Lib.), *A. solani*, *F. oxysporum* f. sp. *radicis-*

lycopersici (Sacc.) and *Verticillium dahlia* (Bayar et al., 2018), EOs extraction from *Acoraceae* is effective against the *A. niger, A. flavus* and *Rhizopus microspores*, EOs extraction from *Amaranthaceae* is effective against the *Verticillium dahlia, F. oxysporum* f. sp. *melonis and Fusarium culmorum* (Zefzoufi et al., 2020), EOs extraction from *Amaranthaceae* is effective against the *A. flavus, Cladosporium* sp., *A. alternata, F. oxysporum and F. Solani* (Perveen and Bokhari, 2020; Perveen et al., 2018; Aati et al., 2020), and EOs extraction from *Burseraceae* is effective against the *Sphaceloma ampelinum* (Rozwalka et al., 2020).

Antifungal mechanism of EOs

Inhibition the formation of cell membrane: The presence of a cell wall barrier reduces the susceptibility of microorganisms to antimicrobial agents (Lagaert, Beliën and Volckaert, 2009). Essential oil molecules target the main components and associated enzymes of the cell wall, including the outer glycoprotein layer and the inner layer composed of β-(1,3)-D-glucan, β-(1,6)-D-glucan, and chitin. Together, these polymers form a robust network that prevents glycoproteins from leaking out of the cell. Glucan, chitin, and mannan are considered the targets of drug action. Currently, β-(1,3)-D-glucan synthase inhibitors are the most commonly reported inhibitors that affect the normal synthesis and structure of fungal cell walls, leading to cell rupture and content leakage (Dwivedy et al., 2017). Chitin synthase, which is specific to fungi, is also a potential target for fungal inhibitors. Chitin, though a small component of the cell wall, is crucial for maintaining the structural integrity of fungal cells. Anethole, the main active ingredient in fennel essential oil, has been shown to inhibit chitin synthase activity and chitin synthesis in fungal hyphae. Additionally, flavonoids extracted from Taxus chinensis can inhibit chitin synthase activity, and their potency is greater than that of cycloserine (Wang et al., 2022). Mannan glycoprotein is another drug target, as the carboxyl group in the drug can combine with mannose-protein in the cell wall, resulting in a Ca^{2+}-dependent complex that interacts with the cell membrane and changes its permeability, ultimately leading to cell rupture and death.

Damage the integrity of cell membrane: The cell membrane is vital in maintaining the normal activities of microorganisms. EOs can impact the structure and function of the cell membrane by altering its permeability. Research has shown that chrysanthemum essence oil can increase the plasma membrane permeability of Saccharomyces cerevisiae and cause cell contents to leak. Cinnamaldehyde can dissolve in the fatty acyl chain of the cell membrane, which disrupts its outer structure, resulting in increased membrane permeability, outflow of ATP, and cell death (Ju et al., 2022). This is likely due to the lipophilic terpenes and terpenoids in EOs that can penetrate the membrane system, increasing permeability and causing further membrane damage. Essential oil molecules can also interact with phospholipid molecules, changing the structure and fatty acid proportion of the cell membrane. Eugenol can increase the proportion of unsaturated fatty acids in the cell membrane of *Aspergillus niger*.

Similarly, thymol and salicylic acid can inhibit the synthesis of ergosterol in the cell membranes of *Fusarium oxysporum* and *Rhizopus stolonifera*, leading to the destruction of membrane integrity.

Mitochondrial dysfunction: Mitochondria are crucial for the respiration and production of ATP energy in eukaryotes. They play a vital role in maintaining the normal energy metabolism and function of microorganisms. Eukaryotes obtain energy through the tricarboxylic acid (TCA) cycle that occurs in the mitochondria, and any disruption in this process can lead to a slowdown in growth or even death of organisms (Ju et al., 2022). Some active molecules present in EOs can hinder the normal functioning of mitochondria by inhibiting mitochondrial dehydrogenase, which is necessary for ATP biosynthesis. For instance, a combination of eugenol and citral can damage the mitochondrial morphology of *Penicillium roqueforti* and disrupt its energy metabolism, leading to cell death. Similarly, turmeric essential oil can inhibit the activity of mitochondrial ATP enzyme, malate dehydrogenase, and succinate dehydrogenase, and cause damage to the mitochondria of *Aspergillus flavus* (Ju et al., 2022).

Suppress discharge pump: The fungal plasma membrane H+-ATPase has a crucial role in various physiological processes of fungal cells. It is responsible for maintaining a significant electrochemical proton gradient across the cell membrane, which aids in nutrient absorption. Additionally, it regulates cell growth and intracellular pH. If the H+-ATPase activity is hindered, it can cause intracellular acidification and result in cell death. Eugenol and thymol are potent fungicides that can inhibit the activity of H+-ATPase (Ju et al., 2022). Studies have demonstrated that curcumin and perillaldehyde can also impede the activity of H+-ATPase in *Aspergillus flavus* (Hu et al., 2017; Xu et al., 2021). Furthermore, carvacrol and thymol present in thyme essential oil exhibit a synergistic antifungal effect with fluconazole by blocking the overexpression of efflux pump genes CDR1 and MDR1 in Candida albicans. These monoterpenes are highly effective at inhibiting drug delivery pumps.

