JOURNAL OF AGRICULTURAL PRODUCTION

Year: 2023 | Volume: 4 | Issue: 2 | e-ISSN 2757-6620 | agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

2023 • VOLUME 4 • ISSUE 2

agriprojournal.com — agriprojournal@gmail.com
prensip.gen.tr — info@prensip.gen.tr

Journal of Agricultural Production is a peer-reviewed scientific journal published by *Gökhan ARSLAN* twice a year.

(Publisher support: ***Prensip Publishing***)

JOURNAL OF AGRICULTURAL PRODUCTION

2023 • VOLUME 4 • ISSUE 2

TABLE OF CONTENTS

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

https://prensip.gen.tr

REVIEW ARTICLE

Exploring the Link between Soil Microbial Diversity and Nutritional Deficiencies

Ali Yetgin[1,2]✉

[1]Toros AGRI Industry and Trade Co. Inc., Research and Development Center, Mersin/Türkiye
[2]Çukurova University, Institute of Nature and Applied Sciences, Department of Biotechnology, Adana/Türkiye

ARTICLE INFO

Article History
Received: 09.04.2023
Accepted: 10.07.2023
First Published: 24.10.2023

Keywords
Biodiversity
Micronutrients
Nutritional deficiencies
Soil microbial diversity

ABSTRACT

The world is facing a hidden hunger crisis, where millions of people are suffering from nutritional deficiencies despite having access to food. While much research has focused on the quality and quantity of food, recent studies have shown that soil microbial diversity may also play a crucial role in human nutrition. Soil microbes interact with plants in complex ways, influencing the absorption of nutrients and producing compounds that are essential for human health. However, factors such as intensive agriculture, climate change, and soil pollution can lead to a decline in soil microbial diversity, which may contribute to the rise of hidden hunger. In this paper, we explore the link between soil microbial diversity and nutritional deficiencies, examining the latest research on the topic and discussing potential solutions to this pressing global issue. Our findings suggest that promoting soil health and biodiversity could be a key strategy for addressing hidden hunger and improving global nutrition.

Please cite this paper as follows:

Yetgin, A. (2023). Exploring the link between soil microbial diversity and nutritional deficiencies. *Journal of Agricultural Production*, *4*(2), 81-90. https://doi.org/10.56430/japro.1279830

1. Introduction

Hidden hunger, also known as micronutrient deficiency, is a form of undernutrition that occurs when people lack access to essential vitamins and minerals in their diet, even if they are consuming enough calories (Biesalski & Biesalski, 2013). Unlike acute hunger, which is characterized by a severe lack of food, hidden hunger can go unnoticed for years as it does not result in immediate starvation. The term “hidden” refers to the fact that the deficiency is often not visible and does not manifest in the same way as other forms of malnutrition. The hidden hunger crisis is a global challenge, affecting an estimated 2 billion people worldwide and contributing to a range of health problems, including stunted growth, impaired cognitive development, and increased susceptibility to infections (Saltzman et al., 2014).

The hidden hunger crisis disproportionately affects vulnerable populations, particularly women and children in low-income countries who have limited access to nutrient-rich foods, such as fruits, vegetables, and animal products. Inadequate soil quality and poor agricultural practices can also contribute to hidden hunger by reducing the nutrient content of crops. The depletion of soil microbial diversity, which plays a crucial role in carbon, nitrogen, phosphorus nutrient cycling and plant growth, can have negative impacts on the nutritional quality of food and the health of the surrounding ecosystem.

✉ Correspondence
E-mail address: ali1992yetgin@gmail.com

Addressing the hidden hunger crisis requires a multifaceted approach that involves promoting access to nutrient-rich foods, improving agricultural practices, and enhancing public awareness and education (Carducci et al., 2020). Strategies for addressing hidden hunger include fortifying staple foods with essential micronutrients, promoting the cultivation of nutrient-rich crops, such as fruits and vegetables, and promoting sustainable agricultural practices that support soil health and biodiversity. The hidden hunger crisis is a complex and pressing global challenge that requires coordinated efforts to address (Fróna et al., 2019). By promoting soil health and biodiversity, improving agricultural practices, and increasing public awareness and education, we can work to address hidden hunger and promote food security and nutrition for all.

Soil microbial diversity plays a crucial role in maintaining healthy and productive ecosystems. Microbes in soil, such as bacteria, fungi, and protozoa, contribute to nutrient cycling, decomposition of organic matter, and the formation of soil structure (Koshila et al., 2019). They also play a critical role in plant growth and health by helping to solubilize nutrients, fix nitrogen, and promote disease resistance. The importance of soil microbial diversity extends beyond agricultural productivity to ecosystem services such as carbon sequestration, water filtration, and climate regulation. Microbial diversity in soil is also important for maintaining biodiversity and supporting wildlife habitats.

However, soil microbial diversity is under threat due to factors such as land-use change, pollution, and climate change. Loss of microbial diversity can have negative impacts on soil health and productivity, as well as on the health of surrounding ecosystems and human communities that depend on them (Sharma et al., 2011). Therefore, it is crucial to promote practices that support soil microbial diversity, such as reducing tillage, promoting crop rotation, and avoiding the use of synthetic fertilizers and pesticides. By supporting soil health and microbial diversity, we can help to maintain healthy and productive ecosystems, support food security and nutrition, and mitigate the impacts of climate change.

In addition to its role in promoting plant growth and supporting ecosystem services, soil microbial diversity has also been linked to human health. Recent research has suggested that the microbiome of the soil and the human gut are connected and that exposure to diverse soil microbiota can positively influence human health outcomes, including reducing the risk of allergies and autoimmune diseases (Blum et al., 2019). Moreover, soil microbial diversity plays a critical role in addressing the hidden hunger crisis. As mentioned earlier, soil microbial diversity is important for nutrient cycling and can impact the nutrient content of crops. Therefore, promoting practices that support soil microbial diversity can lead to an increase in the nutritional quality of crops, and subsequently, help address the hidden hunger crisis.

Soil microbial diversity is a crucial component of healthy and productive ecosystems that provides a range of benefits, from supporting plant growth to promoting human health. With increasing threats to soil microbial diversity, it is important to promote practices that support soil health and biodiversity, such as reducing tillage, promoting crop rotation, and avoiding the use of synthetic fertilizers and pesticides (Dias et al., 2015). By doing so, we can support sustainable agriculture, address the hidden hunger crisis, and mitigate the impacts of climate change. The study aims to understand how the depletion of soil microbial diversity, which can occur as a result of intensive farming practices and the use of synthetic fertilizers, can impact the nutrient content of crops and contribute to the hidden hunger crisis.

By exploring this link, the study seeks to identify potential strategies for promoting soil health and microbial diversity to improve the nutritional quality of crops and address the hidden hunger crisis. Additionally, the study aims to raise awareness about the importance of soil health and biodiversity in agricultural systems and the role of sustainable agriculture in promoting food security and nutrition (Thrupp, 2000). Ultimately, the study seeks to contribute to a broader understanding of the interconnections between soil health, agriculture, and human nutrition, and to inform policy and practice in support of sustainable and equitable food systems.

2. Hidden Hunger: Causes and Consequences

The consequences of hidden hunger can be severe, particularly for vulnerable populations such as children and pregnant women. Micronutrient deficiencies can lead to a range of health problems, including stunted growth, impaired cognitive development, weakened immune systems, and increased risk of infections and chronic diseases (Brownie, 2006; Bailey et al., 2015). In children, micronutrient deficiencies can result in developmental delays, decreased academic performance, and increased mortality rates. Pregnant women with micronutrient deficiencies are at increased risk of complications during pregnancy and childbirth, including anemia, pre-eclampsia, and low birth weight (Hovdenak & Haram, 2012).

Moreover, the economic costs of hidden hunger are significant. Productivity losses, increased healthcare expenditures, and reduced earning potential can result from the long-term health impacts of micronutrient deficiencies. The economic burden of hidden hunger can be especially challenging for low-income countries and vulnerable populations, exacerbating existing inequalities and hindering development (Redón Lago, 2021). In addition to the human and economic costs, hidden hunger can have wider social implications. Poor nutrition can lead to social exclusion, as individuals may face discrimination or stigma due to their physical appearance or perceived intellectual capacity.

Moreover, hidden hunger can undermine social cohesion and contribute to social unrest, as individuals and communities struggle to meet their basic needs. The consequences of hidden hunger are far-reaching and can have serious impacts on human health, economic development, and social cohesion. Addressing hidden hunger requires a comprehensive approach that involves promoting access to nutrient-rich foods, enhancing agricultural practices, and improving public awareness and education. By addressing hidden hunger, we can promote human well-being, reduce economic burdens, and build more equitable and resilient societies.

To expand on the consequences of hidden hunger, micronutrient deficiencies can also have implications for global sustainability and environmental health. Malnutrition can exacerbate environmental degradation by increasing demand for land use, water, and energy resources. For instance, in low-income countries, the over-reliance on staple crops, which are often low in essential micronutrients, can lead to extensive land use and deforestation, which can further impact soil health and lead to biodiversity loss. Furthermore, the impacts of hidden hunger can extend beyond individual health to affect entire populations and even future generations. For example, maternal malnutrition can lead to adverse outcomes for both mothers and children, including low birth weight, premature birth, and developmental delays. These outcomes can have lasting impacts on individuals' health and well-being, as well as their economic and social opportunities (Jetten et al., 2014; Kansky, 2017).

In addition, hidden hunger can have broader implications for global health security. Micronutrient deficiencies can increase the risk of infectious diseases, particularly in regions with poor water and sanitation. Inadequate nutrient intake can also impact the efficacy of vaccines, leading to reduced immunogenicity and effectiveness (Calder et al., 2022). Addressing hidden hunger requires a coordinated and collaborative approach across multiple sectors, including agriculture, health, education, and social welfare. Efforts to promote access to nutrient-rich foods, improve agricultural practices, and enhance public awareness and education are critical to reducing the prevalence and impact of hidden hunger. By addressing hidden hunger, we can improve individual health and well-being, promote sustainable development, and contribute to a more equitable and resilient future.

Hidden hunger is a global issue that affects millions of people, particularly in low- and middle-income countries. According to the World Health Organization (WHO), over 2 billion people worldwide suffer from micronutrient deficiencies (WHO, 2006). The prevalence of hidden hunger varies by region and is often highest in sub-Saharan Africa, South Asia, and parts of Latin America. The populations most affected by hidden hunger are often those who are already vulnerable due to poverty, limited access to healthcare, and inadequate nutrition. Children, pregnant and lactating women, and the elderly are among the groups most at risk of micronutrient deficiencies. In low-income countries, over 90% of children under the age of 5 and 75% of pregnant women suffer from one or more micronutrient deficiencies (Durkin, 2002; Gernand et al., 2016).

In addition to individual vulnerability, hidden hunger can also disproportionately impact entire communities and regions. Populations living in areas with poor soil quality or limited access to nutrient-rich foods are at increased risk of micronutrient deficiencies (Miller & Welch, 2013). Conflict, displacement, and natural disasters can also exacerbate the prevalence of hidden hunger, as individuals and communities face disrupted food systems and limited access to essential nutrients. Addressing the prevalence of hidden hunger requires a targeted and equitable approach that prioritizes the most vulnerable populations. Efforts to improve nutrition outcomes must be integrated with broader development goals, including poverty reduction, improved water and sanitation, and enhanced economic opportunities. By addressing the root causes of hidden hunger and promoting access to essential nutrients, we can improve the health and well-being of individuals and communities, reduce inequalities, and build more resilient and sustainable societies.

Hidden hunger is not only a problem in low- and middle-income countries but also affects certain populations in high-income countries. In developed nations, hidden hunger can arise due to a lack of diversity in diets, as well as food insecurity and poverty. Vulnerable populations in high-income countries, such as the elderly, low-income families, and immigrant communities, may also be at increased risk of micronutrient deficiencies. Moreover, the impact of hidden hunger goes beyond individual health and can have significant economic consequences. According to the Global Panel on Agriculture and Food Systems for Nutrition, the economic cost of malnutrition worldwide is estimated to be around $3.5 trillion per year, equivalent to 3.5% of global GDP (Agriculture and Food Systems for Nutrition, 2016). These costs are associated with a range of impacts, including decreased productivity, increased healthcare expenditures, and reduced earning potential.

Addressing hidden hunger requires a comprehensive and integrated approach that addresses both individual and systemic factors contributing to micronutrient deficiencies (Burchi et al., 2011). Efforts must focus on promoting access to diverse and nutrient-rich foods, improving agricultural practices and food systems, and enhancing public awareness and education on nutrition and health. Additionally, it is essential to address the root causes of poverty, inequality, and social exclusion that contribute to hidden hunger, as well as to strengthen health systems and improve access to essential health services. The prevalence and impact of hidden hunger are significant and far-

reaching, affecting millions of individuals and communities worldwide. Addressing this issue requires a coordinated and multisectoral approach that prioritizes the most vulnerable populations, promotes sustainable development, and builds more equitable and resilient societies. By working together to address hidden hunger, we can improve individual and population health, reduce economic burdens, and promote a more just and sustainable world.

3. Soil Microbial Diversity and Nutrient Availability

Soil microbial diversity refers to the variety and abundance of microorganisms present in the soil, including bacteria, fungi, and other microscopic organisms. These microorganisms play a critical role in soil health and ecosystem functioning, influencing a range of processes from nutrient cycling to plant growth and disease suppression (Chourasiya et al., 2017). The importance of soil microbial diversity stems from its fundamental role in sustaining plant growth and crop yields. Microorganisms in the soil play a critical role in breaking down organic matter and releasing essential nutrients such as nitrogen, phosphorus, and potassium, making them available for plant uptake. They also help to suppress plant pathogens and promote disease resistance, which is essential for maintaining healthy plant populations.

Furthermore, soil microbial diversity is crucial for maintaining the overall health and resilience of terrestrial ecosystems. Microorganisms in the soil are key drivers of soil carbon sequestration and contribute to the regulation of greenhouse gas emissions, helping to mitigate climate change. They also play a critical role in maintaining soil structure and stability, reducing erosion and improving water infiltration (Adugna, 2016). In addition to their ecological importance, soil microorganisms have significant potential for promoting sustainable agriculture and addressing global food security challenges. For example, microbial inoculants can be used to improve plant growth and nutrient uptake, reduce the need for synthetic fertilizers, and promote the use of sustainable farming practices. The importance of soil microbial diversity lies in its critical role in sustaining plant growth, ecosystem functioning, and overall soil health. By promoting soil microbial diversity and harnessing its potential for sustainable agriculture, we can help to ensure food security, mitigate climate change, and promote more resilient and sustainable ecosystems.

Soil microbial diversity also plays a crucial role in supporting human health and well-being. Microorganisms in the soil are a source of beneficial compounds such as antibiotics, enzymes, and other bioactive molecules, many of which have potential therapeutic applications (Challinor & Bode, 2015). In addition, studies have shown that exposure to soil microbes can help to promote immune system development and function, particularly in early childhood. Moreover, soil microbial diversity is essential for maintaining global biodiversity and ecosystem services. The diversity of microorganisms in the soil is closely linked to the diversity of plant and animal species that depend on them for survival. By supporting healthy soil ecosystems and promoting biodiversity, we can help to preserve the essential ecosystem services that underpin human well-being, such as nutrient cycling, water purification, and climate regulation.

However, soil microbial diversity is under threat from a range of human activities, including intensive agriculture, deforestation, urbanization, and pollution. These activities can disrupt soil ecosystems and reduce microbial diversity, leading to reduced soil fertility, increased soil erosion, and decreased resilience to climate change (Qiu et al., 2021). Soil microbial diversity is a critical component of healthy soils, sustainable agriculture, and overall ecosystem functioning. By promoting soil microbial diversity and protecting soil ecosystems from human-induced threats, we can help to ensure a more resilient, sustainable, and healthy planet for generations to come.

Microbes play a fundamental role in soil nutrient cycling, which is the process by which nutrients such as nitrogen, phosphorus, and carbon are cycled through the soil ecosystem. This process is essential for the growth and survival of plants and other organisms, as it ensures that essential nutrients are available for uptake. In soil nutrient cycling, microbes act as decomposers, breaking down organic matter such as dead plant material and animal waste into simpler forms that can be taken up by plants. Microbes also play a critical role in transforming and recycling nutrients, converting them from one form to another as they move through the soil ecosystem (Prasad et al., 2021).

For example, nitrogen-fixing bacteria convert atmospheric nitrogen into a form that plants can use, while other bacteria and fungi break down organic matter and release nutrients such as phosphorus and potassium into the soil (Van Der Heijden et al., 2008; Rashid et al., 2016). In addition, mycorrhizal fungi form symbiotic relationships with plant roots, helping to enhance nutrient uptake and improve plant growth. Moreover, soil microbial diversity is crucial for maintaining nutrient cycling processes and ensuring the availability of essential nutrients for plant growth. When microbial diversity is reduced, soil nutrient cycling can be disrupted, leading to reduced soil fertility and decreased crop yields. Microbes play a critical role in soil nutrient cycling, which is essential for maintaining healthy soil ecosystems, promoting plant growth, and ensuring sustainable agricultural production. By promoting soil microbial diversity and supporting nutrient cycling processes, we can help to ensure the long-term health and resilience of our soils and ecosystems.

Soil microbial diversity is affected by a range of biotic and abiotic factors, including soil type, climate, land use, and management practices (Barto et al., 2010). Understanding these

factors can help us to promote healthy soil ecosystems and support the important functions of soil microorganisms. Soil type is a key factor affecting soil microbial diversity, as different soils have different physical and chemical properties that can influence the types of microorganisms that thrive in them. For example, soils with high levels of organic matter are often more diverse, as they provide a rich source of nutrients for microorganisms.

Climate also plays a role in soil microbial diversity, as temperature and moisture can affect microbial growth and activity. In general, warmer and wetter climates tend to support more diverse microbial communities, while colder and drier climates are less conducive to microbial activity (Brockett et al., 2012). Land use and management practices can also have a significant impact on soil microbial diversity (Tardy et al., 2015). Intensive agriculture, for example, can lead to soil compaction, erosion, and nutrient depletion, all of which can reduce microbial diversity. Conversely, organic farming practices, such as cover cropping and crop rotation, can promote microbial diversity and support healthy soil ecosystems. Other factors that can affect soil microbial diversity include soil pH, nutrient availability, and the presence of pollutants or other stressors. By understanding the factors that influence soil microbial diversity, we can take steps to promote healthy soil ecosystems and support the essential functions of soil microorganisms, such as nutrient cycling, disease suppression, and carbon sequestration.

4. Linking Soil Microbial Diversity and Hidden Hunger

There is growing evidence of a strong correlation between soil microbial diversity and nutrient availability. Studies have shown that soils with high microbial diversity tend to have higher levels of available nutrients, such as nitrogen, phosphorus, and potassium, compared to soils with low microbial diversity. One reason for this correlation is that soil microorganisms play a crucial role in nutrient cycling, breaking down organic matter and releasing essential nutrients into plant-available forms. Soils with a greater diversity of microorganisms are likely to have a greater capacity for nutrient cycling and a more efficient use of available nutrients (Hooper & Vitousek, 1998; Bhowmik et al., 2017).

In addition, soil microorganisms can also help to create nutrient-rich soil environments through their interactions with plant roots. Certain types of bacteria and fungi form symbiotic relationships with plant roots, known as mycorrhizal associations, which can help to increase nutrient uptake by the plant. Several studies have also shown that soil microbial diversity can be positively correlated with crop productivity (Tautges et al., 2016). For example, soils with high microbial diversity had significantly higher crop yields than soils with low microbial diversity. The evidence suggests that promoting soil microbial diversity can have significant benefits for nutrient availability, crop productivity, and ecosystem health (Chaparro et al., 2012). By supporting healthy soil ecosystems and promoting sustainable management practices, we can help to ensure that soils remain productive and resilient in the face of changing environmental conditions.

Recent research has also shown that soil microbial diversity can have a significant impact on plant health and disease resistance. Certain soil microorganisms, such as rhizobacteria and mycorrhizal fungi, can form beneficial relationships with plants, promoting their growth and helping to protect them against pathogens. Studies have found that soils with higher microbial diversity tend to have a greater abundance and diversity of beneficial microorganisms, leading to healthier and more resilient plant communities (Ambrosini et al., 2016). In addition, soil microbial diversity may be particularly important in mitigating the impacts of climate change, as healthy soil ecosystems can help to store carbon, improve soil water-holding capacity, and reduce the risk of soil erosion.

However, despite the growing recognition of the importance of soil microbial diversity, many agricultural practices continue to rely on inputs such as synthetic fertilizers and pesticides, which can disrupt soil ecosystems and reduce microbial diversity. As a result, there is a growing movement towards more sustainable and regenerative farming practices that promote soil health and support healthy microbial communities. The evidence suggests that soil microbial diversity plays a critical role in nutrient cycling, plant health, and ecosystem functioning. By promoting healthy soil ecosystems and supporting sustainable management practices, we can help to ensure that soils remain productive and resilient in the face of environmental challenges (Lal et al., 2021).

Soil microorganisms contribute to nutrient availability through several mechanisms (Blagodatskaya & Kuzyakov, 2008; Kumar & Verma, 2019). One of the most important mechanisms is nutrient mineralization, in which microorganisms break down organic matter in the soil and release nutrients such as nitrogen, phosphorus, and sulfur into plant-available forms. Microorganisms can also play a role in nutrient immobilization, in which nutrients are taken up by microorganisms and temporarily stored in their biomass. This can help to regulate nutrient availability and prevent nutrient loss through leaching or runoff.

In addition, some microorganisms are capable of fixing atmospheric nitrogen, converting it into a plant-available form that can be used by crops. This process is particularly important in systems with low soil nitrogen availability, such as those found in many tropical soils. Microorganisms can also help to improve soil structure and water-holding capacity, which can further enhance nutrient availability by promoting root growth and nutrient uptake. The mechanisms by which soil microorganisms contribute to nutrient availability are complex

and multifaceted and are influenced by a wide range of biotic and abiotic factors (Jayaraman et al., 2021). Understanding these mechanisms and their interactions is critical for developing sustainable soil management practices that promote healthy soil ecosystems and support optimal plant growth and productivity.

Another important mechanism by which soil microorganisms contribute to nutrient availability is through symbiotic relationships with plants. Certain microorganisms, such as mycorrhizal fungi, form symbiotic associations with plant roots, providing them with nutrients in exchange for carbohydrates produced by the plant (Johnson & Gehring, 2007; Nanjundappa et al., 2019). Mycorrhizal fungi, for example, can increase plant access to soil phosphorus, which is often limiting in many agricultural systems. By extending the reach of plant roots, mycorrhizal fungi can access soil nutrients that are otherwise unavailable to plants and can also help to protect plants against environmental stressors such as drought and disease.

In addition to mycorrhizal fungi, other soil microorganisms such as nitrogen-fixing bacteria and rhizobacteria can also form beneficial relationships with plants, contributing to nutrient availability and promoting plant growth. The contributions of soil microorganisms to nutrient availability are diverse and complex and are influenced by a wide range of factors including soil type, climate, and management practices (Van Der Heijden et al., 2008; Fierer, 2017). As such, developing effective strategies for promoting soil microbial diversity and enhancing nutrient availability requires a holistic and context-specific approach that takes into account the unique characteristics of each soil system.

Soil degradation can have significant impacts on soil microbial diversity and nutrient availability, with potentially far-reaching consequences for agricultural productivity and ecosystem functioning. One of the primary impacts of soil degradation is the loss of soil organic matter, which is a key source of nutrients for soil microorganisms. As soil organic matter declines, microbial biomass and activity can also decrease, leading to reduced nutrient cycling and decreased plant productivity. Soil degradation can also result in changes in soil structure and compaction, which can reduce water infiltration and air exchange in the soil, leading to reduced microbial activity and nutrient availability. Similarly, soil erosion can lead to the loss of topsoil and nutrient-rich organic matter, further reducing soil fertility and microbial diversity (Xiao et al., 2017).

In addition to physical changes in the soil, soil degradation can also lead to chemical changes that can impact soil microbial communities. For example, the use of synthetic fertilizers and pesticides can disrupt soil ecosystems and reduce microbial diversity, while high levels of soil acidity or salinity can also inhibit microbial activity and nutrient availability. The impacts of soil degradation on microbial diversity and nutrient availability are complex and multifaceted, and are influenced by a wide range of biotic and abiotic factors (Prashar et al., 2014). As such, developing effective strategies for mitigating the impacts of soil degradation and promoting soil health requires a holistic and context-specific approach that takes into account the unique characteristics of each soil system.

In addition to the direct impacts on soil microbial communities and nutrient availability, soil degradation can also have indirect impacts on ecosystem services such as carbon sequestration and water filtration (Faucon et al., 2017). For example, declines in soil organic matter and microbial activity can reduce the ability of soils to sequester carbon, potentially exacerbating climate change. Similarly, soil degradation can reduce water infiltration and increase the risk of soil erosion, leading to decreased water quality and increased flood risk. Given the interconnected nature of soil health, it is essential to take a holistic approach to addressing soil degradation and promoting soil health (Louwagie et al., 2011; Keesstra et al., 2018). This may involve a combination of strategies, such as reducing tillage and increasing the use of cover crops to promote soil organic matter and microbial diversity, as well as reducing the use of synthetic fertilizers and pesticides to minimize chemical impacts on soil ecosystems. In addition to these on-farm strategies, it is also important to consider broader policy and institutional changes that can support soil health and promote sustainable land use practices. For example, policies that support conservation agriculture and promote the use of agroforestry and other land-use systems that promote soil health and biodiversity can play a key role in mitigating the impacts of soil degradation and promoting sustainable land use practices.

5. Solutions for Addressing the Hidden Hunger Crisis

Agricultural practices play a crucial role in enhancing soil health and promoting microbial diversity. One of the most effective strategies for promoting soil health is reducing tillage. Reduced tillage practices can help to maintain soil structure and promote soil organic matter accumulation, which in turn can support microbial diversity and nutrient cycling. In addition, reduced tillage practices can help to reduce soil erosion and conserve soil moisture, leading to improved water infiltration and plant growth. Another key strategy for promoting soil health and microbial diversity is the use of cover crops (Vukicevich et al., 2016). Cover crops can help to reduce soil erosion, improve soil structure, and promote nutrient cycling. They can also provide a habitat and food source for soil microorganisms, which can help to maintain microbial diversity and activity. In addition, cover crops can help to suppress weeds and reduce the need for synthetic herbicides.

In addition to reducing tillage and using cover crops, there are a variety of other practices that can promote soil health and microbial diversity. These may include crop rotation, intercropping, agroforestry, and the use of compost and other organic amendments. Each of these practices can help to maintain soil organic matter and promote nutrient cycling, while also providing additional benefits such as improved pest and disease management and increased biodiversity. Promoting soil health and microbial diversity requires a multifaceted approach that considers the unique characteristics of each soil system (Nannipieri et al., 2003; Hartmann et al., 2015). By reducing tillage, using cover crops, and adopting a range of other practices that support soil health, farmers can help to maintain soil fertility and productivity, while also promoting the long-term sustainability of their agricultural systems.

In addition to reducing tillage and using cover crops, there are a variety of other practices that can promote soil health and microbial diversity. One such practice is the use of organic amendments such as compost, manure, and other organic materials (Hue & Silva, 2000). These amendments can help to increase soil organic matter, which in turn can promote microbial diversity and nutrient cycling. They can also improve soil structure and water holding capacity, leading to improved plant growth and productivity. Another strategy for promoting soil health and microbial diversity is crop rotation. By rotating crops, farmers can help to break pest and disease cycles, promote nutrient cycling, and maintain soil organic matter. This can lead to improved soil health and increased crop productivity over time.

Intercropping and agroforestry are other strategies that can promote soil health and microbial diversity. Intercropping involves planting different crops together in the same field, which can help to promote biodiversity and reduce pest and disease pressure. Agroforestry involves planting trees or shrubs in agricultural fields, which can provide additional habitat for soil microorganisms and improve soil structure and nutrient cycling (Smith et al., 2013). Reducing the use of synthetic fertilizers and pesticides can help to promote soil health and microbial diversity. These chemicals can have negative impacts on soil microorganisms, which can lead to decreased nutrient cycling and reduced soil fertility over time. By reducing or eliminating the use of these chemicals, farmers can promote the long-term sustainability of their agricultural systems and maintain soil health and productivity. Promoting soil health and microbial diversity requires a holistic approach that considers the unique characteristics of each soil system (Lehmann et al., 2020). By adopting a range of practices that support soil health and microbial diversity, farmers can help to maintain soil fertility and productivity, while also promoting the long-term sustainability of their agricultural systems.

Dietary interventions and food fortification are important strategies to address hidden hunger, particularly in populations that are at risk for nutrient deficiencies. Dietary interventions involve promoting the consumption of nutrient-dense foods, such as fruits, vegetables, whole grains, and lean protein sources (Flock & Kris-Etherton, 2011; Smethers & Rolls, 2018). These foods are rich in a variety of essential vitamins and minerals and can help to ensure that individuals are meeting their daily nutrient requirements. Food fortification is another important strategy for addressing hidden hunger. Fortification involves adding essential vitamins and minerals to commonly consumed foods, such as wheat flour, rice, and salt. This can be a cost-effective way to ensure that individuals are receiving adequate amounts of essential nutrients in their diets, particularly in populations where access to a variety of nutrient-dense foods is limited.

In addition to these strategies, it is important to ensure that individuals have access to clean water and sanitation facilities. Poor water quality and sanitation can increase the risk of nutrient deficiencies, as well as other health problems such as diarrhea and other infectious diseases. A combination of dietary interventions, food fortification, and improvements in water and sanitation can help to address hidden hunger and improve overall nutrition and health outcomes (Burchi et al., 2011; Gödecke et al., 2018).

Policy and institutional interventions are crucial to addressing hidden hunger, particularly at the national and global levels. One important policy intervention is the development and implementation of nutrition-specific policies and programs, such as nutrition education and counseling, micronutrient supplementation, and food fortification programs (Mason et al., 2014). These interventions can help to ensure that individuals are receiving the necessary nutrients for good health and wellbeing, particularly in vulnerable populations such as pregnant women, children, and the elderly. Institutional interventions are also important to address hidden hunger. These interventions can include strengthening health systems to ensure that individuals have access to appropriate health services, including diagnosis and treatment of nutrient deficiencies. They can also involve improving food systems to ensure that nutrient-rich foods are available and accessible to all individuals.

At the global level, international organizations such as the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) play a critical role in addressing hidden hunger (Dalmiya & Schultink, 2003; Amoroso, 2016). These organizations work to raise awareness of the issue, provide technical assistance to countries, and develop global guidelines and standards for addressing nutrient deficiencies. In addition to these interventions, it is important to address the underlying social, economic, and environmental determinants of hidden hunger. This can involve addressing poverty, improving access to education and employment opportunities, and promoting sustainable agriculture and food

systems. By addressing these underlying determinants, it may be possible to prevent nutrient deficiencies from occurring in the first place, and to promote overall health and wellbeing for all individuals.

6. Conclusion

The link between soil microbial diversity and nutritional deficiencies is an important area of research that requires further exploration. The depletion of soil microbial diversity through practices such as intensive farming and the use of synthetic fertilizers has been shown to have negative impacts on the nutritional quality of crops and the health of the surrounding ecosystem. By better understanding the relationship between soil microbial diversity and the nutritional content of food, we can develop more sustainable and effective strategies for addressing hidden hunger and promoting public health. This research highlights the importance of promoting soil health and biodiversity in our agricultural systems as a means of promoting food security and addressing global nutrition challenges.

Conflict of Interest

The author declared no conflict of interest.

References

Adugna, G. (2016). A review on impact of compost on soil properties, water use and crop productivity. *Academic Research Journal of Agricultural Science and Research*, *4*(3), 93-104. https://doi.org/10.14662/ARJASR2016.010

Agriculture and Food Systems for Nutrition. (2016). *The cost of malnutrition: Why policy action is urgent*. Global Panel. https://glopan.org/sites/default/files/pictures/CostOfMalnutrition.pdf

Ambrosini, A., de Souza, R., & Passaglia, L. M. (2016). Ecological role of bacterial inoculants and their potential impact on soil microbial diversity. *Plant and Soil*, *400*, 193-207. https://doi.org/10.1007/s11104-015-2727-7

Amoroso, L. (2016). The second international conference on nutrition: Implications for hidden hunger. *Hidden Hunger*, *115*, 142-152. https://doi.org/10.1159/000442100

Bailey, R. L., West Jr, K. P., & Black, R. E. (2015). The epidemiology of global micronutrient deficiencies. *Annals of Nutrition and Metabolism*, *66*(Suppl. 2), 22-33. https://doi.org/10.1159/000371618

Barto, E. K., Alt, F., Oelmann, Y., Wilcke, W., & Rillig, M. C. (2010). Contributions of biotic and abiotic factors to soil aggregation across a land use gradient. *Soil Biology and Biochemistry*, *42*(12), 2316-2324. https://doi.org/10.1016/j.soilbio.2010.09.008

Bhowmik, A., Cloutier, M., Ball, E., & Bruns, M. A. (2017). Underexplored microbial metabolisms for enhanced nutrient recycling in agricultural soils. *AIMS Microbiology*, *3*(4), 826-845. https://doi.org/10.3934/microbiol.2017.4.826

Biesalski, H. K., & Biesalski, H. K. (2013). *Hidden hunger*. Springer Berlin Heidelberg.

Blagodatskaya, E., & Kuzyakov, Y. (2008). Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: Critical review. *Biology and Fertility of Soils*, *45*, 115-131. https://doi.org/10.1007/s00374-008-0334-y

Blum, W. E. H., Zechmeister-Boltenstern, S., & Keiblinger, K. M. (2019). Does soil contribute to the human gut microbiome? *Microorganisms*, *7*(9), 287. https://doi.org/10.3390/microorganisms7090287

Brockett, B. F. T., Prescott, C. E., & Grayston, S. J. (2012). Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biology and Biochemistry*, *44*(1), 9-20. https://doi.org/10.1016/j.soilbio.2011.09.003

Brownie, S. (2006). Why are elderly individuals at risk of nutritional deficiency? *International Journal of Nursing Practice*, *12*(2), 110-118. https://doi.org/10.1111/j.1440-172X.2006.00557.x

Burchi, F., Fanzo, J., & Frison, E. (2011). The role of food and nutrition system approaches in tackling hidden hunger. *International Journal of Environmental Research and Public Health*, *8*(2), 358-373. https://doi.org/10.3390/ijerph8020358

Calder, P. C., Berger, M. M., Gombart, A. F., McComsey, G. A., Martineau, A. R., & Eggersdorfer, M. (2022). Micronutrients to support vaccine immunogenicity and efficacy. *Vaccines*, *10*(4), 568. https://doi.org/10.3390/vaccines10040568

Carducci, B., Oh, C., & Bhutta, Z. A. (2020). Addressing hidden hunger in school-aged children and adolescents within the context of the food system. *Hidden Hunger and the Transformation of Food Systems*, *121*, 21-30. https://doi.org/10.1159/000507499

Challinor, V. L., & Bode, H. B. (2015). Bioactive natural products from novel microbial sources. *Annals of the New York Academy of Sciences*, *1354*(1), 82-97. https://doi.org/10.1111/nyas.12954

Chaparro, J. M., Sheflin, A. M., Manter, D. K., & Vivanco, J. M. (2012). Manipulating the soil microbiome to increase soil health and plant fertility. *Biology and Fertility of Soils*, *48*, 489-499. https://doi.org/10.1007/s00374-012-0691-4

Chourasiya, D., Sharma, M. P., Maheshwari, H. S., Ramesh, A., Sharma, S. K., & Adhya, T. K. (2017). Microbial diversity and soil health in tropical agroecosystems. In T. K. Adhya, B. B. Mishra, K. Annapurna, D. K. Verma & U. Kumar (Eds.), *Advances in soil microbiology: Recent trends and future prospects: Volume 2: Soil-*

microbe-plant interaction (pp. 19-35). Springer. https://doi.org/10.1007/978-981-10-7380-9_2

Dalmiya, N., & Schultink, W. (2003). Combating hidden hunger: The role of international agencies. *Food and Nutrition Bulletin*, *24*(4), 69-77. https://doi.org/10.1177/15648265030244s203

Dias, T., Dukes, A., & Antunes, P. M. (2015). Accounting for soil biotic effects on soil health and crop productivity in the design of crop rotations. *Journal of the Science of Food and Agriculture*, *95*(3), 447-454. https://doi.org/10.1002/jsfa.6565

Durkin, M. (2002). The epidemiology of developmental disabilities in low-income countries. *Mental Retardation and Developmental Disabilities Research Reviews*, *8*(3), 206-211. https://doi.org/10.1002/mrdd.10039

Faucon, M. P., Houben, D., & Lambers, H. (2017). Plant functional traits: Soil and ecosystem services. *Trends in Plant Science*, *22*(5), 385-394. https://doi.org/10.1016/j.tplants.2017.01.005

Fierer, N. (2017). Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*, *15*(10), 579-590. https://doi.org/10.1038/nrmicro.2017.87

Flock, M. R., & Kris-Etherton, P. M. (2011). Dietary guidelines for Americans 2010: Implications for cardiovascular disease. *Current Atherosclerosis Reports*, *13*(6), 499-507. https://doi.org/10.1007/s11883-011-0205-0

Fróna, D., Szenderák, J., & Harangi-Rákos, M. (2019). The challenge of feeding the world. *Sustainability*, *11*(20), 5816. https://doi.org/10.3390/su11205816

Gernand, A. D., Schulze, K. J., Stewart, C. P., West Jr, K. P., & Christian, P. (2016). Micronutrient deficiencies in pregnancy worldwide: Health effects and prevention. *Nature Reviews Endocrinology*, *12*(5), 274-289. https://doi.org/10.1038/nrendo.2016.37

Gödecke, T., Stein, A. J., & Qaim, M. (2018). The global burden of chronic and hidden hunger: Trends and determinants. *Global Food Security*, *17*, 21-29. https://doi.org/10.1016/j.gfs.2018.03.004

Hartmann, M., Frey, B., Mayer, J., Mäder, P., & Widmer, F. (2015). Distinct soil microbial diversity under long-term organic and conventional farming. *The ISME Journal*, *9*(5), 1177-1194. https://doi.org/10.1038/ismej.2014.210

Hooper, D. U., & Vitousek, P. M. (1998). Effects of plant composition and diversity on nutrient cycling. *Ecological Monographs*, *68*(1), 121-149. https://doi.org/10.2307/2657146

Hovdenak, N., & Haram, K. (2012). Influence of mineral and vitamin supplements on pregnancy outcome. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *164*(2), 127-132. https://doi.org/10.1016/j.ejogrb.2012.06.020

Hue, N. V., & Silva, J. A. (2000). Organic soil amendments for sustainable agriculture: Organic sources of nitrogen, phosphorus, and potassium. In J. A. Silva & R. S. Uchida (Eds.), *Plant nutrient management in Hawaii's soils, approaches for tropical and subtropical agriculture* (pp. 133-144*)*. College of Tropical Agriculture and Human Resources.

Jayaraman, S., Naorem, A. K., Lal, R., Dalal, R. C., Sinha, N. K., Patra, A. K., & Chaudhari, S. K. (2021). Disease-suppressive soils-beyond food production: A critical review. *Journal of Soil Science and Plant Nutrition*, *21*, 1437-1465. https://doi.org/10.1007/s42729-021-00451-x

Jetten, J., Haslam, C., Haslam, S. A., Dingle, G., & Jones, J. M. (2014). How groups affect our health and well-being: The path from theory to policy. *Social Issues and Policy Review*, *8*(1), 103-130. https://doi.org/10.1111/sipr.12003

Johnson, N. C., & Gehring, C. A. (2007). Mycorrhizas: Symbiotic mediators of rhizosphere and ecosystem processes. In Z. G. Cardon & J. L. Whitbeck (Eds.), *The Rhizosphere* (pp. 73-100). Academic Press. https://doi.org/10.1016/B978-012088775-0/50006-9

Kansky, J. (2017). Benefits of well-being: Health, social relationships, work, and resilience. *Journal of Positive Psychology and Wellbeing*, *1*(2), 129-169.

Keesstra, S., Mol, G., De Leeuw, J., Okx, J., Molenaar, C., De Cleen, M., & Visser, S. (2018). Soil-related sustainable development goals: Four concepts to make land degradation neutrality and restoration work. *Land*, *7*(4), 133. https://doi.org/10.3390/land7040133

Koshila Ravi, R., Anusuya, S., Balachandar, M., & Muthukumar, T. (2019). Microbial interactions in soil formation and nutrient cycling. In A. Varma & D. K. Choudhary (Eds.), *Mycorrhizosphere and pedogenesis* (pp. 363-382). Springer. https://doi.org/10.1007/978-981-13-6480-8_21

Kumar, A., & Verma, J. P. (2019). The role of microbes to improve crop productivity and soil health. In V. Achal & A. Mukherjee (Eds.), *Ecological wisdom inspired restoration engineering* (pp. 249-265). Springer. https://doi.org/10.1007/978-981-13-0149-0_14

Lal, R., Bouma, J., Brevik, E., Dawson, L., Field, D. J., Glaser, B., Hatano, R., Hartemink, A. E., Kosaki, T., Lascelles, B., Monger, C., Muggler, C., Ndzana, G. M., Norra, S., Pan, X., Paradelo, R., Reyes-Sánchez, L. B., Sandén, T., Singh, B. R., Spiegel, H., Yanai, J., & Zhang, J. (2021). Soils and sustainable development goals of the United Nations: An International Union of Soil Sciences perspective. *Geoderma Regional*, *25*, e00398. https://doi.org/10.1016/j.geodrs.2021.e00398

Lehmann, J., Bossio, D. A., Kögel-Knabner, I., & Rillig, M. C. (2020). The concept and future prospects of soil health. *Nature Reviews Earth & Environment*, *1*(10), 544-553. https://doi.org/10.1038/s43017-020-0080-8

Louwagie, G., Gay, S. H., Sammeth, F., & Ratinger, T. (2011). The potential of European Union policies to address soil degradation in agriculture. *Land Degradation & Development*, *22*(1), 5-17. https://doi.org/10.1002/ldr.1028

Mason, J. B., Shrimpton, R., Saldanha, L. S., Ramakrishnan, U., Victora, C. G., Girard, A. W., McFarland, D. A., & Martorell, R. (2014). The first 500 days of life: Policies to support maternal nutrition. *Global Health Action*, *7*(1), 23623. https://doi.org/10.3402/gha.v7.23623

Miller, D. D., & Welch, R. M. (2013). Food system strategies for preventing micronutrient malnutrition. *Food Policy*, *42*, 115-128. https://doi.org/10.1016/j.foodpol.2013.06.008

Nanjundappa, A., Bagyaraj, D. J., Saxena, A. K., Kumar, M., & Chakdar, H. (2019). Interaction between arbuscular mycorrhizal fungi and *Bacillus* spp. in soil enhancing growth of crop plants. *Fungal Biology and Biotechnology*, *6*, 1-10. https://doi.org/10.1186/s40694-019-0086-5

Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G., & Renella, G. (2003). Microbial diversity and soil functions. *European Journal of Soil Science*, *54*(4), 655-670. https://doi.org/10.1046/j.1351-0754.2003.0556.x

Prasad, S., Malav, L. C., Choudhary, J., Kannojiya, S., Kundu, M., Kumar, S., & Yadav, A. N. (2021). Soil microbiomes for healthy nutrient recycling. In A. N. Yadav, J. Singh, C. Singh & N. Yadav (Eds.), *Current trends in microbial biotechnology for sustainable agriculture* (pp. 1-21). Springer. https://doi.org/10.1007/978-981-15-6949-4_1

Prashar, P., Kapoor, N., & Sachdeva, S. (2014). Rhizosphere: Its structure, bacterial diversity and significance. *Reviews in Environmental Science and Bio/Technology*, *13*, 63-77. https://doi.org/10.1007/s11157-013-9317-z

Qiu, L., Zhang, Q., Zhu, H., Reich, P. B., Banerjee, S., van der Heijden, M. G., Sadowsky, M. J., Ishii, S., Jia, X., Shao, M., Liu, B., Jiao, H., Li, H., & Wei, X. (2021). Erosion reduces soil microbial diversity, network complexity and multifunctionality. *The ISME Journal*, *15*(8), 2474-2489. https://doi.org/10.1038/s41396-021-00913-1

Rashid, M. I., Mujawar, L. H., Shahzad, T., Almeelbi, T., Ismail, I. M., & Oves, M. (2016). Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. *Microbiological Research*, *183*, 26-41. https://doi.org/10.1016/j.micres.2015.11.007

Redón Lago, A. (2021). *Hidden hunger in the current world: Causes, consequences and solutions to a global public health challenge and a particular look at Spain* (Bachelor's thesis, Barcelona University).

Saltzman, A., Birol, E., Wiesman, D., Prasai, N., Yohannes, Y., Menon, P., & Thompson, J. (2014). *2014 global hunger index: The challenge of hidden hunger*. The International Food Policy Research Institute.

Sharma, S. K., Ramesh, A., Sharma, M. P., Joshi, O. P., Govaerts, B., Steenwerth, K. L., & Karlen, D. L. (2011). Microbial community structure and diversity as indicators for evaluating soil quality. In E. Lichtfouse (Ed.), *Biodiversity, biofuels, agroforestry and conservation agriculture* (pp. 317-358). Springer. https://doi.org/10.1007/978-90-481-9513-8_11

Smethers, A. D., & Rolls, B. J. (2018). Dietary management of obesity: Cornerstones of healthy eating patterns. *Medical Clinics of North America*, *102*(1), 107-124. https://doi.org/10.1016/j.mcna.2017.08.009

Smith, J., Pearce, B. D., & Wolfe, M. S. (2013). Reconciling productivity with protection of the environment: Is temperate agroforestry the answer? *Renewable Agriculture and Food Systems*, *28*(1), 80-92. https://doi.org/10.1017/S1742170511000585

Tardy, V., Spor, A., Mathieu, O., Lévèque, J., Terrat, S., Plassart, P., Regnier, T., Bardgett, R. D., van der Putten, W. H., Roggero, P. P., Seddaiu, G., Bagella, S., Lemanceau, P., Ranjard, L., & Maron, P. A. (2015). Shifts in microbial diversity through land use intensity as drivers of carbon mineralization in soil. *Soil Biology and Biochemistry*, *90*, 204-213. https://doi.org/10.1016/j.soilbio.2015.08.010

Tautges, N. E., Sullivan, T. S., Reardon, C. L., & Burke, I. C. (2016). Soil microbial diversity and activity linked to crop yield and quality in a dryland organic wheat production system. *Applied Soil Ecology*, *108*, 258-268. https://doi.org/10.1016/j.apsoil.2016.09.003

Thrupp, L. A. (2000). Linking agricultural biodiversity and food security: The valuable role of agrobiodiversity for sustainable agriculture. *International Affairs*, *76*(2), 265-281. https://doi.org/10.1111/1468-2346.00133

Van Der Heijden, M. G., Bardgett, R. D., & Van Straalen, N. M. (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, *11*(3), 296-310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

Vukicevich, E., Lowery, T., Bowen, P., Úrbez-Torres, J. R., & Hart, M. (2016). Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. *Agronomy for Sustainable Development*, *36*, 1-14. https://doi.org/10.1007/s13593-016-0385-7

WHO. (2006). *Guidelines on food fortification with micronutrients*. World Health Organization. https://www.who.int/publications/i/item/9241594012

Xiao, H., Li, Z., Chang, X., Huang, J., Nie, X., Liu, C., Liu, L., Wang, D., Dong, Y., & Jiang, J. (2017). Soil erosion-related dynamics of soil bacterial communities and microbial respiration. *Applied Soil Ecology*, *119*, 205-213. https://doi.org/10.1016/j.apsoil.2017.06.018

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

https://prensip.gen.tr

RESEARCH ARTICLE

Analysis of the Factors Affecting the Tendency of Fishery Consumption: Case of Iğdır Province

Emine Aşkan✉

Iğdır University, Faculty of Agriculture, Department of Agricultural Economics, Iğdır/Türkiye

ARTICLE INFO

Article History
Received: 27.06.2023
Accepted: 22.08.2023
First Published: 24.10.2023

Keywords
Consumption
Fish
Ordered probit model
Socio-demographic characteristics

ABSTRACT

In the research, socio-economic and demographic factors affecting the fish consumption amount of consumers in Iğdır province were tried to be determined. The main material of the research is the survey data obtained from 325 consumers residing in the province of Iğdır and determined by using the commensurate sampling method. In the research, the factors affecting the fish consumption amount of the consumers were analyzed with the sequential probit model. In the sequential probit model created, the fish consumption levels of the consumers were used as the addict variable, and the inaddict variables were determined as gender, income level, frequency of fish consumption, type of fish consumed, annual red meat consumption and type of fish consumption. The model results; The variables of gender, income level, frequency of fish consumption, type of fish consumed, annual red meat consumption and fish consumption pattern were all found to be statistically significant. According to the ordinal probit model results, it was determined that the gender of the consumer decreased the fish consumption level. As expected in the research, it was defined that there was a negative relationship between income level and fish consumption level, and a positive relationship between fish consumption frequency and consumption amount. With respect to the model conculsions, it was defined that there is a positive relationship between the amount of red meat consumption of consumers and the amount of fish consumption.

Please cite this paper as follows:

Aşkan, E. (2023). Analysis of the factors affecting the tendency of fishery consumption: Case of Iğdır province. *Journal of Agricultural Production*, *4*(2), 91-99. https://doi.org/10.56430/japro.1319991

1. Introduction

Today, the rapidly increasing world population, global warming and pandemic processes have increased the tendency to healthy and balanced nutrition. Animal proteins play an active role in a healthy and balanced diet.

Today, it is stated that a healthy person should consume 1 g of protein per kilogram of body weight per day, of which 42% should be of animal origin (WHO, 2020). In developed countries, less developed while the amount of daily protein consumption per capita has doubled compared to developing countries or developing countries, the rate of protein intake from animal products in developing countries is around 20% (Béné et al., 2007; Belton et al., 2016). In developed countries, this rate is around 65% (Anonymous, 2013). Fish, which is among the animal proteins, meets the body's basic nutritional needs with its rich protein content and polyunsaturated fatty acids in its structure. In addition, fish play an active role on human physiology and metabolic functions (Yücel, 2001; Kaya et al., 2004; Roos et al., 2007; Marques et al., 2019).

Production of fishery products is not distributed homogeneously throughout the world. China is the leader in world aquaculture production, accounting for 35% of the total production (62.8 million tons), followed by India, Indonesia, Vietnam and Peru. In aquaculture, the total production of sea and inland water fishing has been at the level of 90 million tons in recent years. In the amount of hunting production, China has

✉Correspondence
E-mail address: 25emineaskan@gmail.com

the largest share (14.6%), followed by Indonesia, Peru, India, Russia, USA and Vietnam. While 51% of the aquaculture production is obtained through hunting, 52% of the fishing is carried out in the Asian continent (FAO, 2022). Of the world aquaculture production, which was 177.8 million tons in 2020, 87.5 million tons (49%) were obtained from aquaculture. 33 million tons of aquaculture production was obtained from the sea and 54 million tons from inland waters (FAO, 2023).

In aquaculture, China produced 49.62 million tons in 2020, 57% of the world's total production. Türkiye became the largest producer country in the European continent in 2021 with 799851 tons. In addition to being surrounded by the sea on three sides due to its location in Türkiye, it has an important aquaculture potential with its inland waters, lakes and dam lakes. Aquaculture constitutes 59% of Türkiye's aquaculture production. 71% of aquaculture production took place in seas and 29% in inland waters. 89% of aquaculture production in Türkiye consists of sea bass (33%), sea bream (28%) and trout (Turkish Salmon) (28%). Trout production constitutes 98.6% of the fish produced by aquaculture from inland waters. 36.4% of the production carried out by aquaculture in the seas was realized in Muğla, 16.6% of the production in inland waters was realized in Elazig. While almost all of the aquaculture production in the seas consists of sea bass and sea bream, only trout in inland waters. The most important share in fishing from the seas belongs to the Black Sea with 77.5%, the Aegean with 12.6%, the Mediterranean with 5.1% and the Marmara Region with 4.9%. The most important species caught from the seas in Türkiye is anchovy (TEPGE, 2021).

Seventy-two percent of the 157 million tons of aquatic products consumed in 2019 were consumed in the Asian Continent. China, Indonesia, India, USA and Japan come first in seafood consumption. Looking at the world in general, it has been designated that 17% of animal protein needs are met from fish in 2019 and this figure corresponds to 7% of all protein consumed (FAO, 2022). While the world fish consumption was 9 kg per capita in 1961, it reached 20.5 kg in 2019. In 2019, 75% of the per capita aquaculture consumption was met from fish. While the annual per capita consumption of fisheries products in Türkiye was 6.3 kg in 2019, it was determined as 6.5 kg in 2021 (TUIK, 2023). Per capita aquaculture consumption in Türkiye is lower than the world average.

In addition to being related to factors such as consumption amount, consumption habits, production amount and price of fishery products, consumer purchasing power, the way it is presented to the market and consumption time, the consumption of fishery products in Türkiye also varies by region. Although the average annual fish consumption per capita in Türkiye is low in Eastern Anatolia, Southeastern Anatolia and Central Anatolia regions, it is quite high in the Black Sea and other coastal regions (Aydın & Karadurmuş, 2013; Ercan & Şahin, 2016). Similarly, per capita consumption in regions near the sea, for example, is 28.08 kg per year in Giresun and Trabzon (Aydın & Karadurmuş, 2013), 25.8 kg per year in Mersin; 21.5 kg per year (Demirtaş et al., 2014; Şen & Şahin, 2017), 15 kg per year in İzmir, 13 kg per year in Tokat, 12.4 kg per year in Isparta, 6.5 kg per year in Erzurum, 4.13 kg per year in Kahramanmaraş, 3.8 kg per year in Niğde (Hatırlı et al., 2004; Erdal & Esengün, 2008; Çaylak, 2013; Ercan & Şahin, 2016; Bashimov, 2017; Uzundumlu, 2017), and 3.4 kg per year in Ankara (Gül Yavuz et al., 2015).

Iğdır is a province located in the Eastern Anatolia Region of Türkiye and in the easternmost part of Türkiye. Azerbaijan (Nakhchivan), Iran and Armenia are border neighbors. The province of Iğdır is completely within the basin of the Aras River. The important streams that join the Aras River within the provincial borders are the Gaziler Stream, Buruksu Stream in the west, and the Aşağı and Orta Karasu Streams in the east. There is a trout facility in Tuzluca district of Iğdır province and its production capacity is around 25 tons in year. The aim of our research is to determine the amount of fish consumption and the socio-demographic and behavioral characteristics of the factors affecting the consumption level of the consumers living in the city center of Iğdır, where it is far from the sea and there is very little aquaculture.

2. Materials and Methods

The basic material of the survey is the original data picked up through questionnaires from 325 consumer living in the urban area of Iğdır. The survey took place between the autumn and winter of 2022. The sample size was decided by the simple random sample method (Topçu & Dağdemir, 2017). In accordance with Article 10/1 of the Iğdır University Scientific Research and Publication Ethics Directive, the survey complies with scientific research and publication ethics.

2.1. Econometric Analysis

In the study, the factors impressive the fish consumption level were analyzed with the help of Ordered Probit Model estimation. In cases where the addict variable is categorical or ordinal, ordered logit or probit probability estimators can be used. Maximum Similarity functions are used in both methods. Although the ordinal probit model is rested on the normal probability dispensation, the ordinal logit model is derived from the standardized logistic probability dispensation. The feature that distinguishes the ordinal probit model from the ordinal logit model is the normal dispensation of errors. In the ordered probit model, it is assumed that there is a continuous but unobservable latent addict variable behind the observable, interval and ordered categories (y). The unobservable, latent addicted variable (y*) is explained by the vector of illustrative variables and the error term. It is assumed that the error term has a normal distribution (Greene, 2008).

$$n = t[1 + (0{,}02)(b - 1)] * pq/E\text{^}2 \quad (1)$$

In the ordered probit model, it is assumed that there is a continuous but unobservable hidden addict variable behind the observable, interval and ordered categories (y). The unobservable, hidden addicted variable (y*) is explained by the vector of explanatory variables and the error term. It is assumed that the error term has a normal distribution (Greene, 2012).

$$Y^* = X'\beta + \varepsilon\varepsilon \sim N[0,1] \quad (2)$$

Here, y*; unobservable addicted variable, x; vector of explanatory variables, β; parameter vector to be estimated and ε; indicates the error term. The relationship between the addict variable (y) and the unobserved addict variable (y*) is considered as a function of the threshold values (μj) that take separate values according to the consumers and are estimated using the regression coefficients (β). In the research, the fish consumption level of the consumers was classified in four different sequential categories (Y= 0, 1, 2, 3). According to this; The relationship between the addict variable (y) of the model and the unobserved addict variable (y*) will be as follows (Chen et al., 2002; Greene, 2012).

$$if 0 < y^* \leq \mu, \qquad y = 0 \quad (3)$$

$$if \mu, < y^* \leq \mu, \qquad y = 1 \quad (4)$$

$$if \mu, < y^x \leq \mu, \qquad y = 2 \quad (5)$$

$$if \mu, \leq y^x \qquad y = 3 \quad (6)$$

The "μ" in the equaty are the threshold values that are estimated in the model and form the lower and upper limits of the values that y will take (Greene, 2008). The ordinal categories of the addict variable used in the model, "Y=0" variable 1-3 kg fish consumption level, "Y=1" variable 4-6 kg fish consumption level, "Y=2" variable 7-10 kg fish consumption level and "Y=3" variable represents the fish consumption level of more than 10 kg.

In the ordered probit model, the error term is assumed to be normally distributed. The probability that consumers will be in one of the 4 categories of fish consumption level is as follows: (Greene, 2012).

$$\text{Prob } (y = 0|x) = \Phi\ (-x\beta), \quad (7)$$

$$\text{Prob } (y = 1|x) = \Phi\ (\mu 1 - x\beta) - \Phi\ (-x\ \beta), \quad (8)$$

$$\text{Prob } (y = 2|x) = \Phi\ (\mu 2 - x'\ \beta) - \Phi\ (\mu 1 - x'\ \beta), \quad (9)$$

$$\text{Prob } (y = 3|x) = 1 - \Phi\ (\mu 3 - x\beta) \quad (10)$$

For these likelinesses to be positive; It should be $0 < \mu 1 < \mu 2$. Φ denotes the cumulative normal distribution function. It is inconvenient to directly interpret the coefficients of the variables of the ordered probit model estimated using the maximum likelihood method (Akbay et al., 2007). The effects of explanatory variables on probabilities are not the same as parameter (β) estimates because they depend on the values of explanatory variables. It is not clear how to interpret the coefficients without additional computation in the ordered probit model. This entailes estimating the marginal effects of interpretive variables so as to determine the effect on probabilities. The marginal effects of the variables are calculated for each likelihood as follows (Greene, 2012).

$$\partial prob(y = 0|x))/\ \partial x = \emptyset(x\text{^}'\beta)\beta, \quad (11)$$

$$\partial prob(y = 1|x))/\ \partial x = [\emptyset(x\text{^}'\beta)\beta, -\emptyset(\mu 1 - x\text{^}'\beta)\beta], \quad (12)$$

$$\partial prob(y = 2|x))/\ \partial x = [\emptyset(\mu 1 - x\text{^}'\beta)\beta, , -\emptyset(\mu 2 - x\text{^}'\beta)]\beta, \quad (13)$$

$$\partial prob(y = 3|x))/\ \partial x = [\emptyset(\mu 3 - x\text{^}'\beta)\beta, \quad (14)$$

The ordered probit model, which is one of the limited addict variable model types, was estimated using the Limdep Econometric Computer Program.

3. Results and Discussion

In the econometric analysis made for the fish consumption desire and tendency of the participants in Iğdır province, the annual fish consumption amount is the addict variable. The average age of the consumers participating in the survey is 30.25, the youngest consumer is 15, and the oldest is 75 years old.

Table 1. Variable definitions and instance statistics (Case=325, Missing=0).

Variable	**Explanation**
Gender (Q1) (Binary)	1: Female; 0: Male
Household (person) (Q3)	1: 2-5 2: 5-7 3: 7-10 4:10-15 5: 15
Education (year) (Q4)	1: Illiterate 2: Primary Education 3: Secondary Education 4: High School 5: University
Occupation (Q5)	1: Farmer 2: Self-Employed 3: Officer 4: Worker 5: Retired 6: Student 7: Housewife
Income (₺/month) (Q6) (Binary)	1: Fixed Income 0: Others
Income level (₺/month) (Q7)	1: 5000 ₺> 2: 5001₺-10000 ₺ 3: 10001₺-15000 ₺ 4-15000 ₺<

Table 1. (continued)

Variable	Explanation
Income status (₺/month) (Q8)	1: 15000 ₺<; 0: 15000 ₺>
The most readily available type of meat (Q9)	1: Cattle 2: Sheep 3: Goat 4: White Meat 5: Fish
Frequency of consuming fish (Q10)	3: Once A Week 2: Every 15 Days 1: Once A Month 0: Less
Are there any places where fish are sold regularly in the region? (Q11) (Binary)	1: Yes 0: No
Where do you buy the fish? (Q12)	1: Marketplace 2: Fish State 3: Fish Market 4: Peddler
Where do you buy the fish? (Q13)	1: Marketplace + Peddler 0: Others
The most consumed type of fish (Q14)	1: Trout 2: Bream 3: Perch 4: Horse Mackerel 5: Anchovy 6: Sardines 7: Acorn
The most consumed fish species) (Binary) (Q15)	1: Anchovy + Horse Mackerel 0: Others
Annual fish consumption amount (kg/year) (Q16)	0: 1-3 Kg 1: 4-6 Kg 2: 6-10 Kg 3: 10 Kg<
Annual red meat consumption amount (kg/year) (Q17)	1: 1-3 Kg 2: 4-6 Kg 3: 6-10 Kg 4: 10-15 Kg 5: 15-20 Kg 6: 20kg<
Annual white meat consumption amount (kg/year) (Q18)	1: 1-3 Kg 2: 2-6 Kg 3: 6-10 Kg 4: 10-15 Kg 5: 15-20 Kg 6: 20kg<
How to consume fish (Q19)	1: Fresh 2. Canned 3: Salty 4: Brine 5: Other
How to consume fish (Q20) (Binary)	1: Fresh 0: Others
What is your method of cooking fish? (Q21)	1: Frying 2: Grid 3: Steaming 4: Others
What is your method of cooking fish? (Q22)	1: Fry + Grill 0: Others
Are you fishing? (Q23)	1: Yes 0: No
Do you care about being balanced and healthy? (Q24)	1: Yes 0: No

While the number of household members is 48.31%, families with 2 to 5 individuals, 32.61% are families with 5-7 individuals, 11.69% have 7-10 individuals, and 6.77% have 10-15 individuals. families and 0.62% are families with 15 or more individuals.

When the education level is examined in the research, university with 31.38% and high school graduates with 31.38% are in the first place, primary education is in the second place with 16.32%, secondary education is in the third place with 16.00% and 4.92% is in the first place. and illiterate consumers take the fourth place.

When the occupational status is examined, self-employment with a rate of 22.46% and students with a rate of 22.46% are in the first place, while civil servants with a rate of 15.69% and a worker with a rate of 15.69% take the second place. In case of occupation, 13.85% of the participants are housewives, 7.39% are self-employed and 2.46% are retirees.

When the income status is examined, it is determined that 17.85% of the participants have an income level of less than 5000 ₺ and 17.85% of them have an income level between 5 001 and 10 000 ₺, while 61.84% of them have an income level between 10 001-15 000 ₺, It has been determined that 2.46% of

them have an income level of over 15 000 ₺. Within the scope of the research, the most easily obtained meat type in the region is white meat with a rate of 32.31%. In the preferences of consumers, beef is in the second place with 31.38%, sheep meat is in the third place with 27.38%, goat meat is in the fourth place with 7.69%, and fish is in the fifth place with 1.23%.

When the frequency of fish consumption was analyzeed, it was defined that 9.54% of the participants consumed fish less than once a week, 19.69% every 15 days, 36.62% once a month and 34.15% less than once a month. In Türkiye, 50% of the consumers in Erzincan (Karakaya et al., 2020) and in another study conducted in Mexico, 24.24% of the individuals reported that every fortnight (Pérez-Ramírez et al., 2015), South Korea In the study conducted in the province of Ardahan, Türkiye, 7.3% of the households were found to be more than once a week (Lee & Nam, 2019), and in another study conducted in Serbia, 52.24% of the participants (Djordjevic et al., 2015) 26.16% (Kılıç et al., 2019), in the study conducted in Mersin, 42.00% (Şen & Şahin, 2017), and in the study conducted in the USA, 24% of the individuals (Hicks et al., 2008) It was determined that they consumed fish once.

In the research region, it was defined that 31.69% of the participants bought fish from the markets where fish are sold, 27.08% from the market place, 25.23% from the fish market and 16.00% from the peddlers. In the study handled in Ankara, it was determined that consumers who prefer to consume fresh fish do not buy fish from fixed consumption places (Gül Yavuz et al., 2015).

When the most consumed fish species were examined, 28.31% of the participants were trout, 28.00% of sea bream, 16.92% of anchovy, 11.69% of sea bass, 8.31% of horse mackerel and 6% of participants. It was determined that 77 of them consumed bonito. In a study handled in Ankara, it was determined that 56.10% of consumers consume anchovy (Gül Yavuz et al., 2015). In the study handled in Mersin, it was determined that the consumers consumed the most (27%) sea bream and the second (21%) anchovy (Şen & Şahin, 2017).

It was determined that 34.15% of the participants consumed more than 6-10 kg, 28.31% of them 4-6 kg, 23.69% of them 1-3 kg and 13.85% of them consumed more than 10 kg of fish per year.

Considering the annual consumption of red meat, 24.62% of the participants are 10-15 kg, 19.38% are 6-10 kg, 18.15% are 4-6 kg, 17.85% are over 20 kg. and 9.23% consume 1-3 kg of red meat. In the study conducted in the province of Erzincan, it was determined that 54.40% of the consumers and in the study conducted in the province of Mersin, 46.00% of the consumers tended to consume red meat (Şen & Şahin, 2017; Karakaya et al., 2020).

Considering the annual consumption of white meat, 30.46% of the participants are over 20 kg, 24.92% are 10-15 kg, 22.15% are 2-4 kg, 11.38% are 6-10 and again, it was determined that 11.38% consumed white meat between 1-3 kg and 10.77% 15-20 kg.

When the fish consumption pattern was examined, it was determined that 61.54% of the participants preferred to consume fish as fresh, 33.54% preferred grilled, 12.92% steamed and 11.39% preferred fish in other ways. In the study handled in Erzincan, it was determined that 53% of the consumers consumed fish in the pan, 20% on the grill, 17% in the oven and 10% as steamed fish. Karakaya et al. (2020) in the province of Erzincan and Bayraktar (2015) in the provinces of Ankara and Çanakkale found that 62.00% of consumers prefer grilled and steamed and 27.00% fry. In a study conducted in Diyarbakır, it was reported that 44.30% of consumers prefer the frying method (Aydın & Odabaşı, 2017), while in Tunceli, 34.00% of individuals prefer to cook the fish in the oven (Balcı et al., 2016). In the study conducted by Kırıcı et al (2018) in Siirt province, pan-fried consumption as a form of fish consumption is in the 1st place with a rate of 31.70%, Olgunoğlu et al (2014) in Adıyaman and Terin et al. (2016) in Van. In their study, they found that the fish consumption type was frying at a rate of 41.00% and 40.20%, respectively.

When the fish cooking method was examined, it was determined that 42.15% of the participants cooked fish as fried, 33.54% grilled, 12.92% steamed and 11.39% others (sushi, soup, etc.). In the study conducted in Erzincan, 53.00% of the consumers were in the pan (Karakaya et al., 2020), in the study conducted in Mersin, 42.00% was grilled (Şen & Şahin, 2017), and in the study conducted in Diyarbakır 44.30%. fried (Aydın & Karadurmuş 2013), and in a study conducted in Tunceli, it was determined that 34.00% of consumers prefer to consume fish in the oven (Balcı et al., 2016).

The coefficient and unit (marginal) effect results of the sequential probit model created to analyze the factors affecting the fish consumption level of consumers living in the urban area of Iğdır Province are given in Table 2. In the sequential probit model created, the fish consumption levels of the consumers were used as the addict variable, and the inaddict variables were determined as gender, income level, frequency of fish consumption, type of fish consumed, annual red meat consumption and type of fish consumption.