Induce the production of reactive oxygen species (ROS)*:* BNOS is an enzyme that catalyzes the synthesis of nitric oxide (NO) from arginine in bacteria. The resulting NO enhances bacteria's resistance to a wide range of antibiotics, enabling them to coexist with antibiotic-producing microorganisms. By chemically modifying toxic compounds and reducing oxidative stress caused by antibiotics, NO-mediated drug resistance is achieved. Consequently, inhibiting BNOS activity may improve the efficacy of antibacterial therapy. Fungi can also produce NO, which helps protect their hyphae from oxidative damage caused by heat stress. Interestingly, recent research suggests that reactive oxygen species (ROS) are a common lethal effect of many antibiotics in fungi, suggesting that ROS plays a crucial role in cell death. ROS production in microorganisms mostly occurs in mitochondria, which are also major targets of ROS attack. When exposed to foreign drugs, the organism generates a substantial amount of ROS, altering mitochondrial membrane potential and releasing proapoptotic factors such as cytochrome C, making mitochondria the key executor of apoptotic signals.

Effects on respiration and energy metabolism: Respiratory metabolism, also known as cellular respiration, is the process by which cells generate energy through the oxidative breakdown of carbohydrates in the body (Chiranjeevi and Patil, 2020). This energy is necessary for various life activities and is produced through metabolic pathways such as the glycolytic pathway, pentose phosphate pathway, and TCA cycle (Kloska et al., 2023). Thymol has been shown to inhibit the activity of enzymes involved in the tricarboxylic acid cycle in the mitochondria of Rhizopus stolonifera, resulting in the disruption of microbial energy metabolism (Kong et al., 2022). Similarly, perilla essential oil has been found to inhibit the glycolytic pathway of Aspergillus flavus, leading to a disruption of energy metabolism and the death of *A. flavus* (Hu et al., 2022).

Influence on genetic material: The genetic material of all organisms, whether DNA or RNA, plays a significant role in controlling the synthesis and metabolism of proteins, as well as in the growth, development, reproduction, mutation, and other important phenomena (Kumar et al., 2022). The accuracy and stability of their self-replication ensure the continuity of inheritance from one generation to the next. Any damage to DNA or RNA can affect the normal reproduction and self-replication of the genetic material. Studies have demonstrated that *Artemisia argyi's* essential oil can interact with microbial DNA and cause DNA fragmentation, leading to inhibition of gene expression, destruction of the DNA structure, and cell lysis. Additionally, certain essential oil molecules can attach to DNA chains of microorganisms, leading to their inability to grow and reproduce (Zhou et al., 2021).

Detoxification of Plant EOs to Mycotoxins

Research on the antibacterial activity of plant EOs has predominantly focused on inhibiting bacteria and mold growth, with limited exploration into the detoxification of mycotoxins (Pandey et al., 2023). A study on *Aflamomum danielli* essential oil demonstrated its capacity to degrade ochratoxin OTA, achieving degradation rates of 90% to 100% at concentrations of 1,000 to 2,000 mg/kg (Aroyeun, et al., 2009). Investigations into *Thymus daenensis*, *Satureja khozistanica*, and *Satureja macrosiphonia* extracts and EOs revealed their potential to inhibit Fumonisin B1 (AFB1) production, with ethanol extracts showing stronger anti-AFB1 biosynthesis activity than aqueous extracts (Tian et al., 2022; Tian et al., 2019). Notably, *Thymus* daenensis aqueous extract demonstrated a remarkable 97% reduction in AFB1. Studies on Allium cepa extract, Ajowan seed extract, and dialysed Trachyspermum ammi extract showcased effective degradation of AFB1 and other aflatoxins (Gorran et al., 2013). Additional research by Perczak et al. (2019) highlighted the efficacy of palmarosa and lemon oils in reducing deoxynivalenol (DON) levels (Perczak et al., 2019b, Perczak et al., 2019a). Phenolic compounds, especially chlorophorin, caffeic acid, and ferulic acid, demonstrated significant reductions in FB1 production. Cinnamon oil emerged as a potent degrader of FB1, exhibiting a degradation rate of 94.06% under optimal conditions (Xing et al., 2014). Moreover, cinnamon oil emulsion droplets showed protective effects against FB1 and AFB1-induced oxidative stress and toxicity in male rats (Abd El-Hack et al., 2018). While these findings highlight the potential of plant EOs in mycotoxin reduction, challenges remain, including optimizing extraction methods and understanding the toxicity of degradation products.