The model estimated by the maximum likelihood method is statistically significant ($p<0.000$). According to Maddala (1983), the threshold values of the model should be positive and $Mu(1) < Mu(2)$. According to the model results, the threshold value parameters Mu(1) and Mu(2) of the model were positive and statistically significant at the 1% level. The fact that the threshold values are econometrically important indicates that the fish consumption level grouping is accurate.

Table 2: Ordered Probit model results.

Variables	Estimated Coefficients	Marginal Effects			
		Y=0 (1-3 kg)	**Y=1 (4-6 kg)**	**Y=2 (7-10 kg)**	**Y=3 (10< kg)**
Constant	0.21779 (0.2918)	--	--	--	--
Gender	-.25246** (0.0384)	0.07406** (0.0394)	0.02614** (0.0467)	-0.05104** (0.0385)	-0.04916** (0.0432)
Income	-.15235** (0.0310)	0.04462** (0.0323)	0.01602** (0.0419)	-0.03088** (0.0340)	-0.02975** (0.0346)
Frequency of consuming fish	.24565*** (0.0001)	-0.07194*** (0.0002)	-0.02584*** (0.0013)	0.04980*** (0.0002)	0.04798*** (0.0004)
The most consumed type of fish	-.27460* (0.0536)	0.08451* (0.0660)	0.02370** (0.0267)	-0.05879* (0.0663)	-0.04942** (0.0395)
The amount of red meat consumed annually	.20466*** (0.0001)	-0.05994*** (0.0001)	-0.02152*** (0.0001)	0.04149*** (0.0001)	0.03997*** (0.0001)
Fish cooking method	-.17544* (0.0916)	0.05138* (0.0932)	0.01845 (0.1036)	-0.03557* (0.0966)	-0.03426* (0.0942)
Mu(1)	0.85393*** (0.0000)				
Mu(2)	1.98151*** (0.0000)				
Log likelihood function	-406.72780				
Restricted log likelihood	-435.20795				
Chi squared [6 d.f.]	56.96031				
Significance level	0.00000				

Note: ***, **, * ==> Severity: 1%, 5%, 10%. Values in parentheses are p values.

According to the model results; The variables of gender, income level, frequency of fish consumption, type of fish consumed, annual red meat consumption and fish consumption pattern are all statistically significant.

Since the interpretation of the coefficients of the sequential probit model results may cause errors, the comments on the factors affecting the fish consumption level were made by evaluating the marginal effects. Among the marginal effects, except for the coefficient of the fish consumption pattern variable (Y=1), the marginal effects are statistically significant and the comments were made on these variables.

According to the ordinal probit model results, the fact that the gender of the consumer is female reduces the level of fish consumption. Since Iğdır is a male-dominated province and men are more likely to be fed outside the home than women, the fact that the consumer is male increases fish consumption. Being a female consumer increases the probability of consuming 1-3 kg (Y=0) fish by 7.41%, and the probability of consuming 4-6 kg (Y=1) by 2.61%, while the probability of consuming 7-10 kg (Y=2) fish and 10 kg (Y=3) reduces fish consumption by 5.10% and 4.92%, respectively.

There is a negative correlation between income level and fish consumption level. In previous studies, the opposite was found, and a positive relationship was determined between household income level and fish consumption (Akinbode & Dipeolu, 2012; Can et al., 2015; Dauda et al., 2016; Terin, 2019). Since the fish species consumed in Iğdır are cheaper than red meat and the fish caught from rivers and lakes in the region are sold at low prices, they are more preferred by those with low income. While income level above 3000 ₺ increases the probability of 1-3 kg (Y=0) fish consumption by 4.46% and 4-6 kg (Y=1) fish consumption probability by 1.60%, while 7-10 kg (Y=0) fish consumption probability at the same income level increases by 4.46%. Y=2) and over 10 kg (Y=3) reduce the probability of fish consumption by 3.01% and 2.98%, respectively.

As expected, there is a positive relationship between the frequency of fish consumption and the amount of consumption. As the frequency of fish consumption increases as a period, the probability of fish consumption level (Y=0) and (Y=1) decreases by 7.19% and 2.58%, while the probability of being (Y=2) and (Y=3) decreases by 4.98% and (Y=3). increasing by 4.80%

One of the issues examined in the model is the relationship between the type of fish consumed and the level of fish consumption. According to the model results, it is possible that those who consume anchovy and horse mackerel, which are the most common sea fish in Türkiye, have lower fish consumption levels. Those who prefer anchovy and horse mackerel in fish consumption increase the probability of being in the (Y=0) and

(Y=1) groups by 8.45% and 2.37%, respectively, while the probability of being in the (Y=2) and (Y=3) groups is 5%, 88 and 4.94% decrease.

According to the model results, there is a positive relationship between consumers' red meat consumption and fish consumption. Red meat is mainly consumed in the region and there is no comparison with fish meat. While fish meat is consumed more seasonally and according to its availability in the region, red meat is constantly reached and consumed. Therefore, red meat and fish meat are not considered as substitutes for each other. This reveals a positive relationship between them in terms of consumption. The increase in the amount of red meat consumption of consumers (Y=0) and (Y=1) decrease the probability of being in the fish consumption group by 6.00% and 2.15%, respectively, while the probability of being in the fish consumption group (Y=2) and (Y=3) decreases by 4.14%. and increases by 4.00%.

Fish cooking method has also been identified as one of the variables that determine the amount of fish consumption. It has been determined that those who prefer their fish consumption by frying and grilling are less likely to consume fish than those who consume it in other ways. That is, the probability of finding (Y=0) and (Y=1) fish frying and grilling was 5.14% and 1.85%, while the probability of finding (Y=2) and (Y=3) was 3.56% and (Y=1). It decreases by 3.43 percent.

4. Conclusion and Recommendations

In the research, the gender, income, frequency of fish consumption, the most consumed fish species, the amount of red meat consumed annually and the method of cooking fish determine the fish consumption tendency of the consumers. In the study, when the relationship between fish consumption and gender is examined, it has been determined that the level of fish consumption decreases if the consumers are women and the income level is high.

In order to increase the consumption of aquatic products, which are a healthy food source in terms of balanced and healthy nutrition and sustainability, the region should be enriched with facilities where aquaculture products can be produced regularly and fish can be kept. In this way, the consumer group that tends to red meat can be directed to seafood. It is possible to improve the fish consumption habits of the consumers by conducting training activities on healthy and balanced nutrition in the province. The public and private sectors and even professional organizations can play an effective role in changing the consumption preferences and habits of consumers. With all these activities, the aquaculture sector can be developed in all societies with the same ecological characteristics and consumer tendencies.

Conflict of Interest

The author declares no conflict of interest.

References

Anonymous. (2013). *Livestock industry report 2013*. http://www.tigem.com.tr

Akbay, C., Tiryaki, G. Y., & Gül, A. (2007). Consumer characteristics influencing fast food consumption in Turkey. *Food Control*, *18*(8), 904-913. https://doi.org/10.1016/j.foodcont.2006.05.007

Akinbode, S. O., & Dipeolu, A. O. (2012). Double-hurdle model of fresh fish consumption among urban households in South-West Nigeria. *Current Research Journal of Social Sciences*, *4*(6), 431-439.

Aydın, M., & Karadurmuş, U. (2013). Trabzon ve Giresun bölgelerindeki su ürünleri tüketim alışkanlıkları. *Karadeniz Fen Bilimleri Dergisi*, *3*(9), 57-71. (In Turkish)

Aydın, M., & Odabaşı, Y. (2017). Su Ürünleri Tüketim Alışkanlıkları Üzerine Bir Araştırma: Diyarbakır ili örneği. *Turkish Journal of Maritime and Marine Sciences*, *3*(2), 101-112. (In Turkish)

Balcı, M., Birici, N., Şeker, T., Akgün, H., Sesli, A., & Arısoy, G. (2016). Tunceli ili balık tüketim analizi. *Erzincan Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, *9*(Special Issue), 73-85. https://doi.org/10.18185/eufbed.61432 (In Turkish)

Bashimov, G. (2017). Niğde ilinde balık eti tüketim alışkanlığının belirlenmesi. *Türk Tarım ve Doğa Bilimleri Dergisi*, *4*(2), 196-204. (In Turkish)

Bayraktar, S. (2015). Ankara ve Çanakkale'de su ürünleri tüketim tercihleri ve alışkanlıklarının karşılaştırılması (Master's thesis, Çanakkale Onsekiz Mart University). (In Turkish)

Belton, B., Bush, S. R., & Little, D. C. (2016). Are farmed fish just for the wealthy? *Nature*, *538*(7624), 171. https://doi.org/10.1038/538171d

Béné, C., Macfadyen, G., & Allison, E. (2007). *Increasing the contribution of small-scale fisheries to poverty alleviation and food security*. Food and Agriculture Organization of the United Nations (FAO). https://www.fao.org/family-farming/detail/en/c/273564/

Can, M. F., Günlü, A., & Can, H. Y. (2015). Fish consumption preferences and factors influencing it. *Food Science and Technology*, *35*(2), 339-346. https://doi.org/10.1590/1678-457X.6624

Chen, K. Z., Ali, M., Veeman, M. M., Unterschultz, J., & Le, T. (2002). Relative importance rankings for pork attributes by Asian-origin consumers in California: Applying an ordered probit model to a choice-based sample. *Journal*

of Agricultural and Applied Economics, *34*(1), 67-79. https://doi.org/10.1017/S1074070800002157

Çaylak, B. (2013). İzmir ili su ürünleri tüketimi ve tüketici tercihleri üzerine bir araştırma (Master's thesis, Çanakkale Onsekiz Mart University). (In Turkish)

Dauda, A. B., Ojoko, E. A., & Fawole, B. E. (2016). Economic analysis of frozen fish demand in Katsina metropolis, Katsina State, Nigeria. *Journal of Fisheries and Aquatic Science*, *11*(1), 93-99. https://doi.org/10.3923/jfas.2016.93.99

Demirtaş, B., Dağistan, E., Akpınar, M. G., & Sayın, C. (2014). Fish consumption patterns of urban households in Hatay, Turkey. *Journal of Academic Documents for Fisheries and Aquaculture*, *2*, 69-77.

Djordjevic, V., Sarcevic, D., & Petronijevic, R. (2015). The attitudes and habits of Serbian schoolchildren to consumption of fish. *Procedia Food Science*, *5*, 73-76. https://doi.org/10.1016/j.profoo.2015.09.018

Ercan, O., & Şahin, A. (2016). Kahramanmaraş kent merkezinde balık eti tüketim analizi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *19*(1), 51-65. https://doi.org/10.18016/ksujns.57816 (In Turkish)

Erdal, G., & Esengün, K. (2008). Tokat ilinde balık tüketimini etkileyen faktörlerin logit model ile analizi. *Ege Üniversitesi Su Ürünleri Dergisi*, *25*(3), 203-209. (In Turkish)

FAO. (2022). *FishStat Plus-Universal software for fishery statistical time series*. Food and Agriculture Organization of the United Nations (FAO). https://www.fao.org/fishery/en/statistics/software/fishstatj

FAO. (2023). *FishStat Plus-Universal software for fishery statistical time series*. Food and Agriculture Organization of the United Nations (FAO). https://www.fao.org/fishery/en/statistics/software/fishstatj

Gül Yavuz, G., Yasan Ataseven, Z., Gül, U., & Gülaç, Z. N. (2015). Su ürünleri tüketiminde tüketici tercihlerini etkileyen faktörler: Ankara ili örneği. *Yunus Araştırma Bülteni*, *15*(1), 73-82. https://doi.org/10.17693/yunusae.v15i21955.235746 (In Turkish)

Hicks, D., Pivarnik, L., & Mc Dermott, R. (2008). Consumer perceptions about seafood-an Internet survey. *Journal of Food Service*, *19*, 213-226. https://doi.org/10.1111/j.1745-4506.2008.00107.x

Greene, W. H. (2008). *Econometric analysis*. Sixth edition. Prentice-Hall.

Greene, W.H. (2012). *Econometric analysis*. Seventh Edition. Prentice-Hall.

Hatırlı, S. A., Demircan, V., & Aktaş, A. R. (2004). Isparta ilinde ailelerin balık tüketiminin analizi. *Süleyman Demirel Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi*, *9*(1), 245-256. (In Turkish)

Karakaya, E., Sökmen, T. Ö., & Kırıcı, M. (2020). Erzincan ili balık tüketim alışkanlıklarının belirlenmesi. *Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi*, *6*(1), 18-29. (In Turkish)

Kaya, Y., Duyar, H. A., & Erdem, M. E. (2004). Balık yağ asitlerinin insan sağlığı için önemi. *Ege Üniversitesi Su Ürünleri Dergisi*, *21*(3-4), 365-370. (In Turkish)

Kılıç, E. Soylu, M., & Uzmanoğlu, M. S. (2019). Ardahan ili su ürünleri tüketim alışkanlıklarının belirlenmesi. *Türk Tarım –Gıda Bilim ve Teknoloji Dergisi*, *7*(7), 1028-1039. https://doi.org/10.24925/turjaf.v7i7.1028-1039.2536 (In Turkish)

Kırıcı, M., Çam, O., & Karakaya, E. (2018). Siirt ili kent merkezinde balık eti tüketim yapısı ve tüketicilerin satın alma eğilimlerinin belirlenmesi. *Akademik Ziraat Dergisi*, *7*(2), 227-236. http://doi.org/10.29278/azd.476656 (In Turkish)

Lee, M. K., & Nam, J. (2019). The determinants of live fish consumption frequency in South Korea. *Food Research International*, *120*, 382-388. https://doi.org/10.1016/j.foodres.2019.03.005

Maddala, G. S. (1983). *Limited-addict and qualitative variables in econometrics*. Cambridge University Press.

Marques, I., Botelho, G., & Guiné, R. (2019). Comparative study on nutritional composition of fish available in Portugal. *Nutrition & Food Science*, *49*(5), 925- 41. https://doi.org/10.1108/NFS-11-2018-0311

Olgunoğlu, İ. A., Bayhan, Y. K., Olgunoğlu, M. P., Artar, E., & Ukav, İ. (2014). Adıyaman ilinde balık eti tüketim alışkanlıklarının belirlenmesi. *GıdaTeknolojileri Elektronik Dergisi*, *9*(1), 21-25. (In Turkish)

Pérez-Ramírez, M., Almendarez-Hernández, M. A., Avilés-Polanco, G., & Beltrán-Morales, L. F. (2015). Consumer acceptance of eco-labeled fish: A Mexican case study. *Sustainability*, *7*(4), 4625–4642. https://doi.org/10.3390/su7044625

Roos, N., Wahab, M. A., Hossain, M. A., & Thilsted, S. H. (2007). Linking human nutrition and fisheries: Incorporating micronutrient-dense, small indigenous fish species in carp polyculture production in Bangladesh. *Food and Nutrition Bulletin*, *28*(2), 280-293. https://doi.org/10.1177/15648265070282s207

Şen, A., & Şahin, A. (2017). Mersin'de yaşayan tüketicilerin balık tüketim tercihlerini demografik faktörler açısından ele alan bir araştırma. *Afyon Kocatepe Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi*, *19*(1), 33-46.

TEPGE. (2021). *Ürün raporu su ürünleri 2021*. Tarımsal Ekonomi ve Politika Geliştirme Enstitüsü. https://arastirma.tarimorman.gov.tr/tepge/Belgeler/PDF%20%C3%9Cr%C3%BCn%20Raporlar%C4%B1/2021%20%C3%9Cr%C3%BCn%20Raporlar%C4%B1/Su%20%C3%9Cr%C3%BCnleri%20%C3%9Cr%C3%BCn%20Raporu%202021-338%20TEPGE.pdf (In Turkish)

Terin, M., Hamamcı, G., Gül, T., & Terin, S. (2016). Van ili kentsel alanda hanelerin balık tüketim yapısı ve satın alma davranışlarının belirlenmesi. *Ege Journal of Fisheries and Aquatic Sciences*, *33*(3), 241-249. https://doi.org/10.12714/egejfas.2016.33.3.08 (In Turkish)

Terin, M. (2019). Household characteristics influencing fish consumption in Van Province, Turkey. *Italian Journal of Food Science*, *31*(3), 416-426. https://doi.org/10.14674/IJFS-1448

Topçu, Y., & Dağdemir, V. (2017). Turkish consumer purchasing decisions regarding PGI-labelled Erzurum civil cheese. *Alınteri Journal of Agricultural Sciences*, *32*(2), 69-80. https://doi.org/10.28955/alinterizbd.343725

TUIK. (2023). *Dış ticaret istatistikleri*. Türkiye İstatistik Kurumu. https://biruni.tuik.gov.tr/disticaretapp/menu.zul (In Turkish)

Uzundumlu, A. S. (2017). Determining fish consumption behavior among households and the most suitable type of fish in Erzurum Province. *Iranian Journal of Fisheries Sciences*, *16*(2), 684-697

WHO. (2020). *Obesity and overweight*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Yücel, A. (2001). *Meat and fisheries technology*. Uludag University, Faculty of Agriculture, Lecture Notes No: 47. (In Turkish)

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

https://prensip.gen.tr

RESEARCH ARTICLE

Agrotechnical Characteristics of Some Vegetable Seeds Commonly Grown in Denizli-Acıpayam

Zeynep Dumanoglu[1]✉ • Gülsüm Ozturk[2]

[1]Bingol University, Faculty of Agriculture, Department of Biosystem Engineering, Bingöl/Türkiye
[2]Ege University, Faculty of Agriculture, Department of Field Crops, İzmir/Türkiye

ARTICLE INFO

Article History
Received: 12.07.2023
Accepted: 07.09.2023
First Published: 30.12.2023

Keywords
Heirloom seed
Seed properties
Seed size
Vegetable seed

ABSTRACT

Vegetables are not only consumed fresh but also have an important place in nutrition in terms of vitamins, carbohydrates, fats and proteins. Parts of these plants such as roots, stems, leaves, fruits and seeds are used and consumed. This study was carried out in the laboratories of Bingol and Ege Universities in 2022. In the study, some physical properties (length, width, surface area, mean arithmetic and geometric diameters, and sphericity) of seeds belonging to five different vegetables (dill, lettuce, parsley, arugula and tongue grass) were investigated. Since the seeds examined in the study are "heirloom seeds", the values obtained will enable sowing operations to be carried out with minimum seed loss by making use of mechanization applications in small and narrow areas as well as breeding studies.

Please cite this paper as follows:

Dumanoglu, Z., & Ozturk, G. (2023). Agrotechnical characteristics of some vegetable seeds commonly grown in Denizli-Acıpayam. *Journal of Agricultural Production*, *4*(2), 100-104. https://doi.org/10.56430/japro.1326502

1. Introduction

Vegetables are not only consumed fresh but also have an important place in nutrition in terms of vitamins, carbohydrates, fats and proteins. Parts of these plants such as roots, stems, leaves, fruits and seeds are used and consumed. Vegetables are used in human nutrition and they are rich in minerals such as Ca, P and Fe, as well as vitamins A, B, C and D (Vural et al., 2000). Moreover, some of the spices are considered as medicinal plants, are added to food products and are used to enhance the sensory properties of that food product such as taste and flavor. Furthermore, these spice extracts have antimicrobial activity against many microorganisms. This creates a great advantage in terms of human and food health against synthetic preservatives in extending the shelf life of products naturally (Negi, 2012). There are approximately 100 families of aromatic plants within the *Apiaceae* (*Umbelliferae)* family (Al, 2019). Some of these plants, which are used both as vegetables and spices, are given below:

Arugula (*Eruca Sativa* L.): It is an annual gramine plant in the Cruciferae (*Brassicaceae*) family. Arugula is pile-rooted and its stem is rosette-shaped (Vural et al., 2000). The arugula plant can be 10-50 cm tall, its leaves are toothed, and its flowers are white and purple (Keleş, 2015). Arugula, which is a biennial plant, is used for its leaves in the first year and for seed formation in the second year. It is grown in Mediterranean

✉Correspondence
E-mail address: zdumanoglu@bingol.edu.tr

countries as fresh salad and garnish (Bianco & Boari, 1996). In the Far East, it is used as an oil plant for human nutrition and medicine (Eşiyok, 1996; Vural et al., 2000). For cultivation, between 100-150 kg of seeds is taken per decare (Vural et al., 2000). Its leaves contain plenty of vitamin C, and its seeds have stimulant, cough suppressant and aphrodisiac properties (İşbilir, 2008).

Dill (*Anethum graveolens* L.): It is an annual cool climate plant belonging to the *Apiaceae* or *Umbelliferae* family from the *Apiaceae* family (Elik, 2010). The roots of dill are stinger-shaped and white in color, the plant grows up to 90 cm in height and has yellow flowers with umbrella shape and hermaphrodite characteristics. The number of seeds per gram is 500-600. Spread sowing is common in dill planting and the planting norm is 1.5-2 g/m^2 (Eşiyok, 2012). Dill seeds are used as essential oil and spice, and its dried leaves are used as tea. Moreover, it has been stated that it reduces the risk of cancer (Elsayed et al., 2020). Its seeds have carminative, stomachic and diuretic properties, moreover, relieve intestinal spasms and eliminate bad breath. It is generally used as a sweetener in the food industry as well as flavoring various foods such as pickles, salads, sauces and soups. Since it prevents the growth of various bacteria, it has a versatile use as a preservative (Jana & Shekhawat, 2010).

Parsley (*Petroselinum crispum* L.): Parsley is a biennial herb belonging to the genus *Petroselinum* from the *Apiaceae* family. Parsley is used as a spice and medicinal plant for food use and therapeutic purposes. Parsley, which is a Mediterranean plant, is pile-rooted, 120 cm tall and has umbrella-shaped white flowers (Vural et al., 2000; Menglan et al., 2005; Halaç, 2018). In the first year the green parts are grown for leaves and flowers and in the second year the seeds are used (Telli & Üremiş, 2010; Al, 2019). Its seeds are rich in essential oil, while its green leaves are rich in vitamins A, C and E (Ceylan et al., 2005). It is consumed fresh in salads and meals. Parsley is harvested 3-5 times a year (Öztürk et al., 2014; Al, 2019). It has been determined that parsley is effective against inflammatory diseases and protects the liver with its high antioxidant properties (Çağın, 2005; Özbek et al., 2008).

Lettuce (*Lactuca sativa* L.): Lettuce is one of the most important vegetables whose leaves are used. It is produced in many countries around the world (Devlez, 2022). It is considered as one of the most important vegetables of the leafy vegetables group. The seeds are generally 3-6 mm long, 0.8-1.0 mm wide and 0.3-0.6 mm thick, and the color of the seeds can vary between dirty white, yellow, cream, brown and black (Çekim & Özarslan, 2020). It can be grown in about 30-60 days under field and greenhouse conditions in Türkiye and can be produced throughout the year (Karaağaç & Balkaya, 2017).

Cress (*Lepidium sativum* L.): It is an annual *gramineas* plant belonging to the cruciferous family (*Brassicaceae*) whose leaves are consumed as a spice. This plant, whose origin is Asia and North Africa, is consumed as a salad or side dish vegetable due to its pleasant smell and slightly spicy structure (Karaali, 2011). The roots are pile rooted, the stem is rosette-shaped and green in color. Flowers are scattered on the stem and on the lateral and main branches. Erselic flowers are white and purple in color. Seed yield per decare is between 60-70 kg (Vural et al., 2000). Its leaves are rich in calcium, magnesium, phosphorus, potassium, copper and manganese and vitamins A, B6, C and K. Tongue grass is used in the treatment of diseases such as cancer and asthma (Yanmaz et al., 2010; Hazıroğlu, 2022). Since the leaves and seeds of the plant contain secondary metabolites called glucosinolate, they are especially used in cancer treatment (Gil & Macleod, 1980; Yavaşoğlu, 2012).

In this study, some agrotechnical characteristics of seeds belonging to five different vegetables (dill, lettuce, parsley, arugula and cress) were determined. These vegetables, which can be produced not only commercially but also in hobby gardens, are among the products that are mostly produced and consumed. Production can be made in large areas by using mechanization from sowing/planting to harvest. However, in the absence of sufficient facilities, portable agricultural tools can be used. It is much easier to use these tools on narrow, non-uniform, uneven and sloping land with leveling problems or in hobby gardens. Determining the appropriate planting unit, especially for such simple agricultural tools used for sowing and planting, increases the possibility of producers to produce without loss of seed under limited conditions. Therefore, it is predicted that the basic physical properties investigated in the study will be beneficial for the design and production stages of such machines and for manufacturers.

2. Materials and Methods

This study was carried out in the laboratories of Bingol and Ege Universities in 2022. The seeds examined in the study were obtained from Acıpayam district of Denizli province in Türkiye (Figure 1). Five different vegetable seeds have been growing in this region for about 30 years and are known as “heirloom seeds”. These seeds, which are known to have 98-99% germination ability, were sown in the 2021-2022 production period.

Since Acıpayam plain has a sandy soil structure, it creates a suitable environment for growing vegetables and fruits. However, due to the insufficient level of irrigation canals, producers benefit from alternative irrigation methods (www.acipayam.bel.tr/coğrafya-iklimi/).

Figure 1. Map of Türkiye and Denizli province (www.cografyaharita.com).

In this study, some agrotechnical characteristics such as length (mm), width (mm), surface area (mm^2), mean arithmetic and geometric diameters and sphericity of seeds belonging to five different plants were determined (Dumanoglu & Ozturk, 2021). For the examination, 100 seeds were selected randomly for each vegetable. Parameters such as length, width and surface area were measured by using a stereo microscope (Nikon SMZ 745T) with its own software (Dumanoglu & Geren, 2020). Using the data obtained, mean arithmetic diameter ((L+W)/2), geometric diameter ($(L*D^2)^{1/3}$) and sphericity (D_0/L) values were determined (L: Seed length value (mm) W: Seed width value (mm), D: Mean arithmetic diameter (mm); D_0: Mean geometric diameter (mm)) (Mohsenin, 1970; Alayunt, 2000; Kara, 2012, 2017).

Table 1. Classification of the seeds according to their geometric properties and shapes (Yağcıoğlu, 2015).

Classification by geometric properties	Grain width/Grain length (b/a) (mm)	Classification by shape	Length (a), Width (b), Thickness (c) (mm)
Long	< 0.6	Round	a ≈ b ≈ c
Medium	0.6 – 0.7	Oval	a/3 < b ≈ c
Short	> 0.7	Long	c < b < a/3

3. Results and Discussion

In this study, some agrotechnical characteristics of seeds belonging to five different vegetables, which are produced intensively, were investigated.

According to the data obtained (Table 2), it was determined that dill seeds had an average length of 3.835 mm, a width of 1.648 mm, a surface area of 4.711 mm^2, an arithmetic diameter of 2.742 mm, a geometric diameter of 9.828 mm and a sphericity of 2.525. The values found coincide with the seed characteristics stated (its seeds are fragrant, small, 4-5 mm long, 1.5-3.5 mm wide, flat and slight, with gray-brown lines on it) by some researchers (Ceylan, 1997; Rekha et al., 2010; Elsayed et al., 2020).

Table 2. Agrotechnical characteristics of some vegetable seeds.

Seed Properties	Dill	Lettuce	Parsley	Arugula	Cress
Length (mm)	3.835	3.387	2.685	1.686	2.580
Width (mm)	1.648	0.927	1.125	1.333	1.137
Surface area (mm^2)	4.771	2.171	2.423	1.891	2.622
Mean arithmetic diameter (mm)	2.742	2.157	1.905	1.510	1.859
Mean geometric diameter (mm)	9.828	5.366	3.328	1.298	2.992
Sphericity	2.525	1.562	1.219	0.763	1.154

Lettuce seeds were found to have an average length of 3.387 mm, a width of 0.927 mm, a surface area of 2.171 mm^2, an arithmetic diameter of 2.157 mm, a geometric diameter of 5.366 mm and a sphericity of 1.562. Çekim and Özarslan (2020) determined that lettuce seeds had a length of 3.36 mm, a width of 0.84 mm and a thickness of 0.54 mm, and a projection area of 2.79 mm^2 with a sphericity value of 0.34. The values obtained coincide with the data found in this study. Furthermore, it was determined that parsley seeds had 2.685 mm length, 1.125 mm width, 2.423 mm^2 surface area, 1.905 mm arithmetic diameter, 3.328 mm geometric diameter and 1.219 sphericity. Arugula seeds were found to have a length of

1.686 mm, a width of 1.333 mm, a surface area of 1.891 mm^2, an arithmetic diameter of 1.510 mm, a geometric diameter of 1.298 mm and a sphericity of 0.763. Besides, it was determined that cress seeds had 2.580 mm length, 1.137 mm width, 2.622 mm^2 surface area, 1.859 mm arithmetic diameter, 2.992 mm geometric diameter and 1.154 sphericity.

According to the values obtained and the seed characteristics in Table 1, it was determined that dill, lettuce, parsley and cress seeds had a long and oval structure, while arugula seeds had a short and oval structure. Accordingly, dill, parsley, arugula and cress seeds have similar seed properties, thus, it is possible to plant them using the same planting arrangement. However, the morphological structure of lettuce seeds is different from the other four vegetable seeds. Hence, choosing a planting unit suitable for lettuce seeds is important in terms of preventing possible seed losses.

The seed of each plant has its own characteristics. However, knowing these agrotechnical characteristics makes the sowing process more practical and economical. The ancestral seeds used in this study meet the predictions of the producers in terms of yield and quality because they maintain their seed characteristics over time and adapt to the climatic characteristics of the region. It is also expected to contribute to the design of new machinery and tools.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Al, G. E. (2019). *A study on the yield of parsley crops (Petroselinum crispum Mill.) grown in different environments during the harvest timesand on the changes in the yield criteria* (Master's thesis, Tokat Gaziosmanpaşa University).

Alayunt, F. N. (2000). *Biyolojik malzeme bilgisi*. Ege Üniversitesi Ziraat Fakültesi Yayınları. (In Turkish)

Bianco, V. V., & Boari, F. (1996). *Up-to-date development of wild rocket cultivation*. Rocket: A Mediterranean Crop for the World Report of the Workshop. Rome.

Çağın, H. K. (2005). *Bitkilerin gizli dünyası, IV maydanozgiller (Apiaeace)*. Bulut Yayınları. (In Turkish)

Çekim, İ., & Özarslan, C. (2020). Marul tohumunun bazı fiziko-mekanik özelliklerinin belirlenmesi. *ÇOMÜ Ziraat Fakültesi Dergisi*, *8*(1), 89-97. https://doi.org/10.33202/comuagri.619355 (In Turkish)

Ceylan, A. (1997). *Tıbbi bitkiler II*. Ege Üniversitesi Ziraat Fakültesi Yayınları. (In Turkish)

Ceylan, Ş., Mordoğan, N., Yoldaş, F., & Çakıcı, H. (2005). *Azotlu gübrelemenin farklı ekim zamanlarında yetiştirilen maydanoz bitkisinde azot birikimi verim ve bazı kalite özelliklerine etkisi*. Ege Üniversitesi Bilimsel Araştırma Raporu. (In Turkish)

Devlez, E. (2022). *The effect of ultraviolet A and B light application on some quality values in post-harvest lettuce (Lactuca sativa)* (Master's thesis, Bilecik Seyh Edebali University).

Dumanoglu, Z., & Geren, H. (2020). An investigation on determination of seed characteristics of some gluten-free crops (*Amarantus mantegazzianus*, *Chenopodium quinoa* Willd., *Eragrostis tef* [Zucc] Trotter, *Salvia* hispanica L.). *Turkish Journal of Agriculture-Food Science and Technology*, *8*(8), 1650-1655. https://doi.org/10.24925/turjaf.v8i8.1650-1655.3394

Dumanoglu, Z., & Ozturk, G. (2021). A research on improving seed quality (pelleting) in true potato of 101 (Nif) genotype. *Fresenius Environmental Bulletin*, *30*(09), 10983-10968.

Elik, H. (2010). *Diyarbakır ekolojik koşullarında farklı ekim zamanlarının dereotu (Anethum Graveolens L.)'nda bazı agronomik ve teknolojik özellikler üzerine etkisi* (Master's thesis, Çukurova University). (In Turkish)

Elsayed, S. I. M., Glala, A. A., Abdalla, A. M., Abd El Ghafour, A., & Darwish, M. A. (2020). Effect of biofertilizer and organic fertilization on growth, nutrient contents and fresh yield of dill (*Anethum graveolens*). *Bulletin of the National Research Centre*, *44*(1), 1-10. https://doi.org/10.1186/s42269-020-00375-z

Eşiyok, D. (1996). Marketing and utilization of rocket in Turkey. In S. Padulosi & D. Pignone (Eds), *Rocket: A Mediterranean crop for the world* (pp. 86-90). IPGRI.

Eşiyok, D. (2012). *Kışlık ve yazlık sebze yetiştiriciliği*. Sidas Yayınları. (In Turkish)

Gil, V., & Macleod, A. J. (1980). The effects of pH on glucosinolate degradation by a thioglucoside glucohydrolase preparation. *Phytochemsitry*, *19*(12), 2547-2551. https://doi.org/10.1016/S0031-9422(00)83916-3

Halaç, B. (2018). *Hatay ili maydanoz (Petroselinum Crispum (Mill.) Nym. Ex A.W. Hill) üretim alanlarında görülen bazı virüs ve fitoplazma hastalıklarının enfeksiyon oranları ile doğal taşınması ve diğer konukçularının araştırılması* (Master's thesis, Hatay Mustafa Kemal University). (In Turkish)

Hazıroğlu, A. N. (2022). *Tere (Lepidium Sativum L. Subsp. Sativum) bitkisi üzerinde demir sülfat (Feso4.7h2o) toksisitesinin morfolojik, anatomik ve fizyolojik etkileri* (Master's thesis, Van Yüzüncü Yıl University). (In Turkish)

İşbilir, Ş. (2008). *Yaprakları salata-baharat olarak tüketilen bazı bitkilerin antioksidan aktivitelerinin incelenmesi* (Doctoral dissertation, Trakya University). (In Turkish)

Jana, S., & Shekhawat, G. S. (2010). *Anethum graveolens*: An Indian traditional medicinal herb and spice.

Pharmacognosy Reviews, *4*(8), 179-184. https://doi.org/10.4103%2F0973-7847.70915

Kara, M. (2012). *Biyolojik ürünlerin fiziksel özellikleri*. Atatürk Üniversitesi Ziraat Fakültesi Yayınları. (In Turkish)

Kara, M. (2017). *Physical properties of biological products*. Güven Bilimsel.

Karaağaç, O., & Balkaya, A. (2017). Türkiyede yerel sebze çeşitlerinin mevcut durumu ve islah programlarında değerlendirilmesi. *Türkiye Tohumcular Birliği Dergisi*, *5*, 8-15. (In Turkish)

Karaal, G. (2011). *Organik gübre katkılı fındık zurufu kompostunda roka (Eruca Sativa L.) ve tere (Lepidium Sativum L.) yetiştiriciliği* (Master's thesis, Ordu University). (In Turkish)

Keleş, R. (2015). *Topraktan izole edilen bakterilerin tanımlanması ve tanımlanan bakterilerin roka bitkisinin (Eruca Sativa) gelişmesine biyogübre olarak etkilerinin incelenmesi* (Master's thesis, Gebze Teknik University). (In Turkish)

Menglan, S., Fading, Pu., Zehui, P., Watson, M. F., Cannon, J. F. M., Holmes-Smith, I., Kljuykov, E. V., Phillippe, L. R., & Pimenov, M. G. (2005). Apiaceae (Umbelliferae). *Flora of China*, *14*, 1-205.

Mohsenin, N. N. (1970). *Physical properties of plant and animal materials*. Gordon and Breach Science Publishers.

Negi, P. S. (2012). Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. *International Journal of Food Microbiology*, *156*(1), 7-17. https://doi.org/10.1016/j.ijfoodmicro.2012.03.006

Özbek, H., Cengiz, N., Bayram, İ., & Öntürk, H. (2008). Alfa-amanitinle oluşturulmuş böbrek ve karaciğer toksisitesinde alfa-pinen ve silibininin etkisinin sıçanlar üzerinde araştırılması. *Genel Tıp Dergisi*, *18*(4), 159-164. (In Turkish)

Öztürk, M., Temel, M., & Tınmaz, A. B. (2014). *Türkiye'de kekik üretim ve pazarlaması*. II. Tıbbi ve Aromatik Bitkiler Sempozyumu. Yalova. (In Turkish)

Rekha, M. N., Yadav, A. R., Dharmesh, S., Chauhan, A. S., & Ramteke, R. S. (2010). Evaluation of antioxidant properties of dry soup mix extracts containing dill (*Anethum sowa* L.) leaf. F*ood and Bioprocess Technology*, *3*(3), 441-449. https://doi.org/10.1007/s11947-008-0123-5

Telli, S., & Üremiş, İ. (2010). Samandağ (Hatay)'da maydanoz yetiştiriciliğinde karşılaşılan bitki koruma sorunları ve çözüm önerileri. *Mustafa Kemal Üniversitesi Ziraat Fakültesi Dergisi*, *15*(1), 39-48. (In Turkish)

Vural, H., Eşiyok, D., & Duman, İ. (2000). *Kültür sebzeleri (sebze yetiştirme)*. Ege Üniversitesi Basımevi. (In Turkish)

Yağcıoglu, A. (2015). *Ürün işleme*. Ege Üniversitesi Yayınları. (In Turkish)

Yanmaz, R., Yıldırım, E., & Koyuncu, D. (2010). Ülkemiz için yeni bir tere (*Lepidium sativum* var. subsp. *sativum*) çeşit adayı: Dadaş. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, *41*(2), 91-95. (In Turkish)

Yavaşoğlu, M. (2012). *Bazı şifalı bitkilerin ekonomiye kazandırılması: Tere (Lepidium Sativum L.) bitkisinin antioksidan, sitotoksik, enzim aktif özüt ve bileşenlerinin belirlenmesi* (Masters's thesis, Çanakkale Onsekiz Mart University). (In Turkish)

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

https://prensip.gen.tr

RESEARCH ARTICLE

The Investigation of the Amount of Blue Crab Production Regarding Other Crustaceans and Molluscs' Farming in Türkiye

Övgü Gencer✉

Ege University, Faculty of Fisheries, Department of Aquaculture, İzmir/Türkiye

ARTICLE INFO

Article History
Received: 20.08.2023
Accepted: 22.10.2023
First Published: 30.12.2023

Keywords
Blue crab
Blue crab production
Molluscs and crustaceans
Water resources

ABSTRACT

The aim of this study was to examine the factors affecting the catch rate of crustaceans and molluscs and to determine the effect of factors affecting the catch rate of blue crab. In this study, data of 14 variables (octopus, lobster, black mussel, etc.), total crustacean and mollusc content and total freshwater fish were examined from the crustaceans and molluscs caught from the TUIK website between 2012-2021. In addition to the comparison of these amounts, variables such as renewable water resources per capita, renewable surface water, total water withdrawal per capita, and water withdrawal for aquaculture were included in the study. Finally, the study was concluded by comparing seafood, aquaculture and freshwater products. The relationships between blue crab and other crustaceans and molluscs, water related statistics, aquaculture statistics were examined by regression analysis. As a result of the analyzes made, other creatures that affect the hunting rates of the blue crab; It was concluded that there is a category of jumbo shrimp, cuttlefish and other. At the same time, it was concluded that the total amount of fresh water and the amount of freshwater products affect the catch rate of the blue crab.

Please cite this paper as follows:

Gencer, Ö. (2023). The investigation of the amount of blue crab production regarding other crustaceans and molluscs' farming in Türkiye. *Journal of Agricultural Production*, *4*(2), 105-110. https://doi.org/10.56430/japro.1346987

1. Introduction

The blue crab, characterized by its superior meat quality and elevated market value, holds prominence within regions of cultivation, notably within Western nations. Additionally, the blue crab demonstrates commercial viability within Türkiye, with its significance exhibiting an upward trajectory (Millikin & Williams, 1984; Ağbaş et al., 2008; Kaya & Yalçın, 2018). Blue crabs live mostly in the Western Atlantic Ocean and the Gulf of Mexico (Jivoff et al., 2017; Weatherall et al., 2018). In later periods, they were transported to European and Far Eastern waters around large fishing ships and through the ships' ballast water. Over time, the blue crabs that continue their lives here have come to the fore as an important commercial product. (Havens et al., 2008; Nehring et al., 2008). The confluence of its meat's exceptional attributes and its commensurate premium pricing has induced a discernible escalation in global crab capture rates, prompting commensurate investments within the domain of aquaculture (Atar et al., 2001). Within the Turkish context, blue crabs, which densely populate the Beymelek, Akyatan, and Yumurtalık regions, have attained escalating significance (Enzerob et al., 1997).

Blue crabs, inhabitants of various locales, notably the Mediterranean and Aegean coastlines, exhibited a catch of 2.1 tons in the year 2012, as documented by the Turkish Statistical Institute (TUİK). Subsequent years witnessed a notable surge, with figures reaching 8.8 tons in 2017 and approximately 10.5 tons in 2018. However, a discernible decline ensued, as

✉Correspondence
E-mail address: ovgu.gencer@ege.edu.tr

evidenced by a reduced capture of 1.5 tons by the year 2021. An assessment of the cumulative haul of crustaceans and molluscs extracted within the territorial waters of Türkiye reveals a discernible trend. Specifically, the aggregate capture amounted to 80,685 tons in 2012, a metric that saw a consequential contraction to approximately 32,728.30 tons by the year 2021 (TUİK, 2023).

Crustaceans and molluscs, distinguished by their prominence as consumable sustenance, owe their dietary appeal to their elevated protein quotient, mineral abundance, and fatty acid composition (Reichmuth et al., 2009; Bembe et al., 2017). The present endeavor embarks on a scrutiny of the causal factors underlying the fluctuations in the cultivation and capturing of aquaculture produce within environments tainted by contamination. This pursuit further entails a comprehensive inquiry into the repercussions these perturbations wield upon the yields of analogous crustacean and mollusc species. The methodology employed herein encompasses rigorous statistical analyses, applied to the dataset at hand.

The aim of this study was to determine the relationship between blue crab production amounts and the catch rate of other crustaceans and molluscs in Türkiye. In this context, other factors affecting the amount of blue crab production are also wanted to be included in the analysis. In addition, as a result of the study, it is aimed to estimate the blue crab production amounts for the coming years.

2. Materials and Methods

Within the ambit of this study, analytical methodologies were diligently applied to the corpus of data culled from the official online repository of TUİK. Complementing the meticulous examination of the quantum of blue crab extractions spanning the years 2012 to 2021, a comprehensive ensemble of marine organisms was subjected to meticulous analysis. These encompassed, inter alia, octopuses, insect species, sea crayfish, lobsters, calamari, male and jumbo shrimp, karabiga shrimp, cockles, black mussels, cuttlefish, mackerel, as well as sundry other members of the crustacean and mollusc taxa. In parallel to these entities, an assortment of additional variables were seamlessly integrated into the analytical framework. These encompassed the collective volumes of crustaceans and molluscs (10^9 m^3), total freshwater fish (10^9 m^3), renewable water resources per capita (10^9 m^3), renewable surface water (10^9 m^3), total water withdrawal per capita (10^9 m^3), and water allocation designated for aquacultural purposes (10^9 m^3). Moreover, the finalization of the analytical phase was effectuated by means of a comparative investigation. This investigation facilitated the discernment of patterns and dynamics by juxtaposing the quantities of marine produce, aquaculture-derived products, and freshwater commodities across identical temporal intervals.

Statistical computations were performed, founded upon the assemblage of accumulated data. Subsequently, variables affiliated with diverse biological entities identified as crustaceans and molluscs, in conjunction with volumetric measurements pertaining to water quantities, were systematically identified. The relationships between blue crab and other crustaceans and molluscs, water related statistics, aquaculture statistics were examined by regression analysis. The reason for using regression analysis between dependent and independent variables is that the variables are continuous.

3. Results and Discussion

The dataset harnessed for the purposes of this investigation was procured from the online repository maintained by TUİK. Comprising a comprehensive compendium, the dataset enlists a total of 23 distinct variables, encompassing the blue crab among others, spanning the temporal scope from 2012 to 2021.

Table 1. Basic statistics of the data.

	Median	Standard Deviation	Minimum	Maximum
Octopus	259.230	55.507	162.70	361.00
Insect	4.120	3.835	.50	11.50
Crayfish	2.570	2.551	.10	6.90
Lobster	3.450	2.407	1.40	8.00
Calamari	487.550	91.869	367.20	631.40
Male Shrimp	85.610	85.626	26.60	255.10
Jumbo Shrimp	586.900	117.717	451.80	758.80
Karabiga	251.660	72.326	171.60	383.90
Cockles	21.840	27.110	.80	83.40
Black Clam	499.930	296.000	48.70	887.40
Cuttlefish	846.980	126.727	696.80	986.00
Scallop	7.580	6.088	1.30	21.60
Blue Crab	3.570	3.456	.60	10.50
Other	271.170	216.075	25.00	761.90

Table 1. (continued)

	Median	Standard Deviation	Minimum	Maximum
Total Shelled Mollusks	49266.020	14753.546	32728.30	80685.00
Total Freshwater	33549.940	1939.994	30139.00	36134.00
Per Capita Renewable Water Supply	2836.335	125.603	2691.52	3040.81
Renewable Surface Water	171.800	.000	171.80	171.80
Total Water Extraction Per Person	56.307	5.039	48.28	62.21
Water Extraction for Aquaculture	706.388	34.965	646.68	744.08
Seafood	336404.100	54975.635	266078.00	431572.00
Aquaculture Products	303215.800	89632.605	212410.00	471686.00
Freshwater Products	33549.900	1939.959	30139.00	36134.00

Upon reviewing the data presented in Table 1, it becomes evident that blue crabs exhibited an average catch of 3.57 tons (±3.456) over the past decade. The year of least catch yielded 0.6 tons, while the peak year observed a capture of 10.50 tons. Correspondingly, the 10-year average for lobsters, classified within the same category of crustaceans and molluscs as blue crab, stands at 3.45 tons (±2.407), mirroring the pattern seen in blue crab. Notably, the preeminent mean within the crustaceans and molluscs category was attributed to cuttlefish, recording a substantial 846.980 (±126.727). In addition, it is noteworthy that the dataset indicates an average freshwater volume of $33{,}549.940 \times 10^9$ m³ (±1,939.994). Furthermore, it is observed that the amount of renewable surface water remained unaltered and constant throughout the ten-year span, maintaining a volume of 171.800×10^9 m³.

Since the data in the data set are continuous variables and the statistical method of measuring the relationship between continuous variables is regression analysis, the values between the variables were compared with the regression analysis method.

Table 2. Comparison of blue crab with other crustaceans and molluscs.

	Constant	B	R square	
Octopus	10.553	-0.27	0.187	F test: 1.842 p value: 0.212
Insect	4.632	-0.258	0.082	F test: 0.712 p value: 0.423
Crayfish	3.717	-0.057	0.002	F test: 0.014 p value: 0.908
Lobster	4.209	-0.185	0.017	F test: 0136 p value: 0.722
Calamari	-0.429	0.008	0.048	F test: 0.399 p value: 0.545
Male Shrimp	4.583	-0.012	0.086	F test: 0.753 p value: 0.411
Jumbo Shrimp	-9.604	0.022	0.584	F test: 11.250 p value: 0.010
Karabiga	8.661	-0.020	0.179	F test: 1.746 p value: 0.223
Cockles	4.642	-0.049	0.148	F test: 1.393 p value: 0.272
Black Mussel	2.526	0.002	0.032	F test: 0.265 p value: 0.621
Cuttlefish	-13.751	-0.020	0.562	F test: 10.274 p value: 0.013
Crab	3.272	0.039	0.005	F test: 0.038 p value: 0.849
Other	0.423	0.012	0.526	F test: 8.895 p value: 0.018
All Variables	7.564	-0.013	0.223	F test: 7.112 p value: 0.321

Based on the regression outcomes presented in Table 2, it is discernible that a statistically significant association exists between Jumbo shrimp (58.4%), Cuttlefish (56.2%), and other species (52.6%), and blue crab. Conversely, when scrutinizing the associations with the remaining variables, it is evident that the p-values surpass the threshold of 0.05. As such, a lack of statistical significance prevails in relation to these variables. It can be said that when all data are included in the equation at the same time, the p value is greater than 0.05 and the model is not statistically significant.

Table 3. Comparison of blue crab and water related statistics.

	Constant	**B**	**R square**	
Total Crustaceans and Molluscs	-0.417	0.00008	0.119	F test: 1.014 p value: 0.328
Total Fresh Water	51.377	-0.001	0.64	F test: 14.201 p value: 0.005
Renewable Water Per Person	37.473	-0.012	0.189	F test: 1.860 p value: 0.210
Total Water Extraction Per Person	-8.665	0.217	0.100	F test: 0.897 p value: 0.372
Water Extraction for Aquaculture	-18.160	0.031	0.097	F test: 0.858 p value: 0.381
All Variables	62.335	0.143	0.221	F test: 1.321 p value: 0.310

Upon dissecting the data encompassing blue crab catch statistics and water-related metrics, as delineated in Table 3, an observation surfaces. Specifically, among the considered parameters, solely the aggregate proportion of freshwater exhibits a noteworthy linkage with the blue crab catch statistics, as underscored by a p-value of 0.005, which stands below the predetermined significance threshold of 0.05 ($p=0.005<0.05$). Significantly, it can be deduced that a singular modification in this variable accounts for 64% of the variability observed in the recorded blue crab capture quantities. It can be said that when all data are included in the equation at the same time, the p value is greater than 0.05 and the model is not statistically significant.

Table 4. Comparison of blue crab and aquaculture statistics.

	Fixed	**B**	**R square**	
Seafood	7.524	-0.00001	0.035	F test: 0.290 p value: 0.605
Aquaculture Products	1.910	0.00005	0.020	F test: 0.165 p value: 0.696
Freshwater Products	51.378	-0.001	0.640	F test: 14.201 p value: 0.005
All Variables	40.185	-0.000315	0.320	F test: 7.325 p value: 0.371

Upon juxtaposing blue crab, seafood, aquaculture products, and freshwater commodities as presented in Table 4, a noteworthy observation emerges. Specifically, the sole discerned instance of statistical significance pertains to the influence of variations in freshwater products on blue crab catch statistics, substantiated by a p-value of 0.005, which resides below the established threshold of significance (0.05) ($p=0.005<0.05$). This interrelation exhibits a calculated ratio of 0.64. It can be said that when all data are included in the equation at the same time, the p value is greater than 0.05 and the model is not statistically significant.

Table 5. Regression analysis results of variables significantly associated with blue crab.

	Beta Coefficient	R Square	F Test	p value
Fixed	23.226	0.819	5.655	0.042
Freshwater Products	-.001			
Other	0.004			
Cuttlefish	0.002			
Jumbo Shrimp	0.008			

In reference to Table 5, a comprehensive regression analysis was conducted to investigate the interplay between blue crab and the pertinent variables, namely freshwater products, other, cuttlefish, and jumbo shrimp, which have been statistically determined to bear significance in relation to blue crab. The scrutiny of this relationship proves substantiated, signified by the notable R square value of 0.819 and the p-value falling below the established threshold of 0.05. Consequently, it can be asserted that the alterations observed across the aforementioned four variables collectively account for a substantial 81.9% of the variations discerned within the blue crab capture quantities.

Table 6. Blue crab catch estimates for the next 5 years.

	Estimate (ton)
2024	1.51
2025	.61
2026	2.01
2027	8.81
2028	10.51

Utilizing the formulated equation, prognostications for forthcoming years' blue crab catch statistics have been derived, as showcased within Table 6.

4. Conclusion

In conjunction with the documented blue crab catch quantities spanning the interval from 2012 to 2021, as extracted from the TUIK website, a diverse array of marine entities has been embraced for analytical contemplation. This comprehensive roster encompasses octopus, insect species, crayfish, lobsters, calamari, male and jumbo shrimp, karabiga shrimp, cockles, black mussels, cuttlefish, mud crab, and assorted other specimens within the crustacean and mollusc classification. Expanding the analytical purview, an assortment of hydrological metrics has been amalgamated. These encompass a spectrum of parameters, including the aggregate volumes of total crustaceans and molluscs (10^9 m³), total freshwater fish (10^9 m³), per capita renewable water resources (10^9 m³), renewable surface water (10^9 m³), total water withdrawal per capita (10^9 m³), and water withdrawal designated for aquaculture activities (10^9 m³). Furthermore, the ambit of analysis incorporates catch statistics pertaining to overall seafood yields, aquaculture-based products, and freshwater commodities.

When the relationships between the variables in the data set and the blue crab were examined, firstly, each variable and the blue crab were included in the regression analysis one by one. As a result of these analyses, it was determined that there was a significant relationship between jumbo shrimp, cuttlefish and other groups and blue crab. Additionally, the relationship is statistically significant when all variables are taken together.

When the relationship between blue crab and water-related measurements was examined, it was concluded that there were significant relationships only when the Total Fresh Water variable and all variables were analyzed together. The relationship between other variables and blue crab was not statistically significant. At the same time, when the statistics of blue crabs and aquaculture were compared, it was concluded that there was a statistically significant relationship only between the freswater products variable and blue crabs.

The predictions for the next 5 years regarding blue crab statistics are as follows; 1.51; 0.61; 2.01; It was estimated at 8.81 and 10.51 tons.

As inferred from the outcomes of the conducted analysis:

- Within the cohort of 13 analyzed crustaceans and molluscs, noteworthy correlations have been established between the catch rates of jumbo shrimp, cuttlefish, and the other subgroup, and the catch rate of the blue crab variable.

- Amidst the considered hydrological metrics, sole statistical significance has been attributed to the realm of total freshwater statistics, which exhibits a substantial relationship with the blue crab catch rate.

- Upon comparative evaluation vis-à-vis aquatic products, it is deduced that a statistically significant relationship manifests exclusively with the catch rates attributed to freshwater products.

- When the cases in which all variables are included in the equation at the same time in all analyzes are examined, it can be said that the p value is greater than 0.05 and all models are not statistically significant.

In order to gauge the collective relationship of these statistically significant variables, a regression analysis was

employed. The outcome of this analysis unveiled an impressive R square value of 0.819, attesting to the substantial significance of the interrelations among these variables. Drawing from the regression findings, when prognosticating future blue crab catch statistics, the predictive model indicates an ascent. Specifically, the catch rate, which stood at 1.51 tons in 2024, is anticipated to ascend to 10.51 tons by the year 2028.

Furthermore, in subsequent research endeavors, there exists a strategic intent to conduct a comparative analysis. This prospective investigation aims to juxtapose the relationship between freshwater products and the catch statistics of blue crab and other crustaceans and molluscs, utilizing datasets from other nations. By doing so, an overarching study of enhanced scope will be undertaken, thereby facilitating a comprehensive examination of blue crab catch statistics. This envisaged approach is poised to further amplify the depth and scope of the present study.

Conflict of Interest

The author declares that there are no financial interests or personal relationships that may have influenced this work.

References

Ağbaş, E., Erdem, Ü., Atasoy, E. G., Türeli, C., & Duysak, Ö. (2008). Köyceğiz lagün sisteminde bulunan mavi yengeç (*Callinectes sapidus* RATHBUN, 1896)'in bazı morfometrik özellikleri ile et kompozisyonu. *Anadolu Üniversitesi Bilim ve Teknoloji Dergisi*, *9*(1), 65-71. (In Turkish)

Atar, H. H., Ölmez, M., Bekcan, S., & Seçer, S. (2001). Mavi yengecin (*Callinectes sapidus* Rathbun 1896) et verimi ve besin madde içeriği üzerine bir araştırma. *GIDA*, *26*(3), 195-203. (In Turkish)

Bembe, S., Liang, D., & Chung, J. S. (2017). Optimal temperature and photoperiod for the spawning of blue crab, *Callinectes sapidus*, in captivity. *Aquaculture Research*, *48*, 5498-5505. https://doi.org/10.1111/are.13366

Enzerob, R., Enzerob, L., & Bingel, F. (1997). Occurence of blue crab, *Callinectes sapidus* (RATHBUN, 1896) (Crustacea, Brachyura) on the Turkish Mediterranean and the Adjacent Aegean Coast and its size Distribution in the Bay of İskenderun. *Journal of Zoology*, *21*, 113-122.

Havens, K. J., Bilkovic, D. M., Stanhope, D., Angstadt, K., & Hershner, C. (2008). The effects of derelict blue crab traps on marine organisms in the lower York River, Virginia. *North American Journal of Fisheries Management*, *28*(4), 1194-1200. https://doi.org/10.1577/M07-014.1

Jivoff, P. R., Smith, J. M., Sodi, V. L., VanMorter, S. M., Faugno, K. M., Werda, A. L., & Shaw, M. J. (2017). Population structure of adult blue crabs, *Callinectes sapidus*, in relation to physical characteristics in Barnegat Bay, New Jersey. *Estuaries and Coasts*, *40*(1), 235-250. https://doi.org/10.1007/s12237-016-0127-8

Kaya, G. K., & Yalçın, H. (2018). Mersin körfezinde avlanan mavi yengecin (*Callinectes sapidus* Rathbun, 1896) mikrobiyolojik kalitesinin araştırılması. *Türk Tarım-Gıda Bilim ve Teknoloji Dergisi*, *6*(7), 881-886. https://doi.org/10.24925/turjaf.v6i7.881-886.1858 (In Turkish)

Millikin, M. R., & Williams, A. B. (1984). *Synopsis of biological data on the blue crab, Callinectes sapidus Rathburn*. FAO Fisheries Synopsis. https://aquadocs.org/bitstream/handle/1834/20609/tr1opt.pdf?sequence=1&isAllowed=y

Nehring, S., Speckels, G., & Albersmeyer, J. (2008). The American blue crab *Callinectes sapidus* RATHBUN on the German North Sea coast: Status quo and further perspectives. *Senckenbergiana Maritima*, *38*(1), 39-44. https://doi.org/10.1007/BF03043867

Reichmuth, J. M., Roudez, R., Glover, T., & Weis, J. S. (2009). Differences in prey capture behavior in populations of blue crab (*Callinectes sapidus* Rathbun) from contaminated and clean estuaries in New Jersey. *Estuaries and Coasts*, *32*, 298-308. https://doi.org/10.1007/s12237-008-9130-z

TÜİK. (2023). *İstatistik veri portalı*. Türkiye İstatistik Kurumu. https://data.tuik.gov.tr/

Weatherall, T. F., Scheef, L. P., & Buskey, E. J. (2018). Spatial and temporal settlement patterns of blue crab (*Callinectes sapidus* and *Callinectes similis*) megalopae in a drought-prone Texas estuary. *Estuarine, Coastal and Shelf Science*, *214*, 89-97. https://doi.org/10.1016/j.ecss.2018.09.017

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

https://prensip.gen.tr

RESEARCH ARTICLE

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

Ömer Bingöl[1]✉ • Abdulhamit Battal[2] • Mehmet Emre Erez[3]

[1]Van Yüzüncü Yıl University, Faculty of Education, Department of Biology Education, Van/Türkiye
[2]Van Yüzüncü Yıl University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Van/Türkiye
[3]Van Yüzüncü Yıl University, Faculty of Science, Department of Molecular Biology and Genetics, Van/Türkiye

ARTICLE INFO

Article History
Received: 23.09.2023
Accepted: 22.10.2023
First Published: 30.12.2023

Keywords
Cadmium toxicity
Germination
Heavy metal
Seedling
Vigor index

ABSTRACT

Cadmium (Cd) is omnipresent trace element in environmental that is unessential in plants. Cd levels rise because of anthropogenic activity such as the combustion of fossil fuels, phosphate fertilizer manufacturing, mineral fertilizers, batteries technology. It is extremely toxic metal and reduces plant growth. In this context, the aim of this study was to investigate the effect of different concentrations (5/10/20/40 ppm) of Cd on germination of seeds and physiological effects in early developmental stage of tomato *Solanum lycopersicum* Lam. seedlings. 20 ppm (80%) and 40 ppm (83.3%) Cd concentrations caused significantly decrease in germination percentage. All Cd treatments were resulted with decrease in Vigor Index, especially in 20 ppm (42% decrease compared to control). Application of 5 ppm Cd caused decreases in chlorophyll and carotenoid contents in seedlings. Finally, significant decrease in protein content of 5 ppm, 10 ppm and 20 ppm treated seedlings were determined compared to control. As a conclusion, Cd negatively affected germination and physiological parameters of tomato in early developmental stage. Overall, these results indicate that Cd affects different physiologic processes and pathways according to concentration.

Please cite this paper as follows:

Bingöl, Ö., Battal, A., & Erez, M. E. (2023). The effects of cadmium concentrations on germination and physiological parameters in tomato (*Solanum lycopersicum* Lam.). *Journal of Agricultural Production*, *4*(2), 111-116. https://doi.org/10.56430/japro.1365163

1. Introduction

Some heavy metals (Cu, Zn, Fe, etc.) are micronutrients that essential in very small amounts for plant growth, but others (Cd, Co, Pb) are metals that have a toxic effect even at very low concentrations (Verbruggen et al., 2009). The average dietary cadmium (Cd) uptake by populations in low-income countries are below World Health Organization limits of concern. However, Cd intake increases in some developing countries; therefore, managing Cd transfer across the food chain is critical to limiting human exposure. Long-term exposure to Cd via air, water, soil, and food effect on nervous, and respiratory systems leads to cancer (Mahajan & Kaushal, 2018).

Cd toxicity is effective on plant growth because of its great mobility and assimilability. Cd enters plants through the roots and then transported by transporters through shoots and into vascular bundles (such as phloem and xylem) in the ionic state (Dong et al., 2019). The most obvious effect of Cd toxicity on plants is on photosynthesis. Iron (Fe^{3+}) reductase enzyme is inhibited by Cd induction and it has a major impact on the photosynthetic process and its components. Cd causes stomatal

✉Correspondence
E-mail address: omerbingol@yyu.edu.tr

closure and overall photosynthetic suppression by degradation of chlorophyll, which is essential for photosynthesis (Hasan et al., 2009).

One of the most important actions in the plant lifecycle is seed germination. Seed germination physiology is combination of different aspects as dormancy, imbibition, mineral uptake and hormone balance (Guilherme et al., 2015). Toxic amounts of Cd in plants restrict germination, affect growth and production, interfere with physiological processes in seedlings, and thereby reduce agricultural productivity (Raza et al., 2020).

Cd has been shown in recent research to limit seed germination via various mechanisms, despite the fact that some cultivars continue to germinate in the presence of high Cd concentrations. There are two proposed routes for effect of Cd on seed germination. First, Cd reduces hydrolyzing enzyme levels, starch mobilization, and seed imbibition, all of which have a negative impact on metabolic reactivation. Second, Cd can affect signaling pathway by Calcium (Ca), mitogen-activated protein kinases (MAPKs), and transcription factors (TFs). This interaction will naturally trigger the phytohormonal mechanism, especially gibberellic acid, auxin and stress hormone abscisic acid. All these interactions crucial in the seed germination process (Huybrechts et al., 2019).

Cd exposure influences not only germination but also seedling formation; nonetheless, investigations linking the relationship between Cd administration and physiological parameters during seedling formation are still scarce. In the study, germination and physiological parameters were analyzed to understand the responses of tomato seedling to different Cd concentrations.

2. Materials and Methods

2.1. Materials

All chemicals were purchased from Sigma-Aldrich, Thermo Fischer, Carlo Erba, Isolab and Duchefa.

The seeds of *Solanum lycopersicum* cultivar H2274 was used as plant material. Tomato seeds were surface sterilized with sodium hypochlorite (3%) for ten 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. Sterilized seeds are used for germination and seedling for Cd toxicity tests.

2.2. Methods

2.2.1. Determination of cadmium effect on germination and Vigor Index test

Cadmium chloride ($CdCl_2$) was used for Cd exposure. Ten uniform seeds were placed in sterile petri dishes covered by double filter paper for each application as triplicate. Hoagland's solution was used for control, different Cd concentrations (5/10/20/40 ppm) were used to determine Cd effect on germination.

Germination percentage was calculated as following formula at the end of the fifth day when germination was completed in the control group;

Germination Percentage (%) = (Germinated seeds/Sowed seeds) * 100

The vigor level of each treated seed lot was calculated according to formula (Kumar et al., 2012);

Vigor Index = Seedling length (mm) x Germination percentage (%)

2.2.2. Determination of cadmium effect on seedling growth

Surface sterilized seeds were planted in plastic pots (including 180 ml of Hoagland's medium). Seven days old, seedlings were exposed to different Cd treatments (5/10/20/40 ppm) for five days. Seedlings were harvested at the 12^{th} day. Shoot and root length were measured by a ruler. Fresh weight was weighed for shoot and root tissues. Tissues were incubated at 72 °C for 48 hours to measure dry weight. Finally, Relative Water Content (RWC) was calculated following formula (Smart & Bingham, 1974):

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

2.2.3. Determination of cadmium effect on pigments and protein content

Chlorophyll a, b and carotenoids were determined by using spectrophotometer (AE-S90-MD UV-VIS). Leaf samples (approximately 0.1 g) were homogenized using 80% acetone to measure pigment content. Chlorophyll and carotenoids contents were assessed by determining absorbance at 470, 645 and 662 nm (Arnon, 1949). Chlorophyll and carotenoids contents were calculated as mg/g fresh weight (Lichtenthaler & Wellburn, 1983).

Protein content of tomato seedlings was determined using the Bradford technique. 5 ml sodium phosphate buffer (pH 6.1), homogenized and centrifuged for 20 minutes at 2300 g. Bradford reagent was added to the supernatant, and the absorbance at 595 nm was measured. The absorbance was measured on a spectrophotometer using a standard curve produced with BSA (bovine serum albumin, concentration of 0.1 - 1%) (Bradford, 1976).

2.2.4. Statistical analysis

Data were presented as average and standard error of mean (SEM). Experiments were conducted as at least three independent replicates. GraphPad Prism 8 package program

one-way anova Fisher's LSD test was used to compare groups. P value lower than 0.05 was accepted as statistically significant.

3. Results and Discussion

3.1. Determination of Cadmium Effect on Germination

Cd exposure affected germination percentage of tomato plant. The highest germination percentage (96.7 %) was in the control group. 20 ppm (80%) and 40 ppm (83.3%) Cd concentrations caused significantly decrease in germination percentage compared to control (Figure 1A). The germination of soybean, lettuce, and sugar beet (*Beta vulgaris* L.) seedlings was reduced by 8.0%, 19.0%, and 18.0%, respectively, after exposure to 5 mg/L Cd (Li et al., 2013; Guilherme et al., 2015).

In addition, Cd treatments affected significantly Vigor Index parameters; the highest vigor index value was calculated in the control group (Figure 1B). Cd stress causes decrease in the release of starch from cotyledons due to the reduction in amylase activity (Kalai et al., 2016). The lower mobilization of storage in sorghum seeds (*Sorghum bicolor* L.) has been attributed to a restriction of hydrolyzing enzymes, specifically acid phosphatases, proteases, and –amylase so germination was affected (De Lespinay et al., 2010).

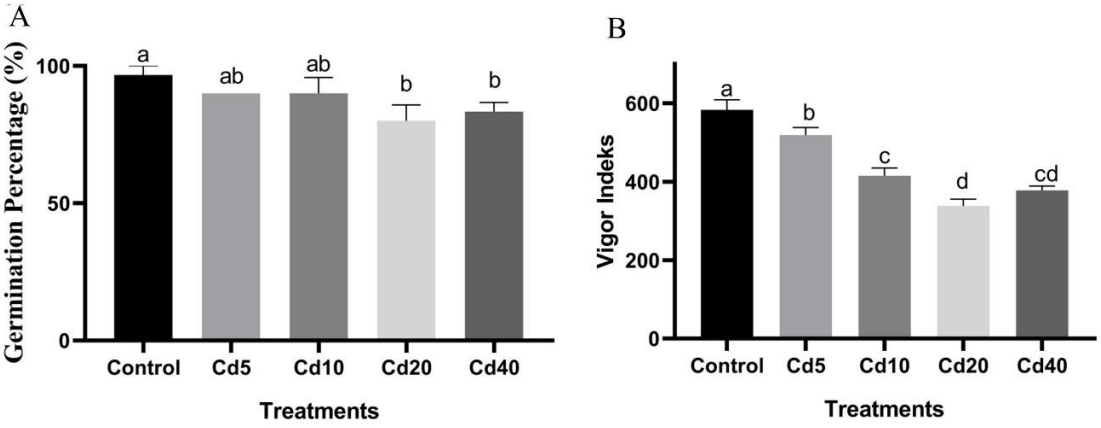

Figure 1. Germination percentage and vigor index of tomato seeds exposed to different Cd concentrations. Different lowercase letters on the columns indicate significantly difference between treatments, P<0.05.