CONCLUSION

In conclusion, plant EOs (EOs) present a promising avenue for mitigating fungal infections and mycotoxin contamination in food and feed. This review explores diverse extraction methods for obtaining EOs, emphasizing the superiority of techniques like microwave-assisted extraction and supercritical CO_2 extraction. Recent reports on plant families used for EO extraction against fungal plant pathogens underscore the efficacy of EOs from Lamiaceae, Myrtaceae, and Apiaceae. Notably, Lamiaceae, with its flagship species like Garden thyme, emerges as a powerful antifungal resource. The antifungal mechanisms of EOs are multifaceted, encompassing the inhibition of cell membrane formation, damage to membrane integrity, induction of reactive oxygen species, and interference with genetic material. Furthermore, EOs demonstrate potential in detoxifying mycotoxins, with various studies highlighting their effectiveness against ochratoxin, aflatoxins, deoxynivalenol, and fumonisin B1. Despite these promising findings, challenges such as optimization of extraction methods and understanding the toxicity of degradation products need to be addressed in future research. Harnessing the full potential of plant EOs can contribute significantly to sustainable and natural strategies for ensuring food and feed safety while minimizing the risks associated with synthetic antifungal agents. Future research directions should focus on refining extraction techniques, exploring new plant sources, and conducting comprehensive toxicity assessments for a holistic understanding of the potential of plant EOs in fungal and mycotoxin management.

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Biological Control of Walnut Brown Apical Necrosis Disease *In Vitro* Conditions

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ABSTRACT: Walnut (*Juglans regia* L.) is widely grown in the world and in our country, economic and medical importance, belonging to the Juglandaceae family which is one of the hard-shelled fruits. Fungal diseases, one of the biotic factors, significantly limit the production of walnut plants. Fungicides are used in the control of fungal diseases. However, the use of bacterial bioagents as an alternative to fungicides has gained importance in recent years. In this study, it was aimed to investigate the effects of some bacterial strains against the brown apical necrosis causative *Fusarium* sp. under *in vitro* conditions, the efficacy of twenty bacterial strains (16 *Bacillus* sp., 3 *Pseudomonas* sp., 1 *Brevibacillus* sp.) in terms of antagonistic properties against agent was determined by dual culture test. In the study, it was determined that the percent inhibition rate values changed between 69.80% and 90.10%. The most effective results in *in vitro* conditions were obtained from *Brevibacillus choshinensis* (TV 53D, 90.10%), respectively by *Pseudomonas fluorescens* (FDG 37, 83.7%) and *Bacillus pumilus* (TV 73F, 82.57%). As a result, it was noted that these isolates are promising for controlling walnut fruit rot disease *in vitro* caused by *Fusarium* sp. It is important to test the promising bacterial strains that are effective against the pathogen in different environmental *in vivo* conditions, in terms of determining their usability as a biopesticide.

Keywords: Biological control, Brown apical necrosis, *Fusarium* sp., *Pseudomonas fluorescens,* Walnut

INTRODUCTION

The Persian walnut tree (*Juglans regia* L. or English walnut) is a widespread monoecious tree species of the Juglandaceae family and the Juglans genus hard-shelled fruit (Germain et al., 1999). Walnut is the most produced and highly economically important nuts globally (Hayes et al., 2015; Khir and Pan, 2019). *J. regia* L. native to the mountain valleys of Central Asia, is grown worldwide in temperate areas (Bernard et al., 2018). World walnut production is estimated to exceed approximately three million tons (in-shell basis) by 2022-23 and is mostly supplied by China, the USA, Iran, and Turkey (Anonymous, 2023). Walnut is in high demand due to their long-term storage, use in the pharmaceutical industry, and valuable wood in Turkey (Bayazit et al., 2016). While our country was at the top of the world in walnut production in the 1970s, it fell to second place in the 1980s, third in the 1990s, and fourth in the 2000s (Aslansoy, 2012). One of the reasons for this decline in walnut production fungi from biotic factors is fungal diseases and Brown Apical Necrosis (BAN) disease significantly affects walnut production (Belisario et al., 2002).

BAN is the most recently described (Belisario et al., 2002) and a critical disease complex of walnut in France, Italy, Spain, and Turkey (Belisario et al., 2004; Moragrega and Özaktan, 2010). It began early walnut fruit drop in our country (Akat et al., 2016). The presence and the economic importance of BAN are related to the occurrence of conducive cultural and environmental conditions promoting the dissemination and colonization of fungal pathogens.

BAN derives the name from its typical symptomology that produces brown to dark-brown spots exclusively at the blossom end of walnut fruit, then the spot enlarges to a patch. Sometimes lesions extend over two-thirds of the whole fruit's inner tissue and surface however, the extent of external and internal lesions are not parallel (Belisario et al., 2002). BAN is caused by present everywhere and polyphagous fungi among which Alternaria and Fusarium were the main genera (Belisario et al., 2010). The commonly isolated species are *A. alternata*, *A. arborescens*, *A. tenuissima*, *F. avenaceum*, *F. graminearum*, *F. semitectum*, *F. culmorum*, and *F. oxysporum* (Belisario et al., 2002; Hong et al., 2006; Scotton et al., 2015; Akat et al., 2016; Moya-Elizando et al., 2021).