3.2. Determination of Cadmium Effect on Seedling Growth

The highest root length was measured in 5 ppm Cd treated tomato seedlings. Probably, tomato seedlings adapted to low concentration of Cd. On the other hand, 10 ppm, 20 ppm and 40 ppm Cd treatments caused significantly decreases in root length compared to control (Figure 2A). The highest shoot length was measured in control seedlings. The lowest shoot length was measured in 40 ppm treated seedlings. Cd treatments caused significantly decreases in shoot length according to control (Figure 2B).

Our results indicated that Cd affected on seedling growth. Especially shoot lengths were more effected by increase in Cd concentrations. Root and shoot length are most sensitive end-points and considered good indicators for metal toxicity (Ahmad et al., 2011).

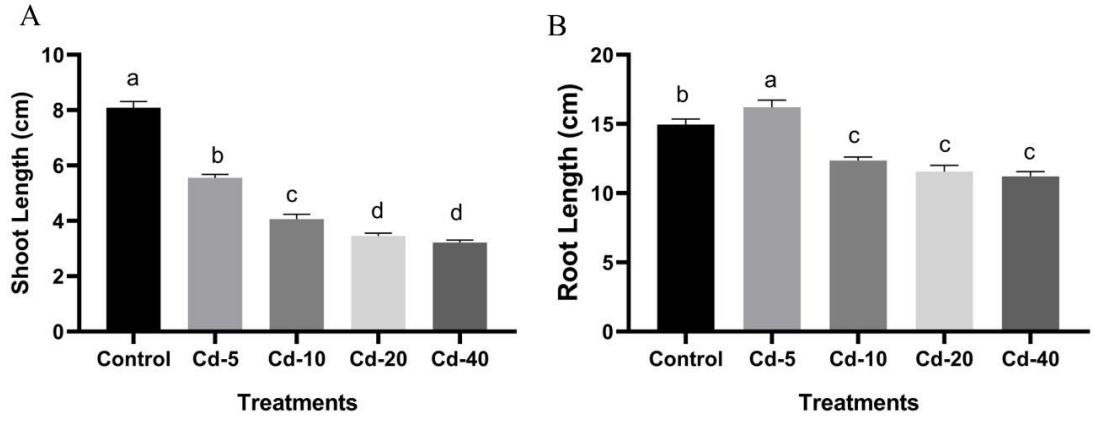

Figure 2. Root and shoot length measurements (cm) exposed to different Cd concentrations. Different lowercase letters on the columns indicate significantly difference between treatments, P<0.05.

3.3. Determination of Cadmium Effect on Pigments and Protein Content

Cd is a toxic heavy metal that can have detrimental effects on plant photosynthesis (Gallego et al., 2012). Considering the effects of chlorophyll in seedling development; 5 ppm Cd treatment caused significantly decrease in pigment content compared to control. (Figure 3A). Cd toxicity has an effect on plants by limiting carbon fixation and lowering chlorophyll content and photosynthetic activity (Gallego et al., 2012). The interaction between Cd and chlorophyll production reduces chloroplast density and causes chlorosis in oilseed crops such as rapeseed (Baryla et al., 2001).

5 ppm, 10 ppm and 20 ppm Cd treatments caused significantly decrease in carotenoid content according to control (Figure 3B). Cd mainly affects photosynthesis metabolism and its pigments, carotenoids and chlorophyll synthesis (Rafiq et al., 2014).

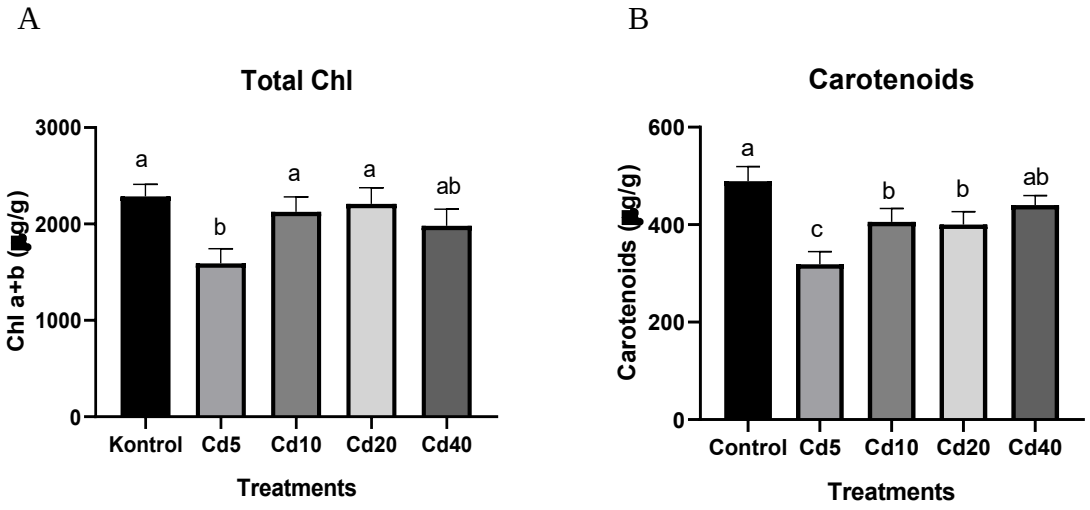

Figure 3. Chlorophyll and carotenoid contents of tomato seedlings exposed to different Cd concentrations. Different lowercase letters on the columns indicate significantly difference between treatments, P<0.05.

The highest protein content was determined in control seedlings. 5 ppm (1301 µg/g FW), 10 ppm (1070 µg/g FW) and 20 ppm (1368 µg/g FW) Cd treatments caused significantly decrease in protein content according to control (1697 µg/g FW) (Figure 4). Metal pollution disrupts plant metabolism through interactions with enzymes and biochemical events that occur within the plant (Ashraf et al., 2011).

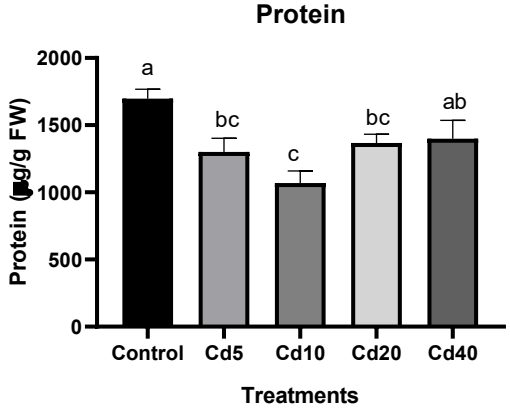

Figure 4. Protein content of tomato seedlings exposed to different Cd concentrations. Different lowercase letters on the columns indicate significantly difference between treatments, P<0.05.

Cd related DNA damage destroys cell membranes and nucleic acids, damages photosynthetic proteins, and reduces protein synthesis, all of which have an impact on organism growth (Abbas et al., 2017). Protein expression of numerous metabolic pathways was negatively affected by Cd entrance in plants. Several proteins abundance decreased associated by oxidative stress response to Cd toxicity (Haider et al., 2021).

4. Conclusion

In conclusion, Cd has reached extremely serious and alarming levels in terms of food safety all over the world, especially with the development of technology. High Cd concentration in plants, which are primary producers, delays their development and photosynthetic activity, which reduces crop productivity. In addition, Cd appears to have a serious effect on germination by acting on enzymes and on early development stage Cd effect primary carbon metabolism and oxidative stress response mechanism, caused to the appearance of chlorosis and changes in protein profile especially in youngest leaves and reduction in growth and yield. However, bioremediation technologies used to decontaminate Cd, understanding Cd accumulator plant mechanisms, and selecting resistant species may provide economically viable and environmentally options for remediating Cd-contaminated soil.

Acknowledgment

The authors are grateful to the Scientific Research Projects Coordination Unit of Van Yüzüncü Yıl University for supporting our project (FYD-2021-9409).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Abbas, T., Rizwan, M., Ali, S., Adrees, M., Zia-ur-Rehman, M., Qayyum, M. F., Ok, Y. S., & Murtaza, G. (2017). Effect of biochar on alleviation of cadmium toxicity in wheat (*Triticum aestivum* L.) grown on Cd-contaminated saline soil. *Environmental Science and Pollution Research*, *25*(26), 25668-25680. https://doi.org/10.1007/s11356-017-8987-4

Ahmad, M. S. A., Ashraf M., Tabassam, Q., Hussain, M., & Firdous, H., (2011). Lead (Pb) induced regulation in growth, photosynthesis, and mineral nutrition in maize (*Zea mays* L.) plants at early growth stages. *Biological Trace Element Research*, *144*(1-3), 1229-1239. https://doi.org/10.1007/s12011-011-9099-5

Arnon, D. I. (1949). Copper enzymes in isolatedchloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiology*, *24*(1), 1-15. https://doi.org/10.1104%2Fpp.24.1.1

Ashraf, M. A., Maah, M. J., & Yusoff, I. (2011). Heavy metals accumulation in plants growing in ex tin mining catchment. *International Journal of Environmental Science and Technology*, *8*, 401-416. https://doi.org/10.1007/BF03326227

Baryla, A., Carrier, P., Franck, F., Coulomb, C., Sahut, C., & Havaux, M. (2001). Leaf chlorosis in oilseed rape plants (*Brassica napus*) grown on cadmium-polluted soil: Causes and consequences for photosynthesis and growtth. *Planta*, *212*(5-6), 696-709. https://doi.org/10.1007/s004250000439

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248-254. https://doi.org/10.1006/abio.1976.9999

De Lespinay, A., Lequeux, H., Lambillotte, B., & Lutts, S. (2010). Protein synthesis is differentially required for germination in *Poa pratensis* and *Trifolium repens* in the absence or in the presence of cadmium. *Plant Growth Regulation*, *61*(2), 205-214. https://doi.org/10.1007/s10725-010-9471-z

Dong, Q., Fang, J., Huang, F., & Cai, K. (2019). Silicon amendment reduces soil Cd availability and Cd uptake of two pennisetum species. *International Journal of Environmental Research and Public Health*, *16*(9), 1624. https://doi.org/10.3390%2Fijerph16091624

Gallego, S. M., Pena, L. B., Barcia, R. A., Azpilicueta, C. E., Iannone, M. F., & Rosales, M. D. (2012). Unravelling cadmium toxicity and tolerance in plant: Insight into regulatory mechanism. *Environmental and Experimental Botany*, *83*, 33-46. https://doi.org/10.1016/j.envexpbot.2012.04.006

Guilherme, M. de F. S., Oliveira, H. M., & Silva, E. D. (2015). Cadmium toxicity on seed germination and seedling growth of wheat *Triticum aestivum*. *Acta Scientiarum Biological Sciences*, *37*(4), 499-511. https://doi.org/10.4025/actascibiolsci.v37i4.28148

Haider, F. U., Liqun, C., Coulter, J. A., Cheema, S. A., Wu, J., Zhang, R., Wenjun, M., & Farooq, M. (2021). Cadmium toxicity in plants: Impacts and remediation strategies. *Ecotoxicology and Environmental Safety*, *211*, 111887. https://doi.org/10.1016/j.ecoenv.2020.111887

Hasan, S. A., Fariduddin, Q., Ali, B., Hayat, S., & Ahmad, A. (2009). Cadmium: Toxicity and tolerance in plants. *Journal of Environmental Biology*, *30*(2), 165-174.

Huybrechts, M., Cuypers, A., Deckers, J., Iven, V., Vandionant, S., Jozefczak, M., & Hendrix, S. (2019). Cadmium and plant development: An agony from seed to seed. *International Journal of Molecular Sciences*, *20*(16), 3971. https://doi.org/10.3390%2Fijms20163971

Kalai, T., Bouthour, D., Manai, J., Ben-Kaab, L. B., & Gouia, H. (2016). Salicylic acid alleviates the toxicity of cadmium on seedling growth, amylases and phosphatases activity in germinating barley seeds. *Archives of Agronomy and Soil Science*, *62*(6), 892-904. https://doi.org/10.1080/03650340.2015.1100295

Kumar, B., Verma, S. K., Ram, G., & Singh, H. P. (2012). Temperature relations for seed germination potential and seedling vigor in Palmarosa (*Cymbopogon martinii*). *Journal of Crop Improvement*, *26*(6), 791-801. https://doi.org/10.1080/15427528.2012.689799

Li, Q., Lu, Y., Shi, Y., Wang, T., Ni, K., Xu, L., & Giesy, J. P. (2013). Combined effects of cadmium and fluoranthene on germination, growth and photosynthesis of soybean seedlings. *Journal of Environmental Sciences*, *25*(9), 1936-1946. https://doi.org/10.1016/S1001-0742(12)60264-2

Lichtenthaler, H., & Wellburn A. (1983). Determinations of total carotenoids, chlorophylls a, and b of leaf extracts in different solvents. *Biochemical Society Transactions*, *11*(5), 591-592. https://doi.org/10.1042/bst0110591

Mahajan, P., & Kaushal, J. (2018). Role of phytoremediation in reducing cadmium toxicity in soil and water. *Journal of Toxicology*, *2018*, 1-16. https://doi.org/10.1155/2018/4864365

Rafiq, M. T., Aziz, R., Yang, X., Xiao, W., Rafiq, M. K., Ali, B., & Li, T. (2014). Cadmium phytoavailability to rice (*Oryza sativa* L.) grown in representative Chinese soils. A model to improve soil environmental quality guidelines for food safety. *Ecotoxicology and*

Environmental Safety, *103*, 101-107. https://doi.org/10.1016/j.ecoenv.2013.10.016

Raza, A., Habib, M., Kakavand, S. N., Zahid, Z., Zahra, N., Sharif, R., & Hasanuzzaman, M. (2020). Phytoremediation of cadmium: Physiological, biochemical, and molecular mechanisms. *Biology*, *9*(7), 177-189. https://doi.org/10.3390/biology9070177

Smart, R. E., & Bingham, G. E. (1974). Rapid estimates of relative water content. *Plant Physiol*ogy, *53*(2), 258-260. https://doi.org/10.1104/pp.53.2.258

Verbruggen, N., Hermans, C., & Schat, H. (2009). Mechanisms to cope with arsenic or cadmium excess in plants. *Current Opinion in Plant Biology*, *12*(3), 364-372. https://doi.org/10.1016/j.pbi.2009.05.001

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

https://prensip.gen.tr

RESEARCH ARTICLE

Zinc Fertilizer Applications to *Ocimum basilicum* L. under Water Stress: Changes in the Total Phenolic and Flavonoid Content, Essential Oil Compounds and Morphological Properties

Funda Ulusu✉

Karamanoğlu Mehmetbey University, Vocational School of Technical Sciences, Department of Crop and Animal Production, Karaman/Türkiye

ARTICLE INFO

Article History
Received: 10.08.2023
Accepted: 27.10.2023
First Published: 30.12.2023

Keywords
Essential oil
Fertilizer
Ocimum basilicum
Water stress

ABSTRACT

Water stress poses a significant challenge for plant growth and productivity, impacting both yield and quality. With the ongoing changes in global climate, mitigating the adverse effects of water deficiency on plants has become crucial. In this study, the focus is on enhancing the tolerance of *Ocimum basilicum* L., a plant highly susceptible to water stress. To achieve this, in this study examined the effects of zinc fertilizer supplementation at varying rates (2.5 - 5 and 10 mg/kg) on *O. basilicum* grown in silty sandy soil and subjected to water stress conditions. Several parameters, including mineral uptake, morphological characteristics, total phenol and flavonoid contents, and essential oil compounds, were evaluated in sweet basil. The results revealed that water stress had a detrimental impact on the morphological properties and secondary metabolites analysed. Estragole emerged as the main compound in the essential oil analysis, with the highest concentration (69.37%) observed in the group treated with 10 mg/kg of zinc fertilizer. Conversely, the lowest concentration (66.14%) was recorded in the water-stressed group without fertilizer. Notably, the application of zinc fertilizer at concentrations of 5 and 10 mg/kg significantly ameliorated the negative effects induced by water stress. Furthermore, zinc exhibited diverse mechanisms of action concerning the uptake of other nutrients from the soil.

Please cite this paper as follows:

Ulusu, F. (2023). Zinc fertilizer applications to *Ocimum basilicum* L. under water stress: Changes in the total phenolic and flavonoid content, essential oil compounds and morphological properties. *Journal of Agricultural Production*, *4*(2), 117-127. https://doi.org/10.56430/japro.1340577

1. Introduction

Plants are exposed to abiotic stress factors such as drought, abnormal temperature changes, salinity, UV, which can cause harmful effects on plant growth many times throughout their lives. Drought, one of these stress factors, negatively affects the phenological, morphological and physiological characteristics of the plant, causing yield and quality losses (Zulkiffal et al., 2021; Pulvento et al., 2022). In this respect, water deficiency limits the growth and productivity of plants more than other stress factors. Due to the global climate change experienced in recent years, the decrease in the amount of water that can be used in agricultural lands causes a lack of nutrients in the products, resulting in serious yield losses (Weisany et al., 2021). In this context, it will be beneficial for the future of agricultural production to develop alternative methods that can increase the drought tolerance of products that are less sensitive to global climate change or to be grown.

✉Correspondence
E-mail address: fulusu@kmu.edu.tr

Ocimum basilicum L. (Sweet basil) is a member of the Lamiaceae family and is renowned for its rich secondary metabolites, including phenolic acids and terpenoids (essential oils), which have been widely studied for their medicinal and aromatic properties (Shahrajabian et al., 2020; Celikcan et al., 2021). This highly valuable plant plays a significant role in various industries such as pharmaceuticals, food, and perfumery, contributing to the economy (Rezaei-Chiyaneh et al., 2021). Sweet basil contains phytochemicals that exhibit diverse therapeutic properties, including antioxidant, anticancer, and antimicrobial effects, making it a crucial component in agricultural practices worldwide (Ahmed et al., 2019; Chu et al., 2022; Perna et al., 2022). However, the sensitivity of basil, especially when exposed to water stress in arid and semi-arid regions, negatively impacts its performance and leads to reduced essential oil production (García-Caparrós et al., 2019). This decline in essential oil output hinders the industrial utilization of the plant. Consequently, several studies have focused on developing strategies to enhance the adaptability and tolerance of basil to abiotic stress factors, resulting in the emergence of various applications and techniques (Farouk et al., 2020; Hozayn et al., 2020; Taha et al., 2020; Kahveci et al., 2021).

While various agricultural practices have successfully enhanced crop yield, improper usage of chemical fertilizers has led to significant issues, causing damage to soil physiology and biochemistry (Ahmadi & Souri, 2020; Zargar Shooshtari et al., 2020). Although plant genetics primarily determine the composition and quantity of phytochemicals, studies have shown that different cultivation techniques employed during plant development can also influence these phytochemicals through distinct mechanisms (Jeffery et al., 2003). Among the commonly employed cultivation techniques, the application of plant nutrients to the soil or directly to the plants remains a popular choice. By utilizing different fertilizers (organic, vermicompost, chemical) at various doses, these techniques have proven to positively impact the phytochemical profiles of medicinal and aromatic plants, offering support for both increased yield and improved quality (Ulusu & Şahin, 2021). It is important to carefully consider and implement appropriate fertilization strategies to maximize the benefits while minimizing any adverse effects on soil health and plant physiology.

Zinc (Zn), a micronutrient, plays a crucial role in vital bio-physicochemical processes within plants, including protein synthesis and gene regulation, among others. Additionally, zinc serves as a cofactor for antioxidant enzymes, helping to mitigate oxidative damage in plant cells during abiotic stress conditions (Marreiro et al., 2017). Moreover, zinc holds a special significance in safeguarding plants against water deficiency stress (Noman et al., 2019). A study demonstrated that the application of zinc fertilizer increased the essential oil production in *Matricaria recutita* L. plants exposed to drought stress (Jeshni et al., 2017). Similarly, in another study, it was reported that the application of zinc fertilizer to *Galanthus elwesii* Hook. significantly enhanced the content of total flavonoids, phenolic compounds, alkaloids, galanthamine, and lycorine (Ay et al., 2023). Furthermore, zinc improves the uptake efficiency of other nutrients such as nitrogen and phosphorus by plants (Shivay et al., 2015), thus, making it vital for minimizing the detrimental effects caused by nutrient deficiencies in plants. In this context, assessing the efficacy of zinc in enhancing the drought tolerance of basil plants holds significant value for agricultural production. Currently, there is limited literature available on the evaluation of various fertilizer applications for medicinal and aromatic plants, particularly in the case of *O. basilicum* (Kalamartzis et al., 2020; Celikcan et al., 2021; Kulak et al., 2021). Thus, this study aims to investigate the potential effects of different dosages of zinc fertilizer on the drought tolerance of basil plants, focusing on alterations in morphological properties and phytochemical components such as total phenolics and essential oils. By examining these aspects, the study seeks to provide valuable insights into optimizing the cultivation practices of basil plants under drought-stress conditions.

2. Materials and Methods

2.1. Plant Material

O. basilicum seeds were obtained from Genta (Simagro Agro & Seed Company) for the experiment. Prior to the planting date, the seeds were stored at 4 °C. To ensure seed sterilization, the basil seeds underwent a surface sterilization process, involving a 1 min incubation in a 1% NaClO solution. The study was conducted in a greenhouse, providing optimal conditions including temperatures ranging from 25 to 30 °C, suitable lighting, and adequate humidity levels. The experiment took place during the 2021-2022 period, following a randomized plot design with three replications to ensure reliable results. For planting, the seeds were spaced 20 cm apart in rows, and the process was carried out in March. The physicochemical properties of the soil samples utilized in the study are detailed in Table 1.

Table 1. The physicochemical properties of the experimental soil.

Physicochemical Properties	Normal Soil	Fertilized Soil
Sand (%)	52.83	51.32
Silt (%)	27.46	26.34
Clay (%)	19.21	19.25
Field capacity (%)	24.58	24.76
pH	7.35	7.13
E.C (mhos/cm)	0.37	0.36
$CaCO_3$ (%)	16.98	17.46
Organic matter (%)	4.12	4.53

Table 1. (continued)

Physicochemical Properties	Normal Soil	Fertilized Soil
N (%)	1.38	1.25
P (%)	8.52	9.67
K (%)	71.37	70.67
Mg (%)	6.26	6.31
Ca (%)	14.14	14.56
Cu (ppm)	3.31	3.31
Fe (ppm)	3.60	3.56
Mn (ppm)	16.25	16.58
Zn (ppm)	1.43	2.25

2.2. Zn Fertilizer and Water Stress Treatments to Plants

After the emergence of plants in all pots, separate applications of zinc fertilizer were administered. The fertilizer was introduced to the soil at three distinct rates (2.5 - 5 and 10 mg/kg) using a stock solution of zinc sulphate (Zn_2SO_4 - 0.22% w/v) (as outlined in Table 2). Pots without zinc fertilizer were designated as the control group for comparative purposes. The plants receiving the fertilization treatment were irrigated every two days with a Zn_2SO_4 solution for a period of 30 days prior to the initiation of water deficiency conditions. Conversely, the control group and without fertilized plants were irrigated solely with distilled water. The drought conditions were maintained for a duration of three weeks. Following the drought period, plant tissues (specifically leaves) were collected for analysis. The freshly harvested tissues were promptly frozen in liquid nitrogen and subsequently stored at -80 °C to maintain their integrity. The drought conditions implemented in this study adhered to the specifications outlined by Bettaieb et al. (2009), as detailed in Table 2. Given that previous research (Kulak et al., 2021) reported 25% of the field capacity to induce stress in basil plants, two distinct water regimes were employed: 100% well-watered (maintaining optimal water levels) and 25% severe water deficit. The control group, on the other hand, was evaluated under 100% field capacity (FC) conditions. The water content at field capacity was expressed as a percentage relative to the maximum pot capacity. To ensure reliability, all measurements were conducted in triplicates.

Table 2. Experimental design of the study.

Treatment	Abbreviation	Water Regime
No-fertilizer + well-watered plants	NF-100 (Control)	100%
No-fertilizer + severe water stressed plants	NF/WS	25%
Zn fertilizer (2.5 mg/kg) + well-watered plants	2.5 ZnF	100%
Zn fertilizer (2.5 mg/kg) + severe water stressed plants	2.5 ZnF/WS	25%
Zn fertilizer (5 mg/kg) + well-watered plants	5.0 ZnF	100%
Zn fertilizer (5 mg/kg) + severe water stressed plants	5.0 ZnF/WS	25%
Zn fertilizer (10 mg/kg) + well-watered plants	10.0 ZnF	100%
Zn fertilizer (10 mg/kg) + severe water stressed plants	10.0 ZnF/WS	25%

NF: No-fertilizer; **ZnF:** Zinc fertilizer; **WS:** Water stress.

2.3. Morphological and Phytochemical Analysis

The effects of all treatments on the morphological characteristics of the plants were evaluated. These morphological properties were plant height (cm), plant weight (g), leaves weight (g), leaves length (cm), leaves width (cm) and root length (cm). In addition, mineral content, total phenolics and flavonoids content, essential oil characterization and ratio were evaluated as phytochemical analysis. All measurements were performed with triplicates.

2.4. Mineral Content Analysis

A comprehensive quantitative elemental analysis was conducted, encompassing nine different elements. The leaves samples, comprising three samples per plant and three plants per replicate, were subjected to rigorous preparation. Prior to analysis, the leaves were carefully washed with deionized water and subsequently dried at a temperature of 70 °C for a duration of 48 hours. The specific analytical techniques employed for each element were as follows: N analysis using the Kjeldahl method, P_2O_5 analysis utilizing spectrophotometric methods, and K_2O, Mg, Fe, Zn, Cu, Mn, and Ca analysis performed through atomic absorption spectroscopy. The methodology employed in this study was based on established protocols outlined in the work of Kulak et al. (2021), ensuring reliable and consistent results.

2.5. Total Phenolics and Flavonoids Content Analysis

To extract the dried and ground leaf tissues, a 5 g sample was mixed with 50 mL of 80% methanol solution in a magnetic stirrer. The mixture was left to stir for 24 hours at room temperature. Subsequently, the solution was filtered using a sterile filter with a pore size of 0.22 µm. The filtrate was then evaporated to dryness using a rotary evaporator set at 40 °C. The resulting green residue, which yielded approximately 9.5% of the initial weight, was stored at +4 °C until further analysis.

The determination of total phenolic content (TPC) was carried out using the Folin-Ciocalteu colorimetric assay, while total flavonoid content (TFC) was assessed using the aluminium chloride assay. The methods utilized in this study were based on the procedures reported by Ulusu et al. (2017) and Ulusu and Şahin (2022). For TPC, the concentration of gallic acid (mg eq. GAE/g DW) was determined using a calibration curve ($y=0.7144x+0.0903$, $R^2=0.9934$) derived from gallic acid standards. On the other hand, TFC was determined by calculating the quercetin equivalent (mg eq. QE/g DW) using a calibration curve ($y=2.0714x-0.0003$, $R^2=0.9925$) generated from quercetin standards.

2.6. Essential Oil Extraction

To extract the essential oil from the dried and ground basil leaves, 1 g of the sample was subjected to hydro-distillation using a Clevenger type apparatus for a duration of 4 h. The solution obtained after hydro-distillation was then subjected to liquid-liquid extraction using n-hexane. The upper phase, containing the essential oil, was carefully collected and transferred to a flask. To remove any remaining water, the solution underwent evaporation, followed by drying with sodium sulphate. The resulting sample was stored at -20 °C until it could be subjected to GC-MS analysis, as described in the methodology outlined by Ulusu and Şahin (2021).

2.7. GC-MS Conditions

The essential oil analysis and identification were conducted using an Agilent 7890A Gas Chromatograph (GC). For the analysis, an HP-5MS capillary column with dimensions of 30 m × 0.25 mm × 0.25 µm was employed, with helium used as the carrier gas at a flow rate of 0.8 mL/min. The injector temperature was set to 240 °C. The oven temperature was initially set at 40 °C and then ramped up to 240 °C at a rate of 4 °C/min. The split ratio used was 1:10. In the electron pulse (EI) mode, the mass spectrometer operated at 70 eV. The scan range for mass detection spanned from 15 to 550 amu. To identify the components, present in the essential oil, reference compounds from the Wiley275 and NIST08 libraries were utilized, allowing for accurate identification based on comparison with known compounds.

2.8. Statistical Analysis

The experimental design involved triplicate replicates for each treatment group, with a total of eight plants analysed. The collected data, which included both fertilizer application and water stress variables, were subjected to statistical analysis using two-way ANOVA. Post-hoc analysis was conducted using Duncan's test. The statistical analysis was carried out using SPSS software, specifically version 24.0 by IBM Corp. located in Armonk, NY, USA. Statistical significance was set at $p<0.05$.

3. Results and Discussion

3.1. Morphological Properties

The response of *O. basilicum* to severe drought conditions in soil treated with different doses of Zn fertilizer was evaluated in terms of morphological characteristics (plant height, plant weight, leaves weight, leaves length, leaves width and root length). Water stress significantly negatively affected all morphological parameters studied in the plants. NF-WS had shorter plant height and leaves length, lighter plant weight (DW) and smaller leaves width compared to the other treatment groups (Table 3). Amending the soil with zinc fertilizer generally affected all morphological parameters positively. In addition, it has been noted that zinc fertilizer has a healing effect in terms of related parameters in plants exposed to water stress. Compared to the control group, shorter plant height and lower plant dry weight were noted at 2.5 ZnF-WS. Water stress caused significant losses in plant and leaf dry weight, while zinc applications supported the increase in plant and leaves dry weight. The dry weight gain of plant and leaves was more pronounced, especially in plants of the applications of 5.0 ZnF and 10.0 ZnF under non-stress conditions. However, in zinc applications were determined higher values in terms of plant dry weight, leaves dry weight, leaves width and root length parameters, relative to plants applied zinc under water stress conditions. In terms of all morphological parameters, 10.0 ZnF treatment showed the best effect in plants. Drought is one of the major constraints on crop yield, quality and productivity of plants. The effects of drought on plants have been reported in different studies (Jeshni et al., 2017; Avila et al., 2020; Ozturk et al., 2021). The first response observed in plants to water stress is generally seen in their phenological, physiological and morphological characteristics (Galmés et al., 2007; He et al., 2020; Ors et al., 2021). In the researches, different fertilizer treatments were carried out to compensate the losses caused by water stress and to increase the tolerance level of the plant (Javan Gholiloo et al., 2019; Ahanger et al., 2021). For instance, in a study investigating the effects of zinc fertilizer treatment on the qualitative properties and oil yield of canola plants grown in different moisture regimes, it was determined that zinc treatments improved all morphological properties of canola exposed to water stress. Especially, it was stated that it contributed to the increase in grain yield (43.82%), biological yield (73.99%) and harvest index (30.04%) (Shahsavari et al., 2014). Gholinezhad (2017), investigated the effects of iron nano fertilizer and different irrigation levels on morphological properties and essential oil percentage in *Anethum graveolens* L. and different properties investigated with the severity of drought stress decreased significantly, but nano Fe contributed towards compensating for these properties. In line with these results determined in the literature, it is extremely important to determine and understand the strengthening of the tolerance to drought stress of medicinal and aromatic plants used in the treatment of diseases with different applications (Sun et al., 2020).

Table 3. The changes in the morphological properties of the plants corresponding to treatments.

Treatments	Plant height (cm)	Plant weight DW (g)	Leaves length (cm)	Leaves weight DW (g)	Leaves width (cm)	Root length (cm)
Control	22.63±1.05^{c}	1.12±0.02^{f}	3.84±0.13^{ab}	0.38±0.03^{e}	1.42±0.15^{bc}	22.54±1.08^{c}
NF/WS	15.42±1.72^{d}	0.86±0.04^{g}	3.28±0.2^{b}	0.24±0.02^{f}	1.33±0.20^{bc}	24.71±1.14^{bc}
2.5 ZnF	21.54±1.35^{c}	1.64±0.05^{e}	3.81±0.14^{ab}	0.61±0.03^{d}	1.84±0.15^{ab}	24.32±1.36^{bc}
2.5 ZnF/WS	19.21±2.12^{c}	1.06±0.05^{f}	3.86±0.61^{ab}	0.52±0.05^{d}	1.61±0.32^{abc}	32.55±2.22^{a}
5.0 ZnF	32.34±1.02^{b}	2.87±0.12^{c}	4.01±0.52^{a}	1.57±0.04^{b}	1.97±0.35^{ab}	27.67±2.13^{ab}
5.0 ZnF/WS	29.12±3.20^{b}	2.14±0.14^{d}	3.94±0.43^{ab}	1.12±0.04^{c}	1.72±0.4^{ab}	31.41±1.11^{a}
10.0 ZnF	35.47±1.13^{a}	3.52±0.06^{a}	4.22±0.26^{a}	1.84±0.05^{a}	2.44±0.10^{a}	27.40±1.53^{b}
10.0 ZnF/WS	28.45±2.64^{b}	3.07±0.1^{b}	3.83±0.42^{ab}	1.61±0.03^{b}	2.14±0.37^{ab}	30.11±1.25^{a}

DW: Dry weight; **NF**: No-fertilizer; **ZnF**: Zn fertilizer; **WS**: Water stress. The same letters in the same column were not differed statistically (Duncan) (p<0.05).

3.2. Mineral Content

When the literature is reviewed, there is little reference to the effect of water stress on *O. basilicum* leaves mineral composition. In this respect, it is worth mentioning the changes in leaf macro and micronutrient concentrations in this study. The changes in the mineral content of plants in response to treatments are listed in the Table 4. In basil leaves, the macroelement (N, P, K, Mg, Ca) and microelement (Cu, Fe, Mn, Zn) contents determined in the control and treatment groups are in a similar range with the previous study (Kulak et al., 2021). In the Table 4, it is seen that the average phosphorus content in basil leaves varies between 0.38 and 0.62 ppm. Phosphorus content is maximum in control while it is minimum in 10.0 ZnF treatment. Zn treatment significantly affected phosphorus in the tissues and the P concentration decreased with the increase of Zn concentration. That is, a negative correlation was determined between Zn and P. This, in turn, is considered as an interference with P uptake by plants as a result of the interaction of these two nutrients (Keram et al., 2012; Samreen et al., 2017). Furthermore, it is clear that Zn treatment causes a negative effect on the Fe uptake of basil plants (Table 4). The highest dose of Zn fertilizer treatment (10.0 ZnF) to the plant reduced the Fe content by 37% compared to the control. The decrease in Fe content may be due to the antagonistic interaction between Zn and Fe in plant roots. In other words, excess Zn can cause a decrease in Fe uptake by plants. Our findings are supported by some studies in the literature (Sresty & Madhava Rao, 1999; Brunetti et al., 2011).

Table 4. Changes in mineral contents in response to treatments.

Treatments	N (%)	P (%)	K (%)	Mg (%)	Ca (%)
Control	2.36±0.02^{d}	0.62±0.01^{a}	0.80±0.01^{g}	0.52±0.01^{d}	1.64±0.04^{f}
NF-WS	1.57±0.01^{g}	0.58±0.02^{b}	0.58±0.01^{h}	0.44±0.02^{e}	1.21±0.02^{g}
2.5 ZnF	2.32±0.02^{d}	0.54±0.03^{b}	1.38±0.15^{d}	0.61±0.01^{c}	2.06±0.03^{e}
2.5 ZnF-WS	1.88±0.01^{f}	0.56±0.03^{b}	1.06±0.02^{f}	0.56±0.01^{c}	2.42±0.01^{c}
5.0 ZnF	2.42±0.01^{c}	0.48±0.01^{c}	1.45±0.02^{c}	0.58±0.02^{c}	2.57±0.02^{b}
5.0 ZnF-WS	2.04±0.15^{e}	0.46±0.01^{c}	1.32±0.01^{e}	0.50±0.03^{d}	2.22±0.02^{d}
10.0 ZnF	2.81±0.02^{a}	0.38±0.03^{d}	1.66±0.01^{a}	0.72±0.02^{a}	2.74±0.04^{a}
10.0 ZnF-WS	2.76±0.01^{b}	0.40±0.02^{d}	1.57±0.01^{b}	0.66±0.01^{b}	2.72±0.01^{a}
	Cu (ppm)	**Fe (ppm)**	**Mn (ppm)**	**Zn (ppm)**	
Control	8.44±0.11^{f}	514.50±12.11^{b}	53.45±0.41^{e}	47.31±1.17^{d}	
NF-WS	6.12±0.12^{g}	489.54±13.10^{b}	42.21±0.54^{g}	39.04±1.40^{e}	
2.5 ZnF	12.11±0.17^{d}	548.72±12.20^{a}	70.88±0.47^{c}	57.24±1.12^{c}	
2.5 ZnF-WS	10.18±0.14^{e}	515.17±11.02^{b}	63.48±0.70^{d}	57.07±1.41^{c}	
5.0 ZnF	14.24±0.26^{c}	423.59±11.11^{d}	102.57±0.30^{a}	69.14±1.33^{b}	
5.0 ZnF-WS	14.52±0.21^{c}	459.83±15.13^{c}	87.45±0.82^{b}	67.06±1.11^{b}	
10.0 ZnF	17.13±0.11^{a}	324.21±16.02^{f}	62.82±0.49^{d}	81.28±2.61^{a}	
10.0 ZnF-WS	16.54±0.20^{b}	382.16±9.15^{e}	51.93±0.52^{f}	79.05±1.60^{a}	

NF: No-fertilizer; **ZnF**: Zn fertilizer; **WS**: Water stress; **DW**: Dry weight. The same letters in the same column were not differed statistically (Duncan) (p<0.05).

It was observed that Zn fertilizer application positively supported the uptake of other nutrients (N, K, Ca, Cu, Mn) by plants. However, the effect of plants on Mg uptake was non-uniform and an increase was observed at 2.5 ZnF and 5.0 ZnF doses compared to the control, while a sharp decrease was observed at 10.0 ZnF doses. This interaction between nutrients is similarly supported by other studies (Fan et al., 2008; Rietra et al., 2017).

According to the data, basil plants have average Zn contents ranging from 39.04 to 81.28 ppm. The Zn content in plants increased significantly with the application of Zn to the soil, resulting in a maximum increase of 67.08% with 10.0 ZnF, followed by a 46.14% increase with 5.0 ZnF. Zinc uptake by plants has a parallel relationship with zinc treatments to the soil. Data supporting our results were also reported by Samreen et al. (2017). They examined the effect of zinc applied in different doses (1 and 2 μM) to the *Vigna radiata* L. plant using hydroponic culture and investigated that on growth, protein and mineral content and determined that the Zn content in the plants increased by 496.6% at the highest dose (2 μM). Nutritional supplementation to the soil facilitates the uptake of mineral elements by plants, and it is a known fact that these nutrients affect important biochemical reactions (photosynthesis, enzyme activity, protein synthesis, etc.) in plants (Prakash et al., 2020; Maitra et al., 2022). According to the findings, water stress generally prevented nutrient uptake in all applications. However, it was observed in the study that zinc can generally modulate other nutrients in plants exposed to water stress and in other treatment groups. With nutritional supplements, plants tolerate environmental stresses better, thus enabling the desired yield and quality increase in addition to growth and development in agriculture.

3.3. Total Phenolics and Flavonoids Content

TPC and TFC obtained from *O. basilicum* leaves samples under water stress and treated with Zn fertilizer are shown in Table 5. While water stress caused an increase in the total phenol content of basil plants, it caused serious decreases in the flavonoid content. However, zinc fertilizer application turned this loss caused by water stress into gain and had an effect on the increase of flavonoid content. In addition, all fertilizer applications significantly increased the total phenolic and flavonoid contents compared to the control (12.30 mg GAE/g DW, 0.53 mg QE/g DW, respectively) and the effect of fertilizer applications on these contents ranged from 14.27-29.42 mg GAE/g DW, 0.81-1.95 mg QE/g DW, respectively. By analyzing the data presented in Table 5, especially, 10.0 ZnF application increased TPC by 107% and TFC by 267% compared to control. In agreement with our results, it was stated in the literature that Zn fertilizer application contributed to the increase in total phenolic and flavonoid content (Ay et al., 2023). Again, it was stated that the application of Zn to *Chrysanthemum balsamita* L. had an effect on increasing the total phenol content (Derakhshani et al., 2011). In addition, Maity et al. (2023) reported that there is a positive correlation between these phytochemicals of nutrients in their study. In previous studies, similar to our study, different fertilizers containing nutrients increased the rate of valuable phytochemicals such as essential oil, saturated and unsaturated oil, phenolic and flavonoid contents (Siddiqui et al., 2020; Ulusu & Şahin, 2021). The main factors that differentiate the synthesis of phenolic compounds in plants are considered to be the amount of water supplied and the exposure time to drought stress (Albergaria et al., 2020). This phenomenon is supported by many studies. McKiernan et al. (2014) determined that the ratio of phenolic compounds in the leaves of 2 different Eucalyptus species (*E. globulus* Labill. and *E. viminalis* Labill.) exposed to drought decreased. In another study, phenolic compound levels of *Salvia officinalis* L. exposed to moderate water deficiency were found to be significantly higher than the control group (Bettaieb et al., 2011). In a study investigating the phenolic and flavonoid contents of 3 different Achillea plants (*A. nobilis* L., *A. millefolium* L. and *A. filipendulina* Lam.) under moderate and severe drought, a significant increase in phenolic and flavonoid contents was determined under moderate water stress (%50 of field capacity) in plants. However, under severe drought (%25 of field capacity), the levels of these compound contents differed within species (Gharibi et al., 2016). Furthermore, *Melissa officinalis* L. exposed to water stress decreased in polyphenols content, while flavonoid content did not change compared to well-watered ones (Szabó et al., 2017). Similar to our study, it was determined that the phenolic content of two basil varieties exposed to drought stress was higher than the control group (regularly irrigated) (Pirbalouti et al., 2017). When we look at the studies, water stress shows intra and inter-species significant differences in plants' secondary metabolite synthesis mechanisms.

Table 5. Total phenolic and total flavonoids content corresponding to the treatments in *O. basilicum* leaves.

Treatments	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
Control	12.30±0.30^{g}	0.53±0.02^{g}
NF-WS	13.21±0.03^{f}	0.35±0.04^{h}
2.5 ZnF	14.27±0.22^{e}	0.81±0.03^{e}
2.5 ZnF-WS	16.33±0.10^{d}	0.72±0.01^{f}
5.0 ZnF	16.47±0.15^{d}	1.57±0.01^{c}
5.0 ZnF-WS	19.86±0.10^{c}	1.14±0.02^{d}
10.0 ZnF	25.50±0.41^{b}	1.95±0.01^{a}
10.0 ZnF-WS	29.42±0.18^{a}	1.83±0.01^{b}

NF: No-fertilizer; **ZnF**: Zn fertilizer; **WS**: Water stress; **DW**: Dry weight; **GAE**: Gallic acid equivalents; **QE**: Quercetin equivalents. The same letters in the same column were not differed statistically (Duncan) (p<0.05).

3.4. Essential Oil Components

The essential oil components identified in *O. basilicum* leaves are shown in the Table 6. The main compound was estragole (phenylpropene) in the range of 66.14-69.37% which was followed by limonene (monoterpen) in the range of 11.38-14.32%. In *O. basilicum*, the interaction of water stress, zinc fertilizer and treatments significantly affected the content of essential oil components. According to the data, all ZnF treatments significantly increased the estragole content. Similarly, the ZnF-WS interaction contributed to an increase in the content of essential oil compound compared to NF-WS. NF-WS caused a drastic decrease in the percentage of all components. Regarding estragole and limonene, 10.0 ZnF treatment significantly affected the increase in the percentage of these two main compounds. Also, compared to control, all zinc fertilizer treatments and zinc fertilizer - water stress interaction treatments contributed to increases of p-cymene, fenchone, methyle eugenol, germacrene D compounds. In *O. basilicum* leaves, the reduction in essential oil content under drought conditions can be associated with a decrease in dry weight.

Water stress is a critical factor that significantly impacts the content of essential oils, particularly in aromatic plants. When plants experience water stress, there is a noticeable decline in the synthesis of phytochemicals (García-Caparrós et al., 2019). The extent and duration of drought conditions, along with the morphological and physiological state of the plant, as well as the plant species and varieties, can influence the essential oil content. Similar to our study, reductions in essential oil content were observed in *Mentha arvensis* L. (Misra & Srivastava, 2000) and *Salvia officinalis* L. (Govahi et al., 2015), both belonging to the Lamiaceae family, when exposed to water stress. However, it is worth noting that literature reports indicate that drought conditions can also lead to an increase in essential oil content in certain Lamiaceae species. For example, in *Thymus caramanicus* Jalas. (Lamiaceae), water stress (at 20% of field capacity) resulted in an 11.9% rise in carvacrol, the primary component of its essential oil (Bahreininejad et al., 2014). Similar findings have been observed in *Salvia officinalis* L. (Bettaieb et al., 2009) and *Satureja hortensis* L. (Baher et al., 2002), both of which belong to the Lamiaceae family. Furthermore, in other studies investigating the effect of Zn fertilizer application on essential oil yield, a significant increase in essential oil content was reported in parallel with fertilizer treatment in *Mentha piperita* L. (Akhtar et al., 2009), *Origanum majorana* L. (Farsi et al., 2017) and *Moringa peregrina* Forssk. (Soliman et al., 2015) plants. In a study evaluating the effect of Zn fertilizer on the *O. basilicum* essential oil content, it was stated that fertilizer application contributed to the production of essential oil, and the results are similar to our findings (Hanif et al., 2017).

In line with our findings, the effects of Zn fertilizer treatment on vegetative growth parameters and essential oil yield of *O. basilicum* plants are similar.

Table 6. Alterations in essential oil components (%) of *O. basilicum* leaves corresponding to the treatments.

Treatments	p-Cymene	Limonene	Fenchone	Estragole	Exo-fenchyle acetate
RT (min)	13.76	14.57	17.81	20.19	20.81
Control	2.32±0.22^{f}	12.63±0.55^{b}	3.02±0.13^{c}	67.12±1.13^{ab}	8.13±0.40^{ab}
NF-WS	1.87±0.11^{g}	11.38±0.42^{c}	2.78±0.14^{d}	66.14±1.12^{b}	7.24±0.20^{b}
2.5 ZnF	2.34±0.02^{f}	13.54±0.53^{ab}	3.18±0.15^{bc}	68.81±0.80^{ab}	8.66±0.30^{a}
2.5 ZnF-WS	2.98±0.03^{d}	13.11±0.30^{b}	3.06±0.21^{c}	67.86±0.11^{b}	8.12±0.40^{ab}
5.0 ZnF	3.12±0.02^{c}	12.18±0.10^{b}	3.45±0.20^{bc}	69.23±0.57^{a}	8.47±0.32^{a}
5.0 ZnF-WS	2.67±0.17^{e}	12.86±0.44^{b}	3.14±0.15^{bc}	68.70±0.52^{a}	8.82±0.53^{a}
10.0 ZnF	3.31±0.12^{b}	14.32±0.35^{a}	3.66±0.10^{b}	69.37±0.18^{a}	7.54±0.47^{b}
10.0 ZnF-WS	3.56±0.01^{a}	14.18±0.60^{ab}	3.98±0.10^{a}	68.56±0.34^{a}	8.37±0.21^{a}

	Carvacrole	Methyle eugenol	Germacrene D	Total (%)
RT (min)	22.54	25.36	27. 41	
Control	0.54±0.01^{b}	0.14±0.01^{d}	0.43±0.01^{d}	94.33
NF-WS	0.42±0.01^{c}	-	0.21±0.02^{e}	90.04
2.5 ZnF	0.64±0.02^{a}	0.18±0.03^{c}	0.56±0.01^{b}	97.91
2.5 ZnF-WS	0.31±0.01^{d}	0.15±0.02^{cd}	0.48±0.02^{c}	96.07
5.0 ZnF	0.67±0.03^{a}	0.23±0.03^{a}	0.59±0.03^{ab}	97.94
5.0 ZnF-WS	0.52±0.02^{b}	0.20±0.01^{b}	0.55±0.02^{b}	97.46
10.0 ZnF	0.63±0.01^{a}	0.24±0.02^{a}	0.62±0.01^{a}	99.69
10.0 ZnF-WS	0.54±0.01^{b}	0.21±0.01^{b}	0.35±0.04^{f}	99.75

NF: No-fertilizer; **ZnF**: Zn fertilizer; **WS**: Water stress; **DW**: Dry weight; **RT**: Retention time. The same letters in the same column were not differed statistically (Duncan) (p<0.05).

4. Conclusion

In summary, the results of this study indicate that water stress caused serious effects on morphological properties, total phenolics and flavonoids content and essential oil components in *O. basilicum*. In addition, it is seen that Zn application under water stress conditions can reduce the damage caused by water stress. Furthermore, that optimum amount the application of Zn that optimum amount to *O. basilicum* under normal conditions positively affects all the investigated criteria, suggesting that this may be due to the critical roles of Zn in plant nutrition and influencing other nutrients. In general, the results showed that the application of 10 mg/kg Zn fertilizer to *O. basilicum* plant under normal conditions (irrigation based on 100% of field capacity) can both improve the morphological characteristics of the plant and synthesize phytochemicals at an optimum level. Studies involving different experimental groups in *O. basilicum* and evaluating them in terms of their biological activities should be considered.

Conflict of Interest

The author has no conflict of interest to declare.

References

Ahanger, M. A., Qi, M., Huang, Z., Xu, X., Begum, N., Qin, C., Zhang, C., Ahmad, N., Mustafa, N. S., & Ashraf, M. (2021). Improving growth and photosynthetic performance of drought stressed tomato by application of nano-organic fertilizer involves up-regulation of nitrogen, antioxidant and osmolyte metabolism. *Ecotoxicology and Environmental Safety*, *216*, 112195. https://doi.org/10.1016/j.ecoenv.2021.112195

Ahmadi, M., & Souri, M. K. (2020). Growth characteristics and fruit quality of chili pepper under higher electrical conductivity of nutrient solution induced by various salts. *AGRIVITA, Journal of Agricultural Science*, *42*(1), 143-152. http://doi.org/10.17503/agrivita.v42i1.2225

Ahmed, A. F., Attia, F. A., Liu, Z., Li, C., Wei, J., & Kang, W. (2019). Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (*Ocimum basilicum* L.) plants. *Food Science and Human Wellness*, *8*(3), 299-305. https://doi.org/10.1016/j.fshw.2019.07.004

Akhtar, N., Sarker, M. A. M., Akhter, H., & Nada, M. K. (2009). Effect of planting time and micronutrient as zinc chloride on the growth, yield and oil content of *Mentha piperita*. *Bangladesh Journal of Scientific and Industrial Research*, *44*(1), 125-130. https://doi.org/10.3329/bjsir.v44i1.2721

Albergaria, E. T., Oliveira, A. F. M., & Albuquerque, U. P. (2020). The effect of water deficit stress on the composition of phenolic compounds in medicinal plants. *South African Journal of Botany*, *131*, 12-17. https://doi.org/10.1016/j.sajb.2020.02.002

Avila, R. T., de Almeida, W. L., Costa, L. C., Machado, K. L., Barbosa, M. L., de Souza, R. P., Martino, P. B., Juárez, M. A., Marçal, D. M., & Martins, S. C. (2020). Elevated air [CO2] improves photosynthetic performance and alters biomass accumulation and partitioning in drought-stressed coffee plants. *Environmental and Experimental Botany*, *177*, 104137. https://doi.org/10.1016/j.envexpbot.2020.104137

Ay, E. B., Açıkgöz, M. A., Kocaman, B., Mesci, S., Kocaman, B., & Yıldırım, T. (2023). Zinc and phosphorus fertilization in *Galanthus elwesii* Hook: Changes in the total alkaloid, flavonoid, and phenolic content, and evaluation of anti-cancer, anti-microbial, and antioxidant activities. *Scientia Horticulturae*, *317*, 112034. https://doi.org/10.1016/j.scienta.2023.112034

Baher, Z. F., Mirza, M., Ghorbanli, M., & Bagher Rezaii, M. (2002). The influence of water stress on plant height, herbal and essential oil yield and composition in *Satureja hortensis* L. *Flavour and Fragrance Journal*, *17*(4), 275-277. https://doi.org/10.1002/ffj.1097

Bahreininejad, B., Razmjoo, J., & Mirza, M. (2014). Effect of water stress on productivity and essential oil content and composition of *Thymus carmanicus*. *Journal of Essential Oil Bearing Plants*, *17*(5), 717-725. https://doi.org/10.1080/0972060X.2014.901605

Bettaieb, I., Hamrouni-Sellami, I., Bourgou, S., Limam, F., & Marzouk, B. (2011). Drought effects on polyphenol composition and antioxidant activities in aerial parts of *Salvia officinalis* L. *Acta Physiologiae Plantarum*, *33*, 1103-1111. https://doi.org/10.1007/s11738-010-0638-z

Bettaieb, I., Zakhama, N., Wannes, W. A., Kchouk, M., & Marzouk, B. (2009). Water deficit effects on *Salvia officinalis* fatty acids and essential oils composition. *Scientia Horticulturae*, *120*(2), 271-275. https://doi.org/10.1016/j.scienta.2008.10.016

Brunetti, G., Farrag, K., Rovira, P. S., Nigro, F., & Senesi, N. (2011). Greenhouse and field studies on Cr, Cu, Pb and Zn phytoextraction by *Brassica napus* from contaminated soils in the Apulia region, Southern Italy. *Geoderma*, *160*(3-4), 517-523. https://doi.org/10.1016/j.geoderma.2010.10.023

Celikcan, F., Kocak, M. Z., & Kulak, M. (2021). Vermicompost applications on growth, nutrition uptake and secondary metabolites of *Ocimum basilicum* L. under water stress: A comprehensive analysis. *Industrial Crops and Products*, *171*, 113973. https://doi.org/10.1016/j.indcrop.2021.113973

Chu, H. T. T., Vu, T. N., Dinh, T. T. T., Do, P. T., Chu, H. H., Tien, T. Q., Tong, Q. C., Nguyen, M. H., Ha, Q. T., & Setzer, W. N. (2022). Effects of supplemental light spectra on the composition, production and antimicrobial activity of *Ocimum basilicum* L. essential oil. *Molecules*, *27*(17), 5599. https://doi.org/10.3390/molecules27175599

Derakhshani, Z., Hassani, A., Sadaghiani, M. H. R., Hassanpouraghdam, M. B., Khalifani, B. H., & Dalkani, M. (2011). Effect of zinc application on growth and some biochemical characteristics of costmary

(*Chrysanthemum balsamita* L.). *Communications in Soil Science and Plant Analysis*, *42*(20), 2493-2503. https://doi.org/10.1080/00103624.2011.609257

Fan, M.-S., Zhao, F.-J., Fairweather-Tait, S. J., Poulton, P. R., Dunham, S. J., & McGrath, S. P. (2008). Evidence of decreasing mineral density in wheat grain over the last 160 years. *Journal of Trace Elements in Medicine and Biology*, *22*(4), 315-324. https://doi.org/10.1016/j.jtemb.2008.07.002

Farouk, S., Elhindi, K. M., & Alotaibi, M. A. (2020). Silicon supplementation mitigates salinity stress on *Ocimum basilicum* L. via improving water balance, ion homeostasis, and antioxidant defense system. *Ecotoxicology and Environmental Safety*, *206*, 111396. https://doi.org/10.1016/j.ecoenv.2020.111396

Farsi, M., Abdollahi, F., Salehi, A., & Ghasemi, S. (2017). Study of physiological characteristics of marjoram (*Origanum majorana*), as a medicinal plant in response to zinc levels under drought stress conditions. *Environmental Stresses in Crop Sciences*, *10*(4), 559-570. https://doi.org/10.22077/escs.2017.68.1017

Galmés, J., Flexas, J., Savé, R., & Medrano, H. (2007). Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: Responses to water stress and recovery. *Plant and Soil*, *290*, 139-155. https://doi.org/10.1007/s11104-006-9148-6

García-Caparrós, P., Romero, M. J., Llanderal, A., Cermeño, P., Lao, M. T., & Segura, M. L. (2019). Effects of drought stress on biomass, essential oil content, nutritional parameters, and costs of production in six Lamiaceae species. *Water*, *11*(3), 573. https://doi.org/10.3390/w11030573

Gharibi, S., Tabatabaei, B. E. S., Saeidi, G., & Goli, S. A. H. (2016). Effect of drought stress on total phenolic, lipid peroxidation, and antioxidant activity of Achillea species. *Applied Biochemistry and Biotechnology*, *178*, 796-809. https://doi.org/10.1007/s12010-015-1909-3

Gholinezhad, E. (2017). Effect of drought stress and Fe nano-fertilizer on seed yield, morphological traits, essential oil percentage and yield of dill (*Anethum graveolens* L.). *Journal of Essential Oil Bearing Plants*, *20*(4), 1006-1017. https://doi.org/10.1080/0972060X.2017.1362999

Govahi, M., Ghalavand, A., Nadjafi, F., & Sorooshzadeh, A. (2015). Comparing different soil fertility systems in Sage (*Salvia officinalis*) under water deficiency. *Industrial Crops and Products*, *74*, 20-27. https://doi.org/10.1016/j.indcrop.2015.04.053

Hanif, M. A., Nawaz, H., Ayub, M. A., Tabassum, N., Kanwal, N., Rashid, N., Saleem, M., & Ahmad, M. (2017). Evaluation of the effects of Zinc on the chemical composition and biological activity of basil essential oil by using Raman spectroscopy. *Industrial Crops and Products*, *96*, 91-101. https://doi.org/10.1016/j.indcrop.2016.10.058

He, X., Xu, L., Pan, C., Gong, C., Wang, Y., Liu, X., & Yu, Y. (2020). Drought resistance of *Camellia oleifera* under drought stress: Changes in physiology and growth characteristics. *PLoS One*, *15*(7), e0235795. https://doi.org/10.1371/journal.pone.0235795

Hozayn, M., Ali, H., Marwa, M., & El-Shafie, A. (2020). Influence of magnetic water on French basil (*Ocimum basilicum* L. var. Grandvert) plant grown under water stress conditions. *Plant Archives*, *20*(1), 3636-3648.

Javan Gholiloo, M., Yarnia, M., Ghorttapeh, A. H., Farahvash, F., & Daneshian, A. M. (2019). Evaluating effects of drought stress and bio-fertilizer on quantitative and qualitative traits of valerian (*Valeriana officinalis* L.). *Journal of Plant Nutrition*, *42*(13), 1417-1429. https://doi.org/10.1080/01904167.2019.1628972

Jeffery, E. H., Brown, A. F., Kurilich, A. C., Keck, A. S., Matusheski, N., Klein, B. P., & Juvik, J. A. (2003). Variation in content of bioactive components in broccoli. *Journal of Food Composition and Analysis*, *16*(3), 323-330. https://doi.org/10.1016/S0889-1575(03)00045-0

Jeshni, M. G., Mousavinik, M., Khammari, I., & Rahimi, M. (2017). The changes of yield and essential oil components of German Chamomile (*Matricaria recutita* L.) under application of phosphorus and zinc fertilizers and drought stress conditions. *Journal of the Saudi Society of Agricultural Sciences*, *16*(1), 60-65. https://doi.org/10.1016/j.jssas.2015.02.003

Kahveci, H., Bilginer, N., Diraz-Yildirim, E., Kulak, M., Yazar, E., Kocacinar, F., & Karaman, S. (2021). Priming with salicylic acid, β-carotene and tryptophan modulates growth, phenolics and essential oil components of *Ocimum basilicum* L. grown under salinity. *Scientia Horticulturae*, *281*, 109964. https://doi.org/10.1016/j.scienta.2021.109964

Kalamartzis, I., Dordas, C., Georgiou, P., & Menexes, G. (2020). The use of appropriate cultivar of basil (*Ocimum basilicum*) can increase water use efficiency under water stress. *Agronomy*, *10*(1), 70. https://doi.org/10.3390/agronomy10010070

Keram, K. S., Sharma, B., & Sawarkar, S. (2012). Impact of Zn application on yield, quality, nutrients uptake and soil fertility in a medium deep black soil (vertisol). *International Journal of Science, Environment and Technology*, *1*(5), 563-571.

Kulak, M., Jorrín-Novo, J. V., Romero-Rodriguez, M. C., Yildirim, E. D., Gul, F., & Karaman, S. (2021). Seed priming with salicylic acid on plant growth and essential oil composition in basil (*Ocimum basilicum* L.) plants grown under water stress conditions. *Industrial Crops and Products*, *161*, 113235. https://doi.org/10.1016/j.indcrop.2020.113235

Maitra, S., Brestic, M., Bhadra, P., Shankar, T., Praharaj, S., Palai, J. B., Shah, M., Barek, V., Ondrisik, P., & Skalický, M. (2022). Bioinoculants-Natural biological resources for

sustainable plant production. *Microorganisms*, *10*(1), 51. https://doi.org/10.3390/microorganisms10010051

Maity, M., Majumdar, S., Bhattacharyya, D. K., Bhowal, J., Das, A., & Barui, A. (2023). Evaluation of prebiotic properties of galactooligosaccharides produced by transgalactosylation using partially purified β-galactosidase from *Enterobacter aerogenes* KCTC2190. *Applied Biochemistry and Biotechnology*, *195*(4), 2294-2316. https://doi.org/10.1007/s12010-022-04073-6

Marreiro, D. D. N., Cruz, K. J. C., Morais, J. B. S., Beserra, J. B., Severo, J. S., & De Oliveira, A. R. S. (2017). Zinc and oxidative stress: Current mechanisms. *Antioxidants*, *6*(2), 24. https://doi.org/10.3390/antiox6020024

McKiernan, A. B., Hovenden, M. J., Brodribb, T. J., Potts, B. M., Davies, N. W., & O'Reilly-Wapstra, J. M. (2014). Effect of limited water availability on foliar plant secondary metabolites of two Eucalyptus species. *Environmental and Experimental Botany*, *105*, 55-64. https://doi.org/10.1016/j.envexpbot.2014.04.008

Misra, A., & Srivastava, N. (2000). Influence of water stress on Japanese mint. *Journal of Herbs, Spices & Medicinal Plants*, *7*(1), 51-58. https://doi.org/10.1300/J044v07n01_07

Noman, A., Aqeel, M., Khalid, N., Islam, W., Sanaullah, T., Anwar, M., Khan, S., Ye, W., & Lou, Y. (2019). Zinc finger protein transcription factors: Integrated line of action for plant antimicrobial activity. *Microbial Pathogenesis*, *132*, 141-149. https://doi.org/10.1016/j.micpath.2019.04.042

Ors, S., Ekinci, M., Yildirim, E., Sahin, U., Turan, M., & Dursun, A. (2021). Interactive effects of salinity and drought stress on photosynthetic characteristics and physiology of tomato (*Lycopersicon esculentum* L.) seedlings. *South African Journal of Botany*, *137*, 335-339. https://doi.org/10.1016/j.sajb.2020.10.031

Ozturk, M., Turkyilmaz Unal, B., García-Caparrós, P., Khursheed, A., Gul, A., & Hasanuzzaman, M. (2021). Osmoregulation and its actions during the drought stress in plants. *Physiologia Plantarum*, *172*(2), 1321-1335. https://doi.org/10.1111/ppl.13297

Perna, S., Alawadhi, H., Riva, A., Allegrini, P., Petrangolini, G., Gasparri, C., Alalwan, T. A., & Rondanelli, M. (2022). In vitro and in vivo anticancer activity of basil (*Ocimum* spp.): Current insights and future prospects. *Cancers*, *14*(10), 2375. https://doi.org/10.3390/cancers14102375

Pirbalouti, A. G., Malekpoor, F., Salimi, A., Golparvar, A., & Hamedi, B. (2017). Effects of foliar of the application chitosan and reduced irrigation on essential oil yield, total phenol content and antioxidant activity of extracts from green and purple basil. *Acta Scientiarum Polonorum Hortorum Cultus*, *16*(6), 177-186. https://doi.org/10.24326/asphc.2017.6.16

Prakash, N. B., Dhumgond, P., Shruthi, & Ashrit, S. (2020). Slag-based gypsum as a source of sulphur, calcium and silicon and its effect on soil fertility and yield and quality of groundnut in Southern India. *Journal of Soil Science and Plant Nutrition*, *20*(4), 2698-2713. https://doi.org/10.1007/s42729-020-00335-6

Pulvento, C., Sellami, M. H., & Lavini, A. (2022). Yield and quality of *Amaranthus hypochondriacus* grain amaranth under drought and salinity at various phenological stages in southern Italy. *Journal of the Science of Food and Agriculture*, *102*(12), 5022-5033. https://doi.org/10.1002/jsfa.11088

Rezaei-Chiyaneh, E., Amani Machiani, M., Javanmard, A., Mahdavikia, H., Maggi, F., & Morshedloo, M. R. (2021). Vermicompost application in different intercropping patterns improves the mineral nutrient uptake and essential oil compositions of sweet basil (*Ocimum basilicum* L.). *Journal of Soil Science and Plant Nutrition*, *21*, 450-466. https://doi.org/10.1007/s42729-020-00373-0

Rietra, R. P. J. J., Heinen, M., Dimkpa, C. O., & Bindraban, P. S. (2017). Effects of nutrient antagonism and synergism on yield and fertilizer use efficiency. *Communications in Soil Science and Plant Analysis*, *48*(16), 1895-1920. https://doi.org/10.1080/00103624.2017.1407429

Samreen, T., Humaira, Shah, H. U., Ullah, S., & Javid, M. (2017). Zinc effect on growth rate, chlorophyll, protein and mineral contents of hydroponically grown mungbeans plant (*Vigna radiata*). *Arabian Journal of Chemistry*, *10*, S1802-S1807. https://doi.org/10.1016/j.arabjc.2013.07.005

Shahrajabian, M. H., Sun, W., & Cheng, Q. (2020). Chemical components and pharmacological benefits of Basil (*Ocimum basilicum*): A review. *International Journal of Food Properties*, *23*(1), 1961-1970. https://doi.org/10.1080/10942912.2020.1828456

Shahsavari, N., Jais, H. M., & Shirani Rad, A. H. (2014). Responses of canola morphological and agronomic characteristics to zeolite and zinc fertilization under drought stress. *Communications in Soil Science and Plant Analysis*, *45*(13), 1813-1822. https://doi.org/10.1080/00103624.2013.875207

Shivay, Y. S., Prasad, R., Singh, R. K., & Pal, M. (2015). Relative efficiency of zinc-coated urea and soil and foliar application of zinc sulphate on yield, nitrogen, phosphorus, potassium, zinc and iron biofortification in grains and uptake by basmati rice (*Oryza sativa* L.). *Journal of Agricultural Science*, *7*(2), 161-173. https://doi.org/10.5539/jas.v7n2p161

Siddiqui, Y., Munusamy, U., Naidu, Y., & Ahmad, K. (2020). Integrated effect of plant growth-promoting compost and NPK fertilizer on nutrient uptake, phenolic content, and antioxidant properties of *Orthosiphon stamineus* and *Cosmos caudatus*. *Horticulture, Environment, and Biotechnology*, *61*, 1051-1062. https://doi.org/10.1007/s13580-020-00277-z

Soliman, A. S., El-feky, S. A., & Darwish, E. (2015). Alleviation of salt stress on Moringa peregrina using foliar application of nanofertilizers. *Journal of*

Horticulture and Forestry, *7*(2), 36-47. https://doi.org/10.5897/JHF2014.0379

Sresty, T. V. S., & Madhava Rao, K. V. (1999). Ultrastructural alterations in response to zinc and nickel stress in the root cells of pigeonpea. *Environmental and Experimental Botany*, *41*(1), 3-13. https://doi.org/10.1016/S0098-8472(98)00034-3

Sun, Y., Wang, C., Chen, H. Y., & Ruan, H. (2020). Response of plants to water stress: A meta-analysis. *Frontiers in Plant Science*, *11*, 978. https://doi.org/10.3389/fpls.2020.00978

Szabó, K., Radácsi, P., Rajhárt, P., Ladányi, M., & Németh, É. (2017). Stress-induced changes of growth, yield and bioactive compounds in lemon balm cultivars. *Plant Physiology and Biochemistry*, *119*, 170-177. https://doi.org/10.1016/j.plaphy.2017.07.019

Taha, R. S., Alharby, H. F., Bamagoos, A. A., Medani, R. A., & Rady, M. M. (2020). Elevating tolerance of drought stress in *Ocimum basilicum* using pollen grains extract; a natural biostimulant by regulation of plant performance and antioxidant defense system. *South African Journal of Botany*, *128*, 42-53. https://doi.org/10.1016/j.sajb.2019.09.014

Ulusu, F., & Şahin, A. (2021). Investigation on the effects of different concen-trations of some fertilizers on yield, quality and essential and fixed oil composition of Nigella. *Romanian Biotechnological Letters*, *26*(3), 2722-2735. https://doi.org/10.25083/rbl/26.3/2722-2735

Ulusu, F., & Şahin, A. (2022). Changes in cytotoxic capacity, phenolic profile, total phenols and flavonoids of *Nigella damascena* L. seed extracts under different liquid fertilization. *South African Journal of Botany*, *150*, 500-510. https://doi.org/10.1016/j.sajb.2022.08.010

Ulusu, Y., Öztürk, L., & Elmastaş, M. (2017). Antioxidant capacity and cadmium accumulation in parsley seedlings exposed to cadmium stress. *Russian Journal of Plant Physiology*, *64*, 883-888. https://doi.org/10.1134/S1021443717060139

Weisany, W., Mohammadi, M., Tahir, N. A.-r., Aslanian, N., & Omer, D. A. (2021). Changes in growth and nutrient status of maize (*Zea mays* L.) in response to two zinc sources under drought stress. *Journal of Soil Science and Plant Nutrition*, *21*, 3367-3377. https://doi.org/10.1007/s42729-021-00612-y

Zargar Shooshtari, F., Souri, M. K., Hasandokht, M. R., & Jari, S. K. (2020). Glycine mitigates fertilizer requirements of agricultural crops: case study with cucumber as a high fertilizer demanding crop. *Chemical and Biological Technologies in Agriculture*, *7*, 1-10. https://doi.org/10.1186/s40538-020-00185-5

Zulkiffal, M., Ahsan, A., Ahmed, J., Musa, M., Kanwal, A., Saleem, M., Anwar, J., ur Rehman, A., Ajmal, S., & Gulnaz, S. (2021). Heat and drought stresses in wheat (*Triticum aestivum* L.): Substantial yield losses, practical achievements, improvement approaches, and adaptive. *Plant Stress Physiology*, *3*. https://doi.org/10.5772/intechopen.92378

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

https://prensip.gen.tr

RESEARCH ARTICLE

Effects of PGPR Bacteria Applications on Soil Properties, Plant Growth and Yield Values in Karaerik and Narince Grape Varieties

Muhammed Kupe[1] • Fazil Hacimuftuoglu[2]✉ • Elif Yağanoğlu[2]

[1]Atatürk University, Faculty of Agriculture, Department of Horticulture, Erzurum/Türkiye
[2]Atatürk University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Erzurum/Türkiye

ARTICLE INFO

Article History
Received: 06.10.2023
Accepted: 06.11.2023
First Published: 30.12.2023

Keywords
Aggregate stability
PGPR
Soil physics
Vine
Yield

ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that promote plant growth by adhering to the root surfaces in the rhizosphere region of plants. In addition to improving the physical properties of soils, these bacteria increase plant growth and yield by positively affecting nitrogen fixation, phosphorus solubility, water and nutrient uptake of plants. In this study, the effects of bacteria applications on the vegetative development and yield levels of Karaerik and Narince grape varieties, which are important table varieties of Erzincan and Tokat regions, grown in greenhouses in Erzurum central conditions were investigated. In the study, 4 different bacterial combinations (*Pseudomonas chlororaphis* + *Paenibacillus pabuli* + *Bacillus simplex* + *Pseudomonas fluorescens*) that promote plant growth were applied to the plant root zone as a solution. In the study, the effects of PGPR applications on the vegetative growth of vines, some pomological characteristics, yield levels, macronutrient contents of leaves and physical and chemical properties of greenhouse soils were determined. While aggregate stability and porosity values of PGPR treated soils increased, water permeability and bulk density values decreased. Bacterial applications in both grape varieties showed a positive effect on shoot length, shoot diameter, number of nodes, berry width, berry length, cluster width, cluster length, number of seeds, number of clusters, cluster weight, number of berries, berry weight, total yield and macronutrient content of leaves. According to the control group, PGPR applied soils; organic matter content increased by 76.2%, aggregate stability values increased by 49.5% and porosity by 5.5%, while water permeability decreased by 18.3% and bulk density by 3.9%. Depending on the application, it was determined that the yield increased by 42.8% in Karaerik grape variety and 35.7% in Narince grape variety.