Different control methods such as pesticide application, use of resistant varieties, cultural methods, and biological control methods are applied to prevent losses due to fungal diseases (Tekiner et al., 2019). However, BAN disease epidemiological aspects are not fully known, and limited information is available (Moragrega and Özaktan, 2010; Moya-Elizondo et al., 2021) therefore, there is a need for improved phytosanitary programs to control this disease.

The present work aimed to identify the biological control agents bacteria that can be used against BAN disease *in vitro* conditions.

MATERIALS AND METHODS

The fungus was isolated and purified from diseased walnut (*Juglans regia* L.) fruits taken from the Banaz district of Uşak. Pathogenicity test and classical diagnosis of the fungus were made (Figure 1).

Figure 1. Diseased plant samples and fungi in Petri dishes

As the biological control agent bacteria, isolates determined to be effective against different disease agents in previous biological control studies were used (Table 1).

Table 1. Biological control agent bacteria used in the study

Isolate	Host	Microbial Identification System (MIS)	Similarity Index	References
FDG 37	Soil	*Pseudomonas flourescens*	0.222	Güneş et al., 2015
RK 103	Apple Branch	*Bacillus pumilus*	0.626	Kotan R., 2000
TV 3A	Rye	*Pseudomonas flourescens*	-	**In this study**

TV 6F	Wheatgrass	*Bacillus substilis*	0.831	Erman et al., 2010
TV 7F	Wheatgrass	*Bacillus mycoides*	-	**In this study**
TV 13A	Raspberry	*Bacillus megaterium*	-	**In this study**
TV 13C	Raspberry	*Bacillus megaterium*	0.595	Erman et al., 2010
TV 17C	Raspberry	*Bacillus substilis*	0.677	Çakmakcı et al. 2010
TV 20D	Raspberry	*Bacillus cereus*	-	**In this study**
TV 42A	Wheatgrass	*Pseudomonas putida*	0.113	Erman et al., 2010
TV 53D	Taraxacum	*Brevibacillus choshinensis*	0.689	Erman et al., 2010
TV 59D	Taraxacum	*Bacillus megaterium*	-	**In this study**
TV 67C	Raspberry	*Bacillus pumilus*	0.630	Erman et al., 2010
TV 73F	Sedum	*Bacillus pumilus*	-	**In this study**
TV 77B	Kekre	*Bacillus substilis*	-	**In this study**
TV 89E	Gladiolus onion	*Bacillus substilis*	-	**In this study**
TV 93B	Sugar beet	*Bacillus substilis*	-	**In this study**
TV 95A	Peas	*Bacillus megaterium*	-	**In this study**
TV 103B	Wheatgrass	*Paenibacillus polymyxa*	0.514	Tozlu et al., 2016
TV 112G	Wheatgrass	*Bacillus atrophaeus*	-	**In this study**

Pathogenicity Test

The pathogenicity of *Fusarium* sp. was tested on walnut fruit. Walnuts were washed under tap water and subjected to surface sterilization with 70% ethyl alcohol in a sterile cabinet. A 6 mm mycelial disc from the fungus, which was previously developed in PDA medium for 7 days, was applied to the fruit by making a wound and the wound surface was wrapped with parafilm. The samples were placed in plastic boxes (7 l) on the bottom of which sterile moist blotting paper was laid, and the growth of the fungus was monitored for 21 days in a 12-hour light/12-hour dark environment at room temperature. In the control application; a sterile PDA disc was placed on the wound site and wrapped with parafilm. The study was carried out in a randomized plot design with 3 replications. Deterioration and mycelial growth on the fruit surface were evaluated as positive and Koch postulates were completed by reisolation from these areas.

Identification of Bioagent Bacterial Strain by Microbial Identification System (MIS)

Bacteria (TV 3A, TV 7F, TV 13A, TV 20D, TV 59D, TV 73F, TV 77B, TV 89E, TV 93B, TV 95A, TV 112G) were identified according to their fatty acid methyl esters (FAME) profiles using Sherlock Microbial Identification System (Miller 1982). These tests were repeated 3 times, and the highest diagnostic result in terms of percentage was considered the definitive result.

Hypersensitivity Response (HR) Testing of Bacterial Biocontrol Agents

The hypersensitivity test of the bioagent bacteria used for the first time in the study in tobacco was carried out on the fresh leaves of the 5-6 week-old *Nicotiana tabacum* var Samsun variety. The bacterial suspensions (10^8 cfu/ml) were prepared in sterile distilled water and infiltrated into the intercostal area of the leaves of tobacco plants by using a 3-cc syringe without a needle. The inoculated plants were incubated in a completely randomized design on the greenhouse bench for 24-48 h at 20-28°C. The presence of rapid tissue necrosis at the inoculation site was recorded within 24-48 h after infiltration. This test was repeated at least three times for each strain. Sterilized distilled water (sdH_2O) was used as a negative control. The tobacco plants' symptoms were examined. The bacterial isolates which weren't developed any symptoms were evaluated as nonpathogenic, but developed were pathogenic (Klement et al., 1964).