Please cite this paper as follows:

Kupe, M., Hacimuftuoglu, F., & Yağanoğlu, E. (2023). Effects of PGPR bacteria applications on soil properties, plant growth and yield values in Karaerik and Narince grape varieties. *Journal of Agricultural Production*, *4*(2), 128-137. https://doi.org/10.56430/japro.1372396

1. Introduction

Soil is an environment that contains various microorganisms and provides habitat for many plants and animals. In recent years, in order to make agricultural production more efficient and more sustainable, biostimulators applied directly to plants or to the rhizosphere are defined as substances or microorganisms that increase nutrient uptake and product quality, reduce the need for fertilizer, and promote plant growth. Microbial biostimulants include mycorrhizal and non-mycorrhizal fungi, bacterial endosymbionts (such as Rhizobium), and plant growth-promoting rhizobacteria (PGPRs). The type and amount of microbial communities in the soil is an important indicator of the soil quality index and is effective in the management of agricultural systems (Elliott et al., 1996; Mäeder et al., 2002; Hoorman 2016). Among these microorganism communities, the bacteria associated with plant

✉Correspondence
E-mail address: fazil@atauni.edu.tr

roots are called root bacteria. Root bacteria are effective in the decomposition and mineralization of organic residues in the soil. In this process, bacteria give to soil an intense biochemical activity and physical quality. This situation affects both the soil structure and other living things in the soil ecosystem, especially plants (Hacımüftüoğlu & Canbolat, 2022).

Many studies have shown that these bacteria living in the rhizosphere support plant growth with different mechanisms of action. Rhizobacteria that support plant growth are also known as "Probiotic Rhizobacteria" due to the many benefits they provide to the plant (John et al., 2020). These bacteria, called PGPR, which inhabit the plant root surface and rhizosphere soil, can promote plant growth through direct and indirect mechanisms of action (İmriz et al., 2014). While some rhizobacteria (Azotobacter, Azospirillum, Beijerrinckia and Pseudomonas i.e.) play a role in nitrogen fixation (Reis et al., 1994), it is reported that some bacteria increase the synthesis of growth-regulating substances in plants (Zahir et al., 2004). Plant growth-promoting PGPR bacteria, through biological fixation and phytohormone production, promote nitrogen fixation and enable the solubilization of phosphorus and heavy metals in the rhizosphere region. These bacteria, in addition to increasing water and mineral uptake by supporting root development, play an important role in biological control of plant diseases and pests (Mayak et al., 2004; Hynes et al., 2008; Berg & Smalla, 2009; Lugtenberg & Kamilova, 2009; Annapurna et al., 2011; El-Boray et al., 2013; Philippot et al., 2013; Panke-Buisse et al., 2015; Tangolar, 2022). Addition of bacteria to soils plays an important role in nutrient cycling in plant development (Elo et al., 2000).

It has been demonstrated in many studies that the germination rate, root and shoot performance, yield, leaf area, chlorophyll ratio, nitrogen ratio, protein ratio, hydraulic activity and drought tolerance in plants increase with PGPR applications (Dobbelaere et al., 2001; Şahin et al., 2004; Altın & Bora, 2005; Dos santos et al., 2020). Although soil and plant nutrition are the most important factors for crop production, intensive fertilization on the same agricultural land for many years threatens the productivity of agricultural lands. Excessive use of fertilizers in order to obtain more products per unit area is increasing day by day, leading to environmental problems and depletion of natural resources. Intensive farming practices can cause water and wind erosion in agricultural areas, depletion of nutrients, loss of soil organic matter and deterioration of various physical properties of the soil (Ruzzi & Aroca, 2015). This situation in crop production requires more production with less input. Plant growth-promoting rhizobacteria (PGPR) are used to minimize fertilizer application and maximize plant development and nutrition (Cakmakci et al., 2006; Sonneveld & Voogt, 2009; Yildirim et al., 2011; Yadav et al., 2015). Studies have revealed that rhizobacteria have significant potential on vegetative and generative development in horticulture plants. Grapes, one of the most widely grown fruits among horticultural crops, have great potential for sustainable agricultural production. The grapevine root system, which is the most important organs involved in water and nutrient uptake and storage, is the first plant part to be affected by soil properties. The development of the above-ground parts of vines is closely related to the structure and health of the root system (Southey, 1992; Smart, 1995). However, the main issue is to reveal the relationship between the targeted yield and quality levels in the vine and root functionality, soil structure and vine performance.

The purpose of this study; to reveal the effects of PGPR bacteria, which have become increasingly used in organic agriculture in recent years, on Karaerik and Narince grape varieties grown under cover (greenhouse) in Erzurum central conditions. Within the scope of this research, depending on the changes caused by PGPR bacterial applications on the physical and chemical properties of greenhouse soils; nutrient intake, vegetative development levels and productivity of grapevines were investigated.

2. Materials and Methods

In the research, 6-year-old vines of Narince and Karaerik varieties grown on their own roots, which are widely grown in Erzincan and Tokat regions, were used. The vineyards grown under unheated greenhouse conditions were given water once a month during the vegetation period. The total of 24 plants were used in the study. While 3 plants were left for the control groups and PGPR bacteria were applied to 9 plants in two different grape varieties in the experiment.

Harvest was performed on September 21, 2022, at the end of the 90th day of bacterial application. Shoot length values were obtained by measuring the length of the summer shoots randomly selected from the vines on the day of harvest, from the point of attachment to an old branch, to the apical. The average shoot diameter values were determined by measuring the diameter between the 2nd and 3rd node of the branch used in the shoot length measurements with a caliper. For each application, the clusters on the vine were counted one by one, before veraison and during the harvest period. The number of clusters determined in two different periods was in harmony with each other. Two clusters were taken from each vine and weighed on a sensitive scale, and the average cluster weight was determined by dividing the total value by the number of clusters. Width and length values of the clusters were determined with a caliper. The number of berry in the bunch was determined by counting the fully ripened berries in 2 harvested clusters. The berries representing the cluster were selected from the middle part of the cluster and the width and height values were determined with a caliper. Berry weight values were determined in grams by weighing 10 berries taken from the clusters of vines on a 0.1g sensitive scale (Gürsöz, 1993). The number of seeds in 10 berries taken from the

selected clusters was counted and the average value per berry was determined. The yield value was obtained by multiplying the number of clusters on a vine by the average cluster weight (Kupe & Kose, 2015). In order to reproduce the bacteria, 'Nutrient agar' medium was prepared at the rate of 28 g/L, sterilized and poured into petri dishes. The 'Nutrient Agar' medium was placed in a glass balloon and sterile distilled water was added and made up to 1 L. The media were placed in an autoclave and sterilized at 121 °C for 15 minutes and, it was then poured into petri dishes without solidifying at about 40 °C and left to cool at room temperature (Kızıloğlu & Bilen, 1997). Reproduction was made from bacterial cultures kept as stock and kept at -70 °C, pure culture was cultivated on nutrient agar medium and incubated at 28-30 °C for 5 days (Gürgün & Halkman, 1988). Bacteria prepared after incubation were transferred onto nutrient broth medium sterilized at 121 °C for 15 minutes in order to multiply bacterial cultures. It was incubated in a shaker for approximately 48 hours (50-60 rpm) and made ready for planting (Kızıloğlu & Bilen, 1997). In the study, 4 different bacterial combinations (*Pseudomonas chlororaphis + Paenibacillus pabuli + Bacillus simplex + Pseudomonas fluorescens*) that support plant growth were applied to the root zone of the plant in solution form, adjusted to a concentration of 10^8 CFU. In the study, the effects of PGPR applied on the vegetative growth, some pomological characteristics, yield levels and macronutrient contents of the leaves were determined.

According to USDA (1999), the soils used in the research are in the sandy loam class. Sub-samples to be used for basic analyzes were prepared by sieving through 2 mm sieves from soil samples that were duly taken from the greenhouse and air-dried under laboratory conditions. Soil texture was determined by Bouyoucos hydrometer method (Gee & Bauder, 1986), soil reaction (pH) by glass electrode pH meter (McLean, 1982), lime content by Scheibler calcimeter (Nelson, 1982), organic matter content by Smith Weldon method (Nelson & Sommers, 1982), electrical conductivity (EC) value with electrical conductivity instrument (Rhoades, 1982), aggregate stability (AS) using Yoder type wet sieving device (Kemper & Rosenau, 1986), water permeability (Klute & Dirksen, 1986), particle density were determined by pycnometer method (Blake & Hartge, 1986). On the other hand, soil bulk density by the cylinder method (Blake & Hartge, 1986), total porosity was calculated from bulk weight and particle density, P_2O_5, Ca^{++}, Mg^{++}, Na^+ and K^+ contents of the soils were determined by Kacar (2014)'s method. Statistical analysis was performed by ANOVA, and differences between means were tested using Duncan's multiple range test.

3. Results and Discussion

Basic soil analysis results are presented in Table 1. In this study, the texture class of the soils was determined as coarse textured in the sandy clay loam texture class (62% sand, 30% silt, 8% clay). Soil organic matter content (3.55%) is in the well class; pH level was found to be 7.62 and neutral, the EC level of the working soils is 1.23 dS/m without salt, the $CaCO_3$ level was determined as 5.11% in the medium calcareous (Ülgen & Yurtsever, 1995), class. According to the available phosphorus contents, the class of the soils was determined as medium (Ülgen & Yurtsever, 1995), Ca^{++}, Mg^{++}, Na^+, K+ contents were determined as 8.15, 5.38, 1.28, 2.24 me/100 g, respectively. Particle density was determined as 2.68 (g/cm^3) (Table 1).

Table 1. Results of some basic physical and chemical analyzes of the researched soils.

Soil Properties	
Sand (%)	62
Silt (%)	30
Clay (%)	8
Texture class	Sandy loam
Particle density (g/cm^3)	2.68
Organic matter (%)	3.55
pH	7.62
Electrical Conductivity (dS/m)	1.23
$CaCO_3$ (%)	5.11
Ca, Mg, Na, K (me/100 g)	8.15, 5.38, 1.28, 2.24

Vegetative growth, pomological characteristics and yield values of Karaerik and Narince grape cultivars were found to be statistically higher in plants treated with PGPR bacteria than control groups (Tables 2 and 3).

Table 2. Vegetative development parameters of Karaerik and Narince varieties.

Vegetative development parameters	**Karaerik Variety**		**Narince Variety**	
	Control	**Application**	**Control**	**Application**
Shoot Length (cm)	3.80ab	4.80a	2.50b	3.50ab
Shoot Diameter (cm)	1.40b	1.63a	1.16c	1.30bc
Node Number (item)	28.6ab	34.6a	21.6b	27.3b

When vegetative development parameters were examined, it was determined that there was a statistical difference between the application and control groups ($p<0.05$). When the shoot length parameters were examined, the highest value (4.80 cm) was determined in the Karaerik grape variety application group. Depending on the PGPR bacterial application, it was

determined that the shoot length value in the Karaerik grape variety increased by 26.3% compared to the control group, and in the Narince grape variety, there was a 40% increase. When the shoot diameters of the vines were examined, it was determined that there was an increase of 16.4% and 12%, respectively, in Karaerik and Narince grape varieties compared to the control groups, depending on the applications. The highest value was determined in the application group of Karaerik grape variety, with 1.63 cm. When the average number of nodes per shoot of vines was examined, it was determined that the number of nodes increased in parallel with the shoot length. It was determined that PGPR bacterial application increased the number of nodes in Karaerik and Narince grape varieties by 21% and 26%, respectively, compared to the control group (Table 2).

Table 3. Pomological features and yield of Karaerik and Narince varieties.

Pomological features and yield	Karaerik Variety		Narince Variety	
	Control	Application	Control	Application
Berry Width (cm)	2.03b	2.16a	1.70d	1.86c
Berry Size (cm)	2.56b	2.70a	1.73d	1.93c
Cluster Width (cm)	14.87b	18.76a	14.06c	15.46b
Cluster Size (cm)	28.3a	29.03a	22.43c	24.46b
Number of Seeds (item)	3ab	3.33a	2c	2.33bc
Number of Clusters (item)	5.33b	6.66a	4.66b	5.33b
Cluster Weight (g)	629.3b	719.3a	367d	435.6c
Number of Berry (item)	66.30b	82.00a	81.00a	81.30a
Berry Weight (g)	7.10b	7.83a	3.00d	4.03c
Yield (g)	3354.17b	4790.5a	1710.2d	2321.7c

When pomological development and yield parameters were examined, it was determined that there was a statistical difference between the application and control groups ($p<0.05$). When the berry width values were examined, the highest value (2.16 cm) was determined in the Karaerik grape variety application group. Depending on the PGPR bacterial application, it was determined that there was an increase of 6.40% in the berry width value of the Karaerik grape variety compared to the control group, and a 9.41% increase in the Narince grape variety (Table 3). When the berry size was examined, it was determined that there was an increase of 5.46% and 11.5%, respectively, in Karaerik and Narince grape varieties compared to the control groups, depending on the applications. It was revealed that the highest value was in the application group of Karaerik grape variety, with 2.70 cm (Table 3). When Table 3 was examined, it was determined that there was a parallel relationship between cluster width and cluster length values.

Depending on the PGPR application, it was determined that cluster width and cluster length values increased by 26.2% and 2.58%, respectively, in Karaerik grape variety compared to the control groups, and by 10% and 9.1% in Narince grape variety. When the number of seeds in the berry was examined, it was determined that the highest average value (3.33 items) was in the Karaerik grape variety application group. Depending on the PGPR bacterial application, it was determined that the number of seeds in the Karaerik grape variety increased by 11% compared to the control group, and in the Narince grape variety, there was an increase of 16.5% (Table 3). When the number of clusters on the vine was examined, it was determined that there was an increase of 25% and 14.4%, respectively, in Karaerik and Narince grape varieties compared to the control groups. It was determined that the highest average number of clusters was in the application group of the Karaerik grape variety with 6.66 units. When the cluster weight values were examined, the highest value (719.3 g) was determined in the Karaerik grape variety application group. Depending on the PGPR bacterial application, it was determined that there was an increase of 14.3% in the Karaerik grape variety and 18.7% in the Narince grape variety in cluster weight values compared to the control groups. When the number of berries on the cluster was examined depending on the applications, it was determined that there was an increase of 23.7% and 0.37%, respectively, in Karaerik and Narince grape varieties compared to the control groups. It was determined that the highest average number of berries was in the application group of the Karaerik grape variety with 82 item (Table 3). When the berry weight values were examined, the highest value (7.83 g) was determined in the Karaerik grape variety application group. It was determined that there was an increase of 10.3% in Karaerik grape variety and 34.3% in Narince grape variety in berry weight values depending on the treatments compared to the control groups. When the average yields of the vines determined based on the average number of clusters and average cluster weight values were examined, it was determined that the highest yield value was in the Karaerik application group with 4790.5 g. Depending on the applications, it was determined that an

increase of 42.8% occurred in Karaerik grape variety compared to the control group. Similarly, depending on the application, it was determined that there was a 35.8% yield increase in Narince grape variety compared to the control group (Table 3).

Table 4. Macro elements contents of Karaerik and Narince leaves.

Macro elements (%)	Karaerik leaves		Narince leaves	
	Control	**Application**	**Control**	**Application**
Ca	0.28b	0.37a	0.27b	0.36a
Mg	0.23c	0.26b	0.23c	0.31a
Na	0.12d	0.15c	0.23b	0.26a
K	1.42c	1.56a	1.40c	1.51b
N	1.44d	1.56c	1.66b	1.69a

When the macronutrient contents of leaves in Kararerik and Narince grape varieties are compared, depending on the PGPR bacterial application, the Ca, Mg, Na, K and N contents in the Karaerik grape variety are 9%, 3%, 3%, 14%, 12%, and in Narince grape variety are increased by 9%, 8%, 3%, 11%, 3% respectively compared to the control groups. When Table 4 was analysed, it was observed that the highest macro element content was generally found in the leaves of Narince grape varieties treated with PGPR.

3.1. Soil Physical and Chemical Properties

At the end of the 90th day of PGPR bacteria combination application, the physical properties were determined on the soil samples taken from the plant root zone. Aggregate stability, water permeability, bulk density and porosity values of the research soils are given in Table 5.

Table 5. Effects of application on the physical and chemical properties of soils.

	Control	**Application**
AS (%)	41.20	61.60
WP (cm/h)	7.10	5.80
BD (gr/cm^3)	1.54	1.48
Porosity (%)	41.90	44.20
OM (%)	1.43	2.52
pH	7.64	7.78
EC	1.23	1.11
Lime (%)	9.17	9.20
P_2O_5 (kg/da)	6.78	7.45
K_2O (kg/da)	246	246

AS: Aggregate stability, **WP:** Water permeability, **BD:** Bulk density **OM:** Organic matter.

According to the results, it was determined that the PGPR applications applied to the soils had a significant effect on the physical properties of the soils. Rhizobacteria are known to increase microaggregates in soil by binding soil particles together (Ingham, 2009; Hacimuftuoglu, 2020). When aggregate stability and porosity values of bacteria-inoculated soils are examined, it is seen that there is a significant increase compared to the control group soils. According to Table 5, it was determined that aggregate stability and porosity values increased by 49.5% and 5.5%, and water permeability and bulk density values decreased by 18.3% and 3.9%, respectively, in the treated soils compared to the control group. Vandevivere and Baveye (1992) and Abdel Aal et al. (2010) found in their research that the addition of bacteria to soils clogged soil pores and significantly reduced soil permeability, depending on microbial biomass.

When the soil chemical properties were examined, it was determined that the most important effect occurred at the organic matter level. It was determined that the organic matter level, which was 1.43% in the control group, increased to 2.52% in the treated soils with an increase of 76%. Since beneficial bacteria in the soil environment convert organic content into usable nutrients, their presence and activities in the soil are very important (Badalucco & Kuikman, 2001). This situation can positively affect soil aggregation as well as soil productivity (Hayes, 2010; Hoorman, 2016). Development of structure in agricultural lands; is a key factor in soil quality and crop production (Six et al., 2000; Díaz-Zorita et al., 2002; Bronick & Lal, 2005). Soil structure provides soil formation and stabilization by controlling biological activity, plant growth and nutrient cycling (Denis & Caron, 1998). The application of organic matter in the soil provides a more suitable environment for plant growth by providing a positive effect on the soil's aggregate stability, water permeability, air-water balance, and uptake of plant nutrients in the soil (Bronick & Lal, 2005; Ingham, 2009; Hacımüftüoğlu & Küpe, 2022). As a result of the research, it was determined that PGPR bacterial applications increased the organic matter content in soils by increasing bacterial activity and had a positive effect on aggregate stability values. It was determined that PGPR bacterial applications changed the physical properties of the soil, thus positively promoting vegetative and generative development in vines.

When Figure 1 was examined, it was determined that while the aggregate stability and porosity values of the soil increased due to PGPR bacterial applications, the soil bulk density and soil water permeability values decreased. Depending on these changes in soil physical properties, it is seen that the shoot development of the vines also increases.

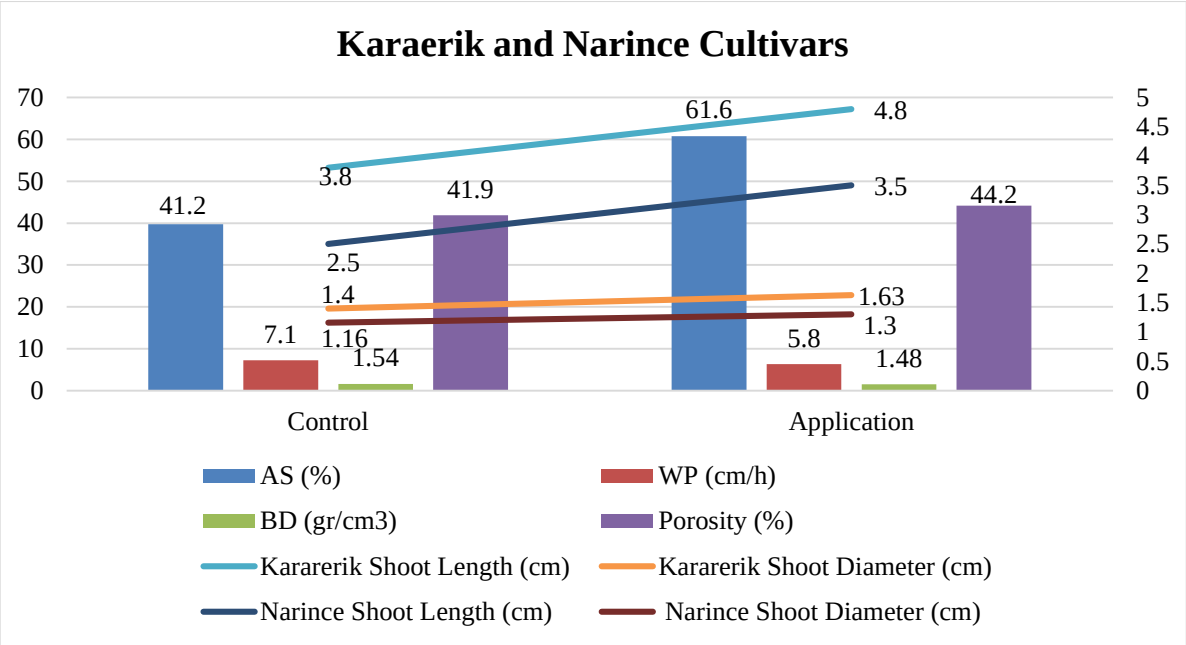

Figure 1. The relationship between soil physical properties and plant vegetative development.

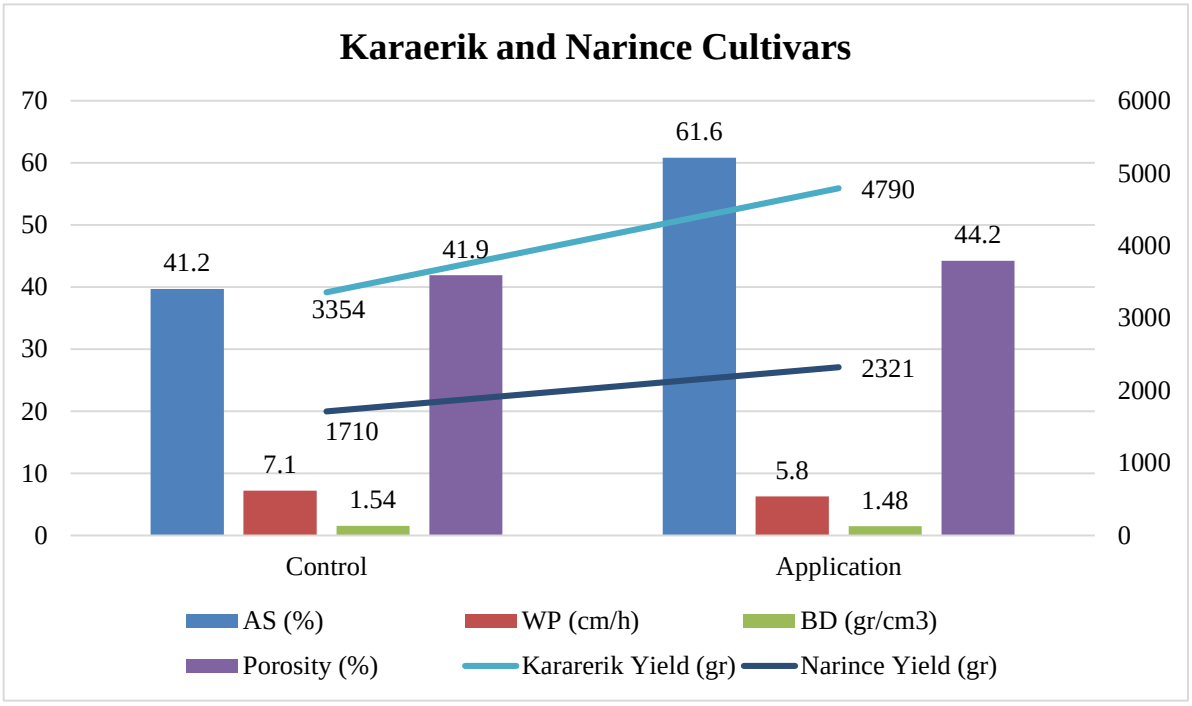

Figure 2. The relationship between soil physical properties and crop yield.

Depending on PGPR bacterial applications, the aggregate stability of soils increased from 41.2% to 61.6%. In parallel with this development, it was determined that the yield value of the Karaerik grape variety increased from 3354 g/vine to 4770 g/vine, and the yield value of the Narince grape variety increased from 1710 grams per vine to 2321 grams (Figure 2).

Soil nutritional status affects all parts of the grapevine, from root growth and distribution through to shoot growth and grape composition (Lanyon et al., 2004). The functional aspects of soil structure, namely water supply (Hamblin, 1986) and aeration (Gupta & Larson, 1982) are the two most important soil characteristics determining suitability of soil for viticulture (Northcote, 1988). These are properties that we need to ascertain their degree of influence on vine performance, with specific attention to root and shoot growth, yield and grape quality. Few studies have been conducted on the effect of soil strength on vine root growth. Among these, Myburgh et al. (1996) found, in an extensive survey of soil conditions in vineyards in all the major grape producing areas across South-eastern and Western Australia, that poor vine performance

(either yield or quality) could often be traced to restricted root development. Rowe (1993) and Wang et al. (2001) demonstrated that, even in situations where water and nutrient availability are non-limiting, the size of the root system has a direct effect on shoot growth and, hence, associated vine balance. As a matter of fact, different studies have stated that PGPR bacterial applications in grapevines have positive effects on vegetative development and mineral uptake (Sabir et al., 2012; Gunes et al., 2015; Korkutal et al., 2020). In this study, it was determined that PGPR bacterial applications stimulated root development in the soil of both grape varieties. In parallel with, a positive effect occurred on shoot length, shoot diameter, number of nodes, grain width, grain length, cluster width, cluster length, number of seeds, number of clusters, cluster weight, number of grains, grain weight and total yield values. It has also been observed that it has a positive effect on the macronutrient content of the leaves.

4. Conclusion

It is known that different rhizobacteria activate different mechanisms on yield parameters. In this study, multiple bacterial combinations, which have been shown to be effective on soil fertility in many studies, were applied to soils. The findings revealed that bacterial treatments significantly increased the degree of soil aggregation, plant vegetative growth and yield parameters. These bacteria play a key role in improving the physical properties of soils for plant root growth and continue to be an important part of organic farming activities. In today's world, where the need for access to plant products comes to the forefront, the importance of bacterial applications is becoming increasingly important for the protection of human health.

It has been determined that PGPR bacteria applications have a significant positive effect on vegetative growth and yield parameters of vines. It was determined that the number of clusters and cluster weights increased as well as the shoot growth of the vines applied. In the light of the data obtained in this study, it is thought that more comprehensive studies in viticulture may be useful to reveal the specific activities and physiological mechanisms of PGPR bacteria applied to soils. We believe that the organic viticulture sector will be more efficient and economical with the widespread use of PGPR bacteria applications in vineyards.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Abdel Aal, G. Z., Atekwana, E. A., & Atekwana, E. A. (2010). Effect of bioclogging in porous media on complex conductivity signatures. *Journal of Geophysical Research: Biogeosciences*, *115*(G3), 1-10. https://doi.org/10.1029/2009JG001159

Altın, N., & Bora, T. (2005). Bitki gelişimini uyaran kök bakterilerinin genel özellikleri ve etkileri. *Anadolu Ege Tarımsal Araştırma Enstitüsü Dergisi*, *15*(2), 87-103. (In Turkish)

Annapurna, K., Ramadoss, D., Vithal, L., Bose, P., & Sajad, A. (2011). *PGPR bioinoculants for ameliorating biotic and abiotic stresses in crop production*. 2nd Asian PGPR Conference. Beijing.

Badalucco, L., & Kuikman, P. J. (2001). Mineralization and immobilization in the rhizosphere. In R. Pinton, Z. Varanini & P. Nannipieri (Eds.), *The Rhizosphere; biochemistry and organic substances at the soil-plant interface* (pp. 159-196). CRC Press.

Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*, *68*(1), 1-13. https://doi.org/10.1111/j.1574-6941.2009.00654.x

Blake, G. R., & Hartge, K. H. (1986). Bulk density. In A. Klute (Ed.), *Methods of soil analysis, Part 1 -Physical and Mineralogical Methods, 2nd Edition, Agronomy Monographs 9, American Society of Agronomy-Soil Science Society of America* (pp. 363-382). Madison.

Bronick, C. J., & Lal, R. (2005). Soil structure and management: A review. *Geoderma*, *124*(1-2), 3-22. https://doi.org/10.1016/j.geoderma.2004.03.005

Cakmakci, R., Dönmez, F., Aydın, A., & Şahin, F. (2006). Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biology and Biochemistry*, *38*(6), 1482-1487. https://doi.org/10.1016/j.soilbio.2005.09.019

Denis, A. A., & Caron, J. (1998). Plant-induced changes in soil structure: Processes and feedback. In N. Van Breemen (Ed.), *Developments in biogeochemistry* (pp. 55-72). Springer. https://doi.org/10.1007/978-94-017-2691-7_3

Díaz-Zorita, M., Perfect, E., & Grove, J.H. (2002). Disruptive methods for assessing soil structure. *Soil and Tillage Research*, *64*(1-2), 3-22. https://doi.org/10.1016/S0167-1987(01)00254-9

Dobbelaere, S., Croonenborghs, A., Thys, A., Ptacek, D., Vanderleyden, J., Dutto, P., & Okon, Y. (2001). Responses of agronomically important crops to inoculation with Azospirillum. *Functional Plant Biology*, *28*(9), 871-879. https://doi.org/10.1071/PP01074

Dos Santos, R. M., Diaz, P. A. E., Lobo, L. L. B., & Rigobelo, E. C. (2020). Use of plant growth-promoting rhizobacteria in maize and sugarcane: Characteristics and applications. *Frontiers in Sustainable Food Systems*, *4*, 136. https://doi.org/10.3389/fsufs.2020.00136

El-Boray, M. S., Shalan, A. M., & Khouri, Z. M. (2013). Effect of different thinning techniques on fruit set, leaf area, yield and fruit quality parameters of *Prunus persica* L. Batsch cv. Floridaprince. *Trends in Horticultural Research*, *3*(1), 1-13. https://doi.org/10.3923/thr.2013.1.13

Elliott, L. F., Lynch, J. M., & Papendick R. I. (1996). The microbial component of soil quality. In G. Stotzky & J. M. Bollag (Eds.), *Soil biochemistry* (pp. 1-21). Marcel Dekker.