Dual Culture Tests

Petri dishes (90 mm) containing 20 ml of PDA were used in dual culture tests, bioagent bacterial isolates were grown on Nutrient Agar (NA) for 24 hours and pathogenic fungus isolate was grown on PDA for 7 days. Then, bioagent bacterial culture was drawn with a sterile stick around the Petri dishes containing PDA, and a 6 mm fungal disc was placed in the middle of the petri dish. Petri dishes were wrapped with parafilm and incubated at 27°C. As a control, a mycelial disc of pathogenic fungus was placed in the middle of the Petri dish. In the control Petri dish, the radial growth of the fungus was measured in mm when the pathogenic fungus covered the entire surface of the Petri dish. The study was carried out in a randomized plot design with 3 replications and 3 Petri dishes were used for each application. The percent inhibition rate (YEO) of the pathogenic fungus colony was calculated using the percent inhibition of radial growth formula specified by Mari et al. (1993).

$$\text{Percent inhibition rate (\%)} = (C-T)\times 100/(C-6)$$

C: Diameter of control plate pathogen

T: Colony diameter of the pathogen in bacterial application

6: Diameter of the pathogen disc

Statistical Analysis

Statistical analysis was performed on the obtained values using JMP 5.0.1 statistical software and the differences between the means were compared at the $P<0.01$ significance level according to the LS Means Student test.

RESULTS AND DISCUSSION

The pathogenicity of the fungus isolated from the walnut fruit was determined and the result was positive (Figure 2).

Pathogen	Control	Re-isolation Petri

Figure 2. Pathogenicity test on walnut fruit and reisolation fungi Petri dish

According to the classical diagnostic results, it was determined that the pathogenic fungus isolate belonged to the genus *Fusarium*. ET 107 code was given and added to the fungus culture collection. Information on the bioagent bacterial isolates that were tried for the first time in this study and the HR test result are given in Table 2. Fatty methyl ester analyzes of bioagent bacterial isolates applicated in this study: 1 of bacterial isolate was *P. fluorescence* (TV 3A), 1 was *B. mycoides* (TV 7F), 3 were *B. megaterium* (TV 13A, TV 59D, TV 95A), 1 was *B. cereus* (TV 20D), 1 was *B. pumilus* (TV 73F), 3 were *B. substilis* (TV 77B, TV 89E, TV 93B) and 1 was *B. atrophaeus*. All bioagent bacteria HR test results were negative.

Table 2. Microbial Identification System and hypersensitivity test results of bioagent bacteria

Isolate	Host	Microbial Identification System (MIS)	Similarity Index	HR
TV 3A	Rye	*Pseudomonas flourescens*	0.562	-
TV 7F	Wheatgrass	*Bacillus mycoides*	0.575	-
TV 13A	Raspberry	*Bacillus megaterium*	0.124	-
TV 20D	Raspberry	*Bacillus cereus*	0.466	-
TV 59D	Taraxacum	*Bacillus megaterium*	0.504	-
TV 73F	Sedum	*Bacillus pumilus*	0.594	-
TV 77B	Kekre	*Bacillus substilis*	0.483	-
TV 89E	Gladiolus onion	*Bacillus substilis*	0.690	-
TV 93B	Sugar beet	*Bacillus substilis*	0.732	-
TV 95A	Peas	*Bacillus megaterium*	0.502	-
TV 112G	Wheatgrass	*Bacillus atrophaeus*	0.421	-

The results of the antifungal activities of the bioagent bacteria tested against the ET 107 isolate in the dual culture tests are given in Table 3 and the Petri dishes views of the most effective bacteria are given in Figure 3.

Table 3. Percent inhibition rates of bioagent bacteria tested against ET 107 isolate

	Bacteria	MIS	PIRG*	
1	TV 53D	*Brevibacillus choshinensis*	A	90.10
2	FDG 37	*Pseudomonas fluorescens*	B	83.70
3	TV 73F	*Bacillus pumilus*	BC	82.57
4	RK 103	*Bacillus pumilus*	BCD	81.37
5	TV 67C	*Bacillus pumilus*	BCDE	80.57

6	TV 7F	*Bacillus mycoides*	BCDE	80.57
7	TV 42A	*Pseudomonas putida*	BCDE	80.17
8	TV 13A	*Bacillus megaterium*	CDE	79.00
9	TV 112G	*Bacillus atrophaeus*	DE	78.60
10	TV 13C	*Bacillus megaterium*	DE	78.20
11	TV 17C	*Bacillus subtilis*	DEF	77.80
12	TV 95A	*Bacillus megaterium*	DEF	77.80
13	TV 6F	*Bacillus substilis*	EF	77.40
14	TV 93B	*Bacillus subtilis*	EF	77.40
15	TV 59D	*Bacillus megaterium*	FG	74.20
16	TV 77B	*Bacillus subtilis*	GH	72.60
17	TV 103B	*Bacillus megaterium*	GH	71.80
18	TV 3A	*Pseudomonas flourescens*	H	70.20
19	TV 20D	*Bacillus cereus*	H	70.20
20	TV 89E	*Bacillus subtilis*	H	69.80
	Control		I	0.0
			CV	**0.032**
			LSD	**3.86**

*There is no statistical difference between the values expressed with similar letters in the same column (p<0.01).

The percent inhibition rates of bioagent bacterial isolates varied between 69.80% and 90.10%. The most effective results in *in vitro* conditions were obtained from *B. choshinensis* (TV 53D, 90.10%), respectively by *P. fluorescens* (FDG 37, 83.7%) and *B. pumilus* (TV 73F, 82.57%). The lowest inhibition rate was obtained from TV 89E (69.80%) (Table 3).

TV 53D	**FDG 37**	**TV 73F**

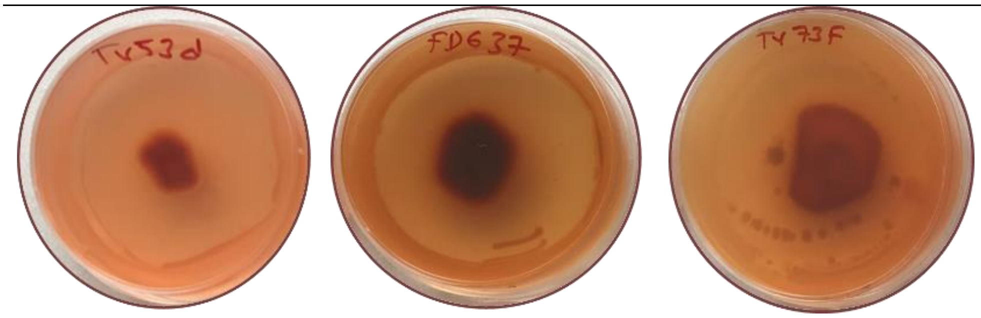

Figure 3. Petri dishes of bacteria most effective against pathogenic fungi *in vitro* conditions

The efficacy of 11 bacterial isolates used for the first time in this study ranged from 69.80% (TV 89E) to 82.57% (TV 73F). The percent inhibition rate in the control, in which only the pathogen isolate ET 107 was applied, was statistically (p<0.01) different from all other tested bacteria. In this study, bioagent bacteria belonging to 1 *Brevibacillus*, 3 *Pseudomonas*, and 16 *Bacillus* genera were used as biological control agents and these bioagents prevented the growth of pathogenic fungi at different rates. Biological control is an effective method of controlling plant diseases in an environmentally friendly way by using microorganisms and bacterial biocontrol agents are used to prevent fungal diseases successfully (Kotan et al., 2009; Abdalla et al., 2014). The effectiveness of bacterial bioagents may be due to various factors (genus of the bioagent bacteria, the competitiveness of the bioagent, the aggressiveness of the pathogen, the host susceptibility and environmental conditions) (Frances et al., 2006). Additionally, there are many studies showing that species belonging to *Pseudomonas* sp., *Pantoea* sp., *Bacillus* sp. and *Brevibacillus* sp. genera can be used as potential bioagent against fungal and bacterial diseases (Kotan et al., 1999; Panwar et al., 2013; Pane and Zaccardelli, 2015; Tekiner et al., 2019; Tozlu and Akça 2022). Studies have shown that bacterial bioagents inhibit the development of the pathogen by producing antibiotic-like substances and hydrolytic enzymes and one of the most important mechanisms for biocontrol of pathogenic fungi (Elshafie et al. 2012; Güneş et al., 2015). It is thought that the bioagent bacteria that were effective in this study inhibited the growth of the fungus by using one of these mechanisms.

In conclusion, TV 53D, FDG 37 and TV 73F bacteria had the potential as bioagents for the control of *Fusarium* sp. under *in vitro* conditions. This study is the first *in vitro* biological control study against BAN disease agent *Fusarium* sp. In future studies, it is of great importance to focus on the determination of the working mechanisms of these bioagents and their *in vivo* applications against *Fusarium* sp. and making them into biological preparations.

Statement of Conflict of Interest

The authors should declare that they are no conflicts of interest.

Authors' Contributions

All authors contributed equally.

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POSTER PRESENTATIONS

Determination of Alternative Methods for Control of Weeds in Medicinal and Aromatic Plants*

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ABSTRACT: As in all cultivated plants, weed competition is one of the leading causes of significant yield loss in medicinal and aromatic plants. In addition to the direct effect of weeds, it creates a risk for human health by mixing with medicinal and aromatic plants and showing toxic effects. The aim of this study is to control weeds by applying different organic materials such as Jute, agril, straw, sawdust and peanut shell on rows of plants such as sage (*Salvia officinalis* L.), izmir thyme (*Origanum onites* L.) and rosemary (*Salvia rosmarinus* L.). For this purpose, these materials were applied according to the strip plots trial design in Silifke district of Mersin province. In the experiment, the strip plots were 18 m long and 50 cm wide. Control plots were created for each product. On the 7th, 14th, 28th and 56th days after the application, it was observed that peanut, sawdust, straw was insufficient to control weeds in rosemary, thyme and sage plants, according to the effectiveness in the counts. In the mulched plots, mulching was performed by *Cynodon dactylon* (L.) Pers. and *Chenopodiu album* L., *Convolvulus arvensis* L., *Lamium purpureum* L., *Malva neglacta* Wallr., *Trifolium repens* L., *Sinapis arvensis* L.

Keywords: Weed, Sage, Rosemary, Thyme, Jut, Agril

INTRODUCTION

Medicinal and aromatic plants are plants that are used as medicine to cure diseases and prevent possible diseases. In addition, it has a wide use in many areas such as nutrition, nutritional supplements, herbal tea, flavor, tea, perfume, body care products and religious ceremonies (Anonymous, 2005; Arslan et al., 2015). Certain dried parts (root-stem, tuber, stem, bark, leaf, flower, fruit, seed) of medicinal and aromatic plants are used (Anonymous, 2012). At the same time, the use of substances with allelopathic effects such as essential oils obtained from thyme, rosemary and sage as an alternative to chemical control in weed control shows how important these medicinal and aromatic plants are. around a dollar. Turkey is located in the middle part of world trade. In Turkey, approximately 20 kinds of medicinal and aromatic plants are cultivated on an area of 1.3-1.5 million decares and production areas until 2015. increased by approximately 40%. Foreign trade of medicinal and aromatic plants was 285 million dollars in exports and 255 million dollars in imports in 2015. Thyme ranks first in exports with a 25% share from medicinal and aromatic plants. Thus, as production increases, trade increases rapidly. Increasing demand for medicinal herbal products is a national and international trend, and the demand for essential oils obtained from plants and used as antioxidants is increasing day by day due to legal restrictions on chemical substances used in food. The recognition of herbal products as safe and healthy products with few side effects, unlike synthetic drugs, is also an important factor that helps to increase the market share (Kartal and Erdem, 2012).

Among the medicinal and aromatic plants, rosemary, sage and thyme are among the important species exported in our country, and their resistance to drought and cold is tolerant compared to many medicinal and aromatic plants. If they are irrigated, it is possible to get two or more crops per year. In order for Turkey to have a say in the world markets in medicinal and aromatic plants, product diversity must be increased and produced in accordance with the desired standards.

The aim of the study is to determine the use of medicinal and aromatic plants and their economic importance from the past to the present, and to investigate alternative control possibilities that can minimize the losses in the production amount and yield of weeds, which is one of the factors limiting the production in the cultivation of medicinal and aromatic plants as well as in other cultural plants.

MATERIAL AND METHOD

In Silifke district of Mersin province, sage (*S. officinalis*) was planted on an area of 840 m2, İzmir thyme (*O. onites*) was planted on an area of 1260 m^2 and rosemary (*R. officinalis*) was planted on an area of 1181 m^2. Jute, agril, straw, sawdust and peanut shell materials were applied on the rows of sage (*Salvia officinalis* L.), izmir thyme (*Origanum onites* L.) and rosemary (*Salvia rosmarinus* L.) plants according to the strip plots experimental design (Figure 1). In the experiment, the strip plots were 18 m long and 50 cm wide. Control plots were created for each product.

Weed density; Density = B (total number of individuals in the total area of the frame thrown) / n (total area of the frame thrown) formula (Güncan, 2001); The frequency of occurrence of weed species was calculated according to the formula (R.S)=100 X (number of fields with one species (n) / total number of fields measured (m) (Odum, 1971).

A

B

C

D	E	F

Figure 1. Materials used in the trial plots (A:control, B:Agril, C:Straw, D:Peanut, E: Sawdust, F: jute series plot

Weed counts were made on the 7th, 14th, 28th and 56th days after the application.

RESULTS AND DISCUSSION

In the Silifke district of Mersin province, 9 broad-leaved and 3 narrow-leaved weed species were found in the rosemary planted field. Information on the detected weed species, their densities and frequency of occurrence are given in Table 1. In the sage field, 10 of the weeds are broad-leaved and 3 are narrow-leaved grasses. Information on the detected weed species, their densities and frequency of occurrence are given in Table 2. In the thyme field, weeds are 9 broad-leaved and 3 narrow-leaved. Information on the detected weed species, their densities and frequency of occurrence are given in Table 3.

Table 1. Weed density (number/m^2) and incidence (%) in the rosemary area in Mersin province

Weed species	Density (piece/m^2)	Frequency of occurrence (%)
Malva neglecta	2.50	65
Sonchus arvensis	6.25	65
Convolvulus arvensis	6.75	85
Mercurialis annua	4.75	65
***Cyperus* sp.**	5.50	35
Trifolium repens	12.5	50
Taraxacum officinale	4.00	50
Fumaria officinalis	3.25	50
***Lamium* sp.**	1.75	35
Sinapis arvensis	1.75	35
Sorghum halepense	0.50	15
Cynodon dactylon	0.50	15

Table 2. Weed density (number/m^2) and incidence (%) in sage field in Mersin province

Weed species	Density (piece/m^2)	Frequency of occurrence (%)
Malva neglecta	8.25	85
Sonchus arvensis	14.25	65
Convolvulus arvensis	8.50	65
Mercurialis annua	2.50	85
***Cyperus* sp.**	8.00	50
Trifolium repens	8.50	80

Taraxacum officinale	0.75	15
Fumaria officinalis	1.25	15
***Lamium* sp.**	3.50	60
Sinapis arvensis	0.75	15
Sorghum halepense	1.25	35
Cynodon dactylon	1.25	15
***Allium* sp.**	0.25	10

Table 3. Weed density (number/m^2) and incidence (%) in the thyme area in Mersin province

Weed species	Density (piece/m^2)	Frequency of occurrence (%)
Malva neglecta	3.00	50
Sonchus arvensis	11.50	65
Convolvulus arvensis	1.25	30
Mercurialis annua	4.25	60
***Cyperus* sp.**	0.50	15
Trifolium repens	7.25	65
Taraxacum officinale	4.50	50
Fumaria officinalis	1.25	15
***Lamium* sp.**	0.50	10
Sinapis arvensis	0.50	10
Sorghum halepense	2.50	35
Cynodon dactylon	2.75	50

In weed control studies, it was observed that peanut, sawdust and straw were insufficient to control weeds in rosemary, thyme and sage plants, according to the effectiveness evaluations made on the 7th, 14th, 28th and 56th days of the application. (Figure 2; Figure 3; Figure 4).

A **B** **C**

Figure 2. Rosemary (A-straw-spread parcel, B-sawdust-laid parcel, C-peanut shell-laid parcel)

A **B** **C**

Figure 3. Thyme (A- straw-laid parcel, B-sawdust-laid parcel, C-peanut shell-laid parcel)

A	B	C

Figure 4. Sage (A-straw-laid parcel, B-sawdust-laid parcel, C-peanut shell-laid parcel)

According to the counts made 14 days after the application, in the mulch-applied plots in the experimental area planted with rosemary, mulching should be carried out such as *C. arvensis, L. purpureum, M. arvensis, T. repens, S. arvensis,* except for the narrow-leaved weeds *C. dactylon* and broad-leaved *C. album*. It has been observed to control broadleaf weeds (Figure 5).

AGRİL	JÜT	CONTROL

Figure 5. Parcel with rosemary agril and jute series

According to the counts made 14 days after the application, it is seen that mulching controls weeds better than other applications in the plots where mulch was applied in the thyme planted trial area (Figure 6).

AGRİL JÜT CONTROL

Figure 6. Parcel with thyme agri and jute series

Likewise, mulch applications in sage plant gave the best results among other applications (Figure 7).

AGRİL JÜT CONTROL

Figure 7. Parcel with sage agril and jute series

Mulch applications gave the best results among the applications in the experiment. Sokat, in the study he carried out in the Izmir thyme fields in Denizli in 2013; 127 different weed species belonging to 31 families (Poaceae, Amaranthaceae, Asteraceae, Chenopodiaceae, Convolvulaceae, Laminaceae) were detected in the experimental area. It has been reported that weed control is achieved in mulch application against these weed species (Sokat, 2013). In our study, the species belonging to the mentioned families were determined in the fields of rosemary, thyme and sage and parallel results were obtained. Arslan and Uygur (2011) stated that no weeds grew in the plots where textile mulch was applied under greenhouse and field conditions in Adana. It has been reported that the application of black polyethylene cover in the production of saplings in Aydın province is long-term effective in the control of annual and perennial weeds (Öğüt, 2007).

Kwiatkowski, et al., 2005, in a study they conducted in Poland in 2004; They reported that *Chenopodium album, Amaranthus retroflexus, Fumaria officinalis, Viola arvensis, Stellaria media, Polygonum persicaria, Polygonum aviculare, Capsella bursa-pastoris, Thlaspi arvense* and Galinsoga parviflora are common weeds in thyme production areas. In our study, it is seen that similar weeds are a problem in thyme fields.

In 2006, Fontana et al. reported that mulching reduces weed density, increases the effect on plant growth (Jacqueline et al., 1991), Fraser and Whish (1997), weed problem in thyme will end with the use of organic mulch, Galambosi et al., (1990).), reports that mulching provides weed control and reduces the labor needed for weed control by 65% - 80% are similar to the results of the study.

CONCLUSION

13 different weed species were determined in the thyme, rosemary and sage cultivation areas in Mersin province. It has been determined that mulching gives the best results in the fight against weeds, and it is foreseen to carry out researches to put these applications into practice in accordance with the production method. In addition, hand hoeing on the row after intermediate ploughing will increase plant growth and indirectly weeds will be combated.

Apart from mulching for weed control in thyme, rosemary and sage, which are important medicinal and aromatic plants and our export products, it is predicted that it would be correct to consider chemical control and olive black juice, cabbage wastes as alternative control and to carry out residue analyzes related to chemical applications in order to avoid any problems in exports in chemical control. It is thought that the creation of these control methods will shed light on the researches of weed problem in medicine and aromatic plants.

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Statement of Conflict of Interest
I declare that there is no conflict between the authors.

Authors' Contributions
All stages of this article, which was produced from my master's thesis, was conducted and written by myself.

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