Elo, S., Maunuksela, L., Salkinoja-Salonen, M., Smolander, A., & Haahtela, K. (2000). Humus bacteria of Norway spruce stands: Plant growth promoting properties and birch, red fescue and alder colonizing capacity. *FEMS Microbiology Ecology*, *31*(2), 143-152. https://doi.org/10.1111/j.1574-6941.2000.tb00679.x

Gee, G. W., & Bauder, J. W. (1986). Particle-size analysis. In A. Klute (Ed.), *Methods of soil analysis: Part 1 physical and mineralogical methods, 5.1, 2nd edition* (pp. 383-411). American Society of Agronomy and Soil Science Society of America. https://doi.org/10.2136/sssabookser5.1.2ed.c15

Gunes, A., Karagoz, K., Turan, M., Kotan, R., Yildirim, E., Cakmakci, R., & Sahin, F. (2015). Fertilizer efficiency of some plant growth promoting rhizobacteria for plant growth. *Research Journal of Soil Biology*, *7*(2), 28-45. https://doi.org/10.3923/rjsb.2015.28.45

Gupta, S. C., & Larson, W. E. (1982). Modeling soil mechanical behavior during tillage. In P. W. Unger, D. M. Van Doren Jr., F. D. Whisler & E. L. Skidmore (Eds.), *Predicting tillage effects on soil physical properties and processes* (pp. 151-178). American Society of Agronomy and Soil Science Society of America. https://doi.org/10.2134/asaspecpub44.c10

Gürgün, V., & Halkman, A. K. (1988). *Mikrobiyolojide sayım yöntemleri*. Gıda Teknolojisi Derneği, San Matbaası. (In Turkish)

Gürsöz, S. (1993). *GAP alanına giren Güneydoğu Anadolu Bölgesi bağcılığı ve özellikle Şanlı Urfa ilinde yetiştirilen üzüm çeşitlerinin ampelografik nitelikleri ile verim ve kalite unsurlarının belirlenmesi üzerinde bir araştırma* (Doctoral dissertation, Çukurova University). (In Turkish)

Hacimuftuoglu, F. (2020). *Farklı tekstürlü topraklara bakteri aşılamasının ve organik polimer uygulamasının toprak fiziksel özellikleri ve mısır bitkisinin (Zea mays l.) gelişimi üzerine etkileri* (Doctoral dissertation, Atatürk University). (In Turkish)

Hacımüftüoğlu, F., & Canbolat, M. Y. (2022). Effects of bacterial inoculation on soil physical properties. *Turkish Journal of Agriculture and Forestry*, *46*(4), 536-549. https://doi.org/10.55730/1300-011X.3024

Hacımüftüoğlu, F., & Küpe, M. (2022). The effects of cattle and sheep manure applications on soil physical properties and rooting and shoot development of grapevines cuttings. *Erzincan University Journal of Science and Technology*, *15*(3), 900-915. https://doi.org/10.18185/erzifbed.1194500

Hamblin, A. P. (1986). The influence of soil structure on water movement, crop root growth, and water uptake. *Advances in Agronomy*, *38*, 95-158. https://doi.org/10.1016/S0065-2113(08)60674-4

Hayes, C. (2010). The basics of beneficial soil microorganisms. *BioWorks Technical Bulletin. Microorganisms*, 7-11.

Hoorman, J. J. (2016). *Role of soil bacteria: Update and revision*. Midwest Cover Crops Council (MCCC).

Hynes, R. K., Leung, G. C., Hirkala, D. L., & Nelson, L. M. (2008). Isolation, selection, and characterization of beneficial rhizobacteria from pea, lentil and chickpea grown in western Canada. *Canadian Journal of Microbiology*, *54*(4), 248-258. https://doi.org/10.1139/W08-008

İmriz, G., Özdemir, F., Topal, İ., Ercan, B., Taş, M. N., Yakışır, E., & Okur, O. (2014). Bitkisel üretimde bitki gelişimini teşvik eden rizobakteri (PGPR)'ler ve etki mekanizmaları. *Elektronik Mikrobiyoloji Dergisi*, *12*(2), 1-19. (In Turkish)

Ingham, E. R. (2009). *Soil biology primer*. Soil and Water Conservation Society.

John, C. J., Kumar S., & Ge, M. (2020). Probiotic prospects of PGPR for green and sustainable agriculture. *Archives of Phytopathology and Plant Protection*, *53*(19-20), 1-16. https://doi.org/10.1080/03235408.2020.1805901

Kacar, B. (2014). *Kolay uygulanabilir bitki analizleri*. Nobel Yayınları. (In Turkish)

Kemper, W. D., & Rosenau, R. C. (1986). Aggregate stability and size distribution. In A. Klute (Ed.), *Methods of soil analysis: Part 1 physical and mineralogical methods, 5.1, 2nd edition* (pp. 425-442). American Society of Agronomy and Soil Science Society of America. https://doi.org/10.2136/sssabookser5.1.2ed.c17

Kızıloğlu, T., & Bilen, S. (1997). *Toprak mikrobiyolojisi laboratuvar uygulamaları*. Atatürk Üniversitesi Ziraat Fakültesi Ders Yayınları. (In Turkish)

Klute, A., & Dirksen, C. (1986). Hydraulic conductivity and diffusivity: Laboratory methods. In A. Klute (Ed.), *Methods of soil analysis: Part 1 physical and mineralogical methods, 5.1, 2nd edition* (pp. 687-734). American Society of Agronomy and Soil Science Society of America. https://doi.org/10.2136/sssabookser5.1.2ed.c28

Korkutal, İ., Bahar, E., & Özakın, T. T. (2020). Aşılı asma (*Vitis vinifera* L.) fidanlarına farklı yöntemlerle uygulanan mikorizaların fidan tutma ve gelişme özellikleri üzerine etkileri. *Mediterranean Agricultural Sciences*, *33*(2), 149-157. https://doi.org/10.29136/mediterranean.496268 (In Turkish)

Kupe, M., & Kose, C. (2015). Karaerik üzüm çeşidinde kış soğuklarından sonra zarar düzeyine bağlı olarak uygun

budama seviyelerinin tespit edilmesi. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, *46*(1), 21-28. (In Turkish)

Lanyon, D. M., Cass, A., & Hansen, D. (2004). *The effect of soil properties on vine performance*. CSIRO Land and Water Technical Report No: 34-04.

Lugtenberg, B., & Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology, 63*, 541-556. https://doi.org/10.1146/annurev.micro.62.081307.162918

Mäeder, P., Fliessbach, A., Dubois, D., Gunst, L., Fried, P., & Niggli, U. (2002). Soil fertility and biodiversity in organic farming. *Science*, *296*(5573), 1694-1697. https://doi.org/10.1126/science.1071148

Mayak, S., Tirosh, T., & Glick, B. R. (2004). Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiology and Biochemistry*, *42*(6), 565-572. https://doi.org/10.1016/j.plaphy.2004.05.009

McLean, E. O. (1982). Soil pH and lime requirement. In A. L. Page (Ed.), *Methods of soil analysis: Part 2 chemical and microbiological properties, 9.2.2, 2nd edition* (pp. 199- 224). American Society of Agronomy and Soil Science Society of America. https://doi.org/10.2134/agronmonogr9.2.2ed.c12

Myburgh, P. A., Cass, A., & Clingeleffer, P. R. (1996). *Root systems and soils in Australian vineyards and orchards - an assessment*. 1996 Barossa Valley Rotary Foundation Fellowship Report. (CRC Soil & Land Management)

Nelson, R. E. (1982). Carbonate and gypsum. In A. L. Page (Ed.), *Methods of soil analysis: Part 2 chemical and microbiological properties, 9.2.2, 2nd edition* (pp. 181-197). American Society of Agronomy and Soil Science Society of America.

Nelson, D. W., & Sommers, L. E. (1982). Total carbon, organic carbon, and organic matter. In A. L. Page (Ed.), *Methods of soil analysis: Part 2 chemical and microbiological properties, 9.2.2, 2nd edition* (pp. 539-579). American Society of Agronomy and Soil Science Society of America. https://doi.org/10.2134/agronmonogr9.2.2ed.c29

Northcote, K. H. (1988). Soil and Australian viticulture. In P. R. Dry & B. G. Coombe (Eds.), *Viticulture: Volume 1-resources* (pp. 61-90). Winetitles.

Panke-Buisse, K., Poole, A. C., Goodrich, J. K., Ley, R. E., & Kao-Kniffin, J. (2015). Selection on soil microbiomes reveals reproducible impacts on plant function. *The ISME Journal*, *9*(4), 980-989. https://doi.org/10.1038/ismej.2014.196

Philippot, L., Spor, A., Hénault, C., Bru, D., Bizouard, F., Jones, C. M., & Maron, P. A. (2013). Loss in microbial diversity affects nitrogen cycling in soil. *The ISME Journal*, *7*(8), 1609-1619. https://doi.org/10.1038/ismej.2013.34

Reis, V., Olivares, F., & Dobereiner, J. (1994). Improved methodology for isolation of *Acetobacter diazotrophicus* and confirmation of its endophytic habitat. *World Journal of Microbiology & Biotechnology*, *10*(4), 401-405. https://doi.org/10.1007/bf00144460

Rhoades, J. D. (1982). Cation exchange capacity. In A. L. Page (Ed.), *Methods of soil analysis: Part 2 chemical and microbiological properties, 9.2.2, 2nd edition* (pp. 149- 157). American Society of Agronomy and Soil Science Society of America. https://doi.org/10.2134/agronmonogr9.2.2ed.c8

Rowe, R. N. (1993). *Grapevine devigoration*. The Australian and New Zealand Wine Industry Journal.

Ruzzi, M., & Aroca, R. (2015). Plant growth-promoting rhizobacteria act as biostimulants in horticulture. *Scientia Horticulturae*, *196*, 124-134. https://doi.org/10.1016/j.scienta.2015.08.042

Sabir, A., Yazici M., Kara Z., & Sahin F. (2012). Growth and mineral acquisition response of grapevine rootstocks (*Vitis* spp.) to inoculation with different strains of plant growth-promoting rhizobacteria (PGPR). *Journal of the Science of Food and Agriculture*, *92*(10), 2148-2153. https://doi.org/10.1002/jsfa.5600

Şahin, F., Çakmakçi, R., & Kantar, F. (2004). Sugar beet and barley yields in relation to inoculation with N2-fixing and phosphate solubilizing bacteria. *Plant and Soil*, *265*(1/2), 123-129.

Six, J., Elliott, E. T., & Paustian, K. (2000). Soil structure and soil organic matter: II. A normalized stability index and the effect of mineralogy. *Soil Science Society of America Journal*, *64*(3), 1042-1049. https://doi.org/10.2136/sssaj2000.6431042x

Smart, R. E. (1995). *Two golden rules of viticulture*. Australian New Zealand Wine Industry Journal.

Sonneveld, C., & Voogt, W. (2009). Nutrient management in substrate systems. In C. Sonneveld & W. Voogt (Eds.), *Plant nutrition of greenhouse crops* (pp. 277-312). Springer. https://doi.org/10.1007/978-90-481-2532-6_13

Southey, J. M. (1992). Root distribution of different grapevine rootstocks on a relatively saline soil. *South African Journal of Enology and Viticulture*, *13*(1), 1-9. https://doi.org/10.21548/13-1-2189

Tangolar, S. (2022). The role of biostimulants in viticulture. In S. Cantürk & İ. S. Gökçen (Eds.), *Current agricultural studies in Türkiye: Research and reviews* (pp. 203-239). İKSAD Yayınevi.

Ülgen, N., & Yurtsever, N., (1995). *Türkiye gübre ve gübreleme rehberi*. Başbakanlık Köy Hizmetleri Genel Müdürlüğü Toprak ve Gübre Araştırma Enstitüsü Müdürlüğü Yayınları. (In Turkish)

USDA. (1999). *Soil taxonomy, a basic system of soil classification for making and interpreting soil surveys, second edition*. United States Department of Agriculture. https://www.nrcs.usda.gov/sites/default/files/2022-06/Soil%20Taxonomy.pdf

Vandevivere, P., & Baveye, P. (1992). Relationship between transport of bacteria and their clogging efficiency in sand columns. *Applied and Environmental Microbiology*, *58*(8), 2523-2530. https://doi.org/10.1128/aem.58.8.2523-2530.1992

Wang, S., Okamoto, G., Hirano, K., Lu, J., & Zhang, C. (2001). Effects of restricted rooting volume on vine growth and berry development of Kyoho grapevines. *American Journal of Enology and Viticulture*, *52*, 248-253. https://doi.org/10.5344/ajev.2001.52.3.248

Yadav, A., Theivasanthi, T., Paul, P. K., & Upadhyay, K. C. (2015). Extracellular biosynthesis of silver nanoparticles from plant growth promoting rhizobacteria *Pseudomonas* sp. *arXiv*:1511.03130. https://doi.org/10.48550/arXiv.1511.03130

Yildirim, E., Turan, M., Ekinci, M., Dursun, A., & Cakmakci, R. (2011). Plant growth promoting rhizobacteria ameliorate deleterious effect of salt stress on lettuce. *Scientific Research and Essays*, *6*(20), 4389-4396. https://doi.org/10.5897/SRE11.219

Zahir, Z. A., Arshad, M., & Frankenberger, W. T. (2004). Plant growth promoting rhizobacteria: Applications and perspectives in agriculture. *Advances in Agronomy*, *81*, 97-168. https://doi.org/10.1016/S0065-2113(03)81003-9

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

https://prensip.gen.tr

RESEARCH ARTICLE

Genome-wide Analysis and Functional Identification of *KCS* Gene Family under Drought and Salt Stresses in *Phaseolus vulgaris* L

Ceren Yılmaz[1] • Merve Yüce[2] • Ahmed Sidar Aygören[1] • Ayşe Gül Kasapoğlu[1] • Selman Muslu[1] • Murat Turan[1] • Emre İlhan[1] • Murat Aydın[3] • Ertan Yıldırım[2]✉

[1]Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetics, Erzurum/Türkiye
[2]Atatürk University, Faculty of Agriculture, Department of Horticulture, Erzurum/Türkiye
[3]Atatürk University, Faculty of Agriculture, Department of Agricultural Biotechnology, Erzurum/Türkiye

ARTICLE INFO

Article History
Received: 05.10.2023
Accepted: 10.11.2023
First Published: 30.12.2023

Keywords
Genome-wide analysis
KCS
Phaseolus vulgaris L
VLCFA

ABSTRACT

β-ketoacyl-CoA synthase (KCS) is an important enzyme that catalyzes the biosynthesis of very-long-chain fatty acids (VLCFAs). In this study, the genome-wide analysis and functional characterization of the *KCS* gene family members in common bean (*Phaseolus vulgaris* L.) plants were conducted, and the response of the identified gene family to abiotic stresses was evaluated. In this study, 19 *KCS* genes were identified and characterized in the *P. vulgaris* genome. The molecular weights of these KCS proteins ranged from 49.14 kDa to 60.57 kDa, their amino acid lengths varied from 437 to 534, and their pI values ranged from 8.81 to 9.47, indicating a basic nature. Segmental and tandem duplications were observed in the *Pvul-KCS* gene family. Phylogenetic analysis revealed that *Pvul-KCS* proteins clustered into three main groups with *Arabidopsis thaliana* and *Glycine max* species. Comparative mapping analysis was also conducted with *A. thaliana* and *G. max*. Expression profile comparisons indicated that these genes had different expression levels in common bean varieties and played a role in the plant's response to biotic and abiotic stresses. This study provides important insights into the biological functions of *KCS* genes in *Phaseolus vulgaris* and offers valuable information for improving drought and salt stress tolerance in common beans.

Please cite this paper as follows:

Yılmaz, C., Yüce, M., Aygören, A. S., Kasapoğlu, A. G., Muslu, S., Turan, M., İlhan, E., Aydın, M., & Yıldırım, E. (2023). Genome-wide analysis and functional identification of *KCS* gene family under drought and salt stresses in *Phaseolus vulgaris* L. *Journal of Agricultural Production*, *4*(2), 138-151. https://doi.org/10.56430/japro.1371633

1. Introduction

Recent environmental developments, such as unstable rainfall patterns, temperature extremes, and salinity, have been linked to variations in soil composition (Verslues et al., 2006). The need for crops to maintain or increase crop yields when faced with unfavorable environmental conditions, such as drought and high salinity, necessitates genetic improvement (Araus et al., 2008) or the use of precursors that interact with these crops and promote plant growth, such as bacteria, hormones, and vitamins (Glick, 2012). From the earliest phases of plant life, the two most common abiotic stressors-salt and drought-effect agricultural yield and production. Salt and drought stress have a negative impact on both the quality and yield of plants (Maggio et al., 2005). Water must be provided to plants in the best possible quantity and quality because it is essential to their effective growth. Extended periods of drought

✉Correspondence
E-mail address: ertanyil@atauni.edu.tr

and excessive salinity can have long-lasting harmful effects on plants, affecting their root and stem growth and resulting in a reduction in the quantity and width of their leaves. Additionally, a halt in plant development may result from cells' reduced water capacity. The effects of salinity and drought stress on photosynthesis, metabolism, and plant growth have been shown in several research (Liu et al., 2016; Ors et al., 2016; Sahin et al., 2018; Ekinci et al., 2020).

The cuticle wax layer, which is made up of long-chain hydrocarbon compounds such alkanes, aldehydes, primary and secondary alcohols, ketones, which are esters, and various other related substances, acts as a barrier to protection for plants when they are subjected to a variety of abiotic challenges (X. Wang et al., 2017). Plant cuticle wax has a very intricate chemical makeup. In the outer cells of the epidermal layer, plant epidermal waxes are synthesized, and transported. The production and release pathways for these waxes involve several distinct organelles and enzymes working in unity (Bernard & Joubès, 2013). All plant species that grow on land have cuticular wax covering their aerial portions, which helps to prevent bacterial and fungal invasion as well as non-stroma transpiration. It coats the fruit. In the growth of fruits and post-harvest storage, it defends against stress from both abiotic and biotic sources by lowering fruit water loss, changing fruit luster, and improving fruit storage quality.

The enzyme that restricts the rate of the synthesis of very long-chain fatty acids (VLCFAs), known as KCS (β-ketoacyl-CoA synthase), provides components for the biosynthesis of cuticular wax (H. Yang et al., 2021). Animals, microbial organisms, and plants all use fatty acids that contain greater than eighteen carbon molecules as biological building blocks for a variety of molecules. For instance, in plants, the epidermis is the primary location of VLCFA synthesis. Here, they play a role in the process of biosynthesis of cuticular waxes. In many seed oils, triacylglycerols are primarily composed of VLCFAs. The *KCS* genes regulate epidermal wax's volume and composition. At every stage of plant growth and development, they actively take part in biochemical and physiological processes that also help plants adapt to stress. VLCFAs are also the main component of triacylglycerols in various plant oils (Ghanevati & Jaworski, 2001). The deletion mutant of the fatty acid elongation gene (FAE1/KCS18) in *Arabidopsis* results in a significant decrease in VLCFA content (Tong et al., 2021). This gene is essential for erucic acid biosynthesis and is involved in the production of VLCFAs in seeds (Kunst et al., 1992). One of the most significant edible legumes in the world is arguably the common bean (*Phaseolus vulgaris* L.), with a global production estimated at 35.5 million hectares in 2020 (http://faostat.fao.org/). Common beans are an important source of the daily requirement for protein in many countries, particularly in Latin America, Africa, and parts of Asia. In North America and Europe, common beans constitute a significant vegetable and legume crop economically. African nations consume considerable amounts of common beans; for instance, per capita common bean consumption in Rwanda, Kenya, and Uganda varies from 50 to 60 kg per year (Broughton et al., 2003; Buruchara et al., 2011). In terms of amino acid content and carbohydrates, vitamins (including A, C, and folate), and biologically significant minerals like Mg, Cu, and Zn, common beans are very nutrient-dense (Broughton et al., 2003; Blair, 2013). Furthermore, through symbiotic nitrogen fixation (SNF), common beans contribute to better soil and environmental health.

One of the key legumes for direct consumption, common beans encounter numerous difficulties as a crop. In their many agroecological contexts, common beans, domesticated from wild ancestors occupying a relatively small ecological niche, are subject to several kinds of stress related restrictions. Abiotic challenges include drought, salinity, chilling, and nutrient deficits, or toxicity in the soil, while biotic stresses on common beans include multiple fungal, bacterial, viral, and insect and worm pests (Assefa et al., 2019). Given the importance of common beans in both agriculture and economics, it is crucial to comprehend the molecular processes through which *KCS* genes in *P. vulgaris* function, particularly in response to abiotic challenges. This study's objectives were to locate *KCS* genes in the common bean genome, describe their characteristics, and analyze how they react to abiotic stressors. This research may provide insights into the functional roles of *KCS* genes in *P. vulgaris* and contribute to the development of stress-tolerant common bean varieties.

2. Materials and Methods

2.1. Identification and Characterization of KCS Genes in *P. vulgaris*

Using the Pfam Accession Number (PF08392) retrieved from the Pfam database, sequence information of the *KCS* gene family found in the *P. vulgaris* genome were obtained from the Phytozome v13 (https://phytozome-next.jgi.doe.gov/) database. To identify all possible KCS homologs in *P. vulgaris* (Schmutz et al., 2014), *G. max* (Valliyodan et al., 2019) and *A. thaliana* (Lamesch et al., 2012), the blastp in the Phytozome v13 database and the Hidden Markov Model (HMM) (http://www.ebi.ac.uk) search with default parameters were used. Additionally, the HMMER database was used to scan the presence of the KCS domain with the sequence information. KCS protein parameters were established by the earlier investigation by Aygören et al. (2023).

2.2. Phylogenetic Analysis

For the phylogenetic research, the Neighbor-joining (NJ) method was performed with a bootstrap value of 1000 replicates. Sequence alignment was performed using the ClustalW algorithm embedded in MEGA (Thompson et al., 1997). Following this, using MEGA v11 phylogenetic tree was

created (Tamura et al., 2011). The "Interactive Tree of Life" (iTOL) web interface was then utilized to model the evolutionary tree (Letunic & Bork, 2011).

2.3. Structure and Physical Location of *Pvul-KCS* Genes, Identification of Gene Duplication and Conserved Motifs, Comparative Mapping Between Other Species

"Gene Structure Display Server v2.0" (http://gsds.gao-lab.org/) (Hu et al., 2015) web interface was used for drawing exon and intron regions of *Pvul-KCS* genes to acquire information about gene structures.

KCS genes' chromosomal sites were found using the Phytozome v13 database. All *P. vulgaris* chromosomes had *Pvul-KCS* genes highlighted, which were then mapped using MapChart software (Voorrips, 2002). The "Multiple Collinearity Scan Toolkit" (MCScanX) database (Y. Wang et al., 2012) was used to search for gene duplication occurrences across *P. vulgaris*, *A. thaliana*, and *G. max*.

The exchange ratios between duplicated pairs of *Pvul-KCS* genes were calculated with values non-homologous (Ka) and homologous (Ks), and ratios non-homologous to homologous (Ka/Ks). The formulation $T=Ka/2\lambda$ was used to estimate the timing of duplication and divergence of each *KCS* gene ($\lambda = 6.56x10^{-9}$) (Z. Yang & Nielsen, 2000; Lynch & Conery, 2003; İlhan et al., 2023).

The "Multiple EM for Motif Elicition (MEME) Tool" (https://meme-suite.org/meme/index.html) was used to find further motifs that are conserved in Pvul-KCS proteins (Bailey et al., 2006). The MEME tool's settings were adjusted by earlier descriptions and discovered motifs were scanned with InterProScan database (Quevillon et al., 2005; Oner et al., 2022).

2.4. Promoter Analyses of the *Pvul-KCS* Gene Family, Intracellular Localization and Prediction of 3D Structures of Proteins

With PlantCARE (Lescot et al., 2002) database, *cis-acting* element analysis was performed in the 2 kb 5' upstream region DNA fragment of each gene of *KCS* (Rakhimzhanova et al., 2023). TBtools software was used to create phenograms (Chen et al., 2020) and WoLF PSORT tool was used to predict intracellular localization (Horton et al., 2007). The protein sequences obtained were visualized in 3D images using the Phyre2 database (Kelley et al., 2015). A single image was obtained by combining the protein images and intracellular localization data.

2.5. In Silico Gene Expression Analysis

The Sequence Read Archive (SRA) datasets in the NCBI database provided the Illumina RNA-seq data. SRR957668 and SRR958469 are for salt stress which are leaf tissue under salt treatment and salt control respectively (Hiz et al., 2014). SRR8284481 and SRR8284480 are for drought stress which are leaf tissue under drought stress treatment and drought stress control respectively (Gregorio Jorge et al., 2020). These accession numbers were used to access the expression level values and these expression values were normalized as demonstrated previously (Muslu et al., 2023). Heatmap graph was produced by using the CIMminer server (Weinstein et al., 1997).

3. Results and Discussion

3.1. P. vulgaris KCS Genes Features

The *P. vulgaris* genome in the Phytozome database v13 was searched for *KCS* gene family members using the PFAM accession number (PF08392). The investigation led to the discovery of 19 *KCS* genes in the common bean genome. The *Pvul-KCS* genes' locations on chromosomes, as well as their start and end positions, molecular weights, polypeptide lengths, isoelectric points, and stability/unstability index, are listed in Table 1.

It was observed that the identified *Pvul-KCS* genes were located on Chr1, Chr3, Chr4, Chr6, Chr7, Chr9, and Chr11 of the common bean genome and the unidentified scaffold_30 (Figure 1). As a result of the data obtained, the molecular weights of KCS genes were found to vary between 49.14 kDa and 60.57 kDa, and amino acid lengths between 437 and 534. The highest molecular weight of *Pvul-KCS-14* was 60.57 kDa, while *Pvul-KCS-16* was 49.14 kDa. *Pvul-KCS-14* contained the highest number of amino acids with 534, while *Pvul-KCS-16* contained the lowest number of amino acids with 437. It was also determined that the identified genes were mostly stable and the instability indices ranged between 29.59 and 49.80. It was observed that all genes were in the alkaline character and pI values ranged between 8.81 and 9.47.

In their study on the barley genome, Tong et al. (2021) found that the molecular weights and isoelectric points of the KCS proteins were 44.30-66.23 kDa and 6.95-10.2, respectively, with the length of these proteins in the range of 398-600 amino acid numbers.

Genome-wide characterization and identification of the *KCS* gene family in different species, Xiao et al. (2016) identified 58 *KCS* gene on *Gossypium hirsutum*, 31 *G. arboreum* and 33 *G. raimondii*, H. Yang et al. (2021) identified 13 *KCS* gene on *Atalantia buxifolia*, 16 *KCS* gene on *Citrus ichangensis*, 21 *KCS* gene on *Citrus medica*, 14 *KCS* gene on *Citrus grandis*, 16 *KCS* gene on *Citrus sinensis*, and 16 *KCS* gene on *Citrus clementina*, You et al. (2014) 38 *KCS* gene on *Linum usitatissimum* L., Lian et al. (2020) 28 *KCS* gene on *Malus domestica*, Xue et al. (2020) 58, 33 and 30 *KCS* genes on *Brassica napus*, *B. rapa* and *B. oleracea*, respectively, Tong

et al. (2021) *Hordeum vulgare* L. 33 *KCS* gene on, Dai et al. (2021) identified 18 *KCS* gene on *Malania oleifera*.

Table 1. Information about *Pvul-KCS* protein.

Gene Name	Pyhtozome ID	Chr No.	Start	End	Number of aa	MW (kDa)	pI	Instability Index
Pvul-KCS-1	Phvul.001G014100	Chr01	1058333	1061570	511	56.76	9.18	Stable
Pvul-KCS-2	Phvul.003G031300	Chr03	3165537	3167339	492	55.66	9.12	Unstable
Pvul-KCS-3	Phvul.003G138226	Chr03	34519665	34522309	516	57.79	9.03	Unstable
Pvul-KCS-4	Phvul.003G160400	Chr03	37852431	37855038	510	57.40	9.08	Stable
Pvul-KCS-5	Phvul.004G076300	Chr04	13184840	13190186	467	52.28	8.81	Stable
Pvul-KCS-6	Phvul.006G184900	Chr06	28607300	28609884	509	57.58	9.00	Stable
Pvul-KCS-7	Phvul.006G215000	Chr06	30773842	30775215	457	51.57	8.85	Unstable
Pvul-KCS-8	Phvul.007G009100	Chr07	657680	659317	483	54.81	9.06	Stable
Pvul-KCS-9	Phvul.007G009200	Chr07	661883	663334	483	54.98	8.92	Stable
Pvul-KCS-10	Phvul.007G027100	Chr07	2080156	2082926	496	56.00	9.15	Unstable
Pvul-KCS-11	Phvul.007G064000	Chr07	5712349	5713797	482	57.16	8.84	Unstable
Pvul-KCS-12	Phvul.007G279500	Chr07	39890399	39893153	519	58.07	9.33	Stable
Pvul-KCS-13	Phvul.009G023600	Chr09	5705803	5708064	512	57.58	9.47	Stable
Pvul-KCS-14	Phvul.009G084500	Chr09	13848281	13854143	534	60.57	9.14	Unstable
Pvul-KCS-15	Phvul.009G199000	Chr09	30198732	30201608	510	57.70	9.22	Stable
Pvul-KCS-16	Phvul.009G249200	Chr09	36980035	36983096	437	49.14	9.23	Unstable
Pvul-KCS-17	Phvul.009G257100	Chr09	37759369	37761230	525	58.57	8.82	Stable
Pvul-KCS-18	Phvul.011G068900	Chr11	6160872	6162607	469	53.31	8.61	Unstable
Pvul-KCS-19	Phvul.L003544	scaffold_30	265129	266550	473	53.59	8.94	Unstable

3.2. Chromosomal Location and Gene Duplication Analysis

KCS genes' chromosomal locations were specified, and the genes were found that unevenly distributed across several chromosomes. The evolution of the *Pvul-KCS* gene family was caused by both tandem and segmental duplications, which can be explained, in accordance with gene duplication studies. Tandem duplications were observed for several gene pairs, while segmental duplications involved genes located on different chromosomes.

As a result of gene duplication analysis, segmental duplication between *Pvul-KCS-2/Pvul-KCS-10*, *Pvul-KCS-8/Pvul-KCS-18* and *Pvul-KCS-18/Pvul-KCS-19* and tandem duplication between *Pvul-KCS-8/Pvul-KCS-9* and their Ka, Ks and Ka/Ks ratios are shown in Table 2. The Ka/Ks number implies positive selection in the evolutionary process when it is larger than 1, purifying selection when it is less than 1, and natural selection in duplication occurrences when it is equal to 1 (Juretic et al., 2005; İlhan, 2018; Kasapoglu et al., 2020).

3.3. Interspecific Phylogenetic Analysis of Pvul-KCS Proteins, Conserved Motif and Gene Structure

To explain the evolutionary relationships of Pvul-KCS proteins and to predict their potential functions, a phylogenetic tree was drawn using KCS-related proteins of *P. vulgaris*, *A. thaliana* and *G. max* species. The Neighbor-Joining (NJ) method was used with MEGA v11 (Molecular Evolutionary Genetic Analysis) software to perform phylogenetic tree analysis of a total of 70 KCS proteins from three plant species (Figure 2). At the phylogenetic tree classification of 19 *Pvul-KCS* gene were divided into 5 groups with *A. thaliana and G. max.*

Table 2. Ka/Ks ratios and segmental-tandem duplications for *P. vulgaris KCS* genes.

Gen 1	Gen 2	Ka	Ks	Ka/Ks	Duplication Type
***Pvul-KCS*-2**	***Pvul-KCS*-10**	1.300	0.0526	0.0404	**Segmental**
***Pvul-KCS*-8**	***Pvul-KCS*-18**	20.5922	0.3774	0.0183	**Segmental**
***Pvul-KCS*-18**	***Pvul-KCS*-19**	2.2121	0.1493	0.0675	**Segmental**
***Pvul-KCS*-8**	***Pvul-KCS*-9**	0.0369	0.0202	0.5478	**Tandem**

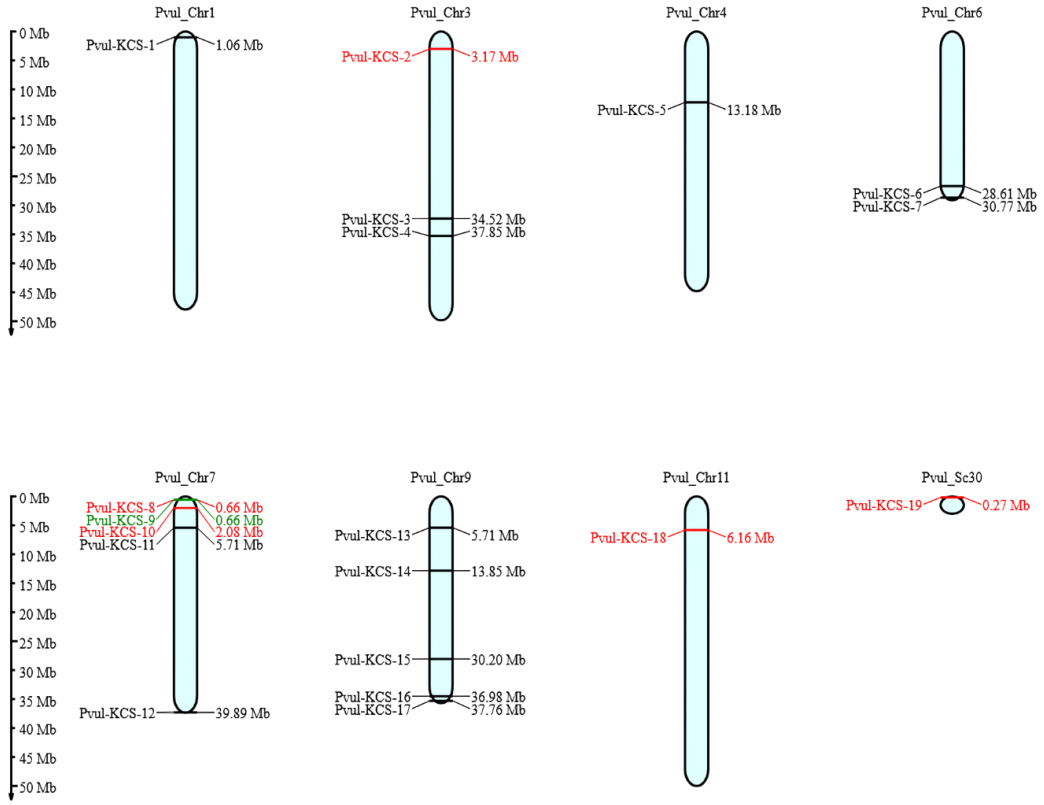

Figure 1. Chromosomal distribution of *Pvul-KCS* genes. Coloured parts indicate segmental duplications.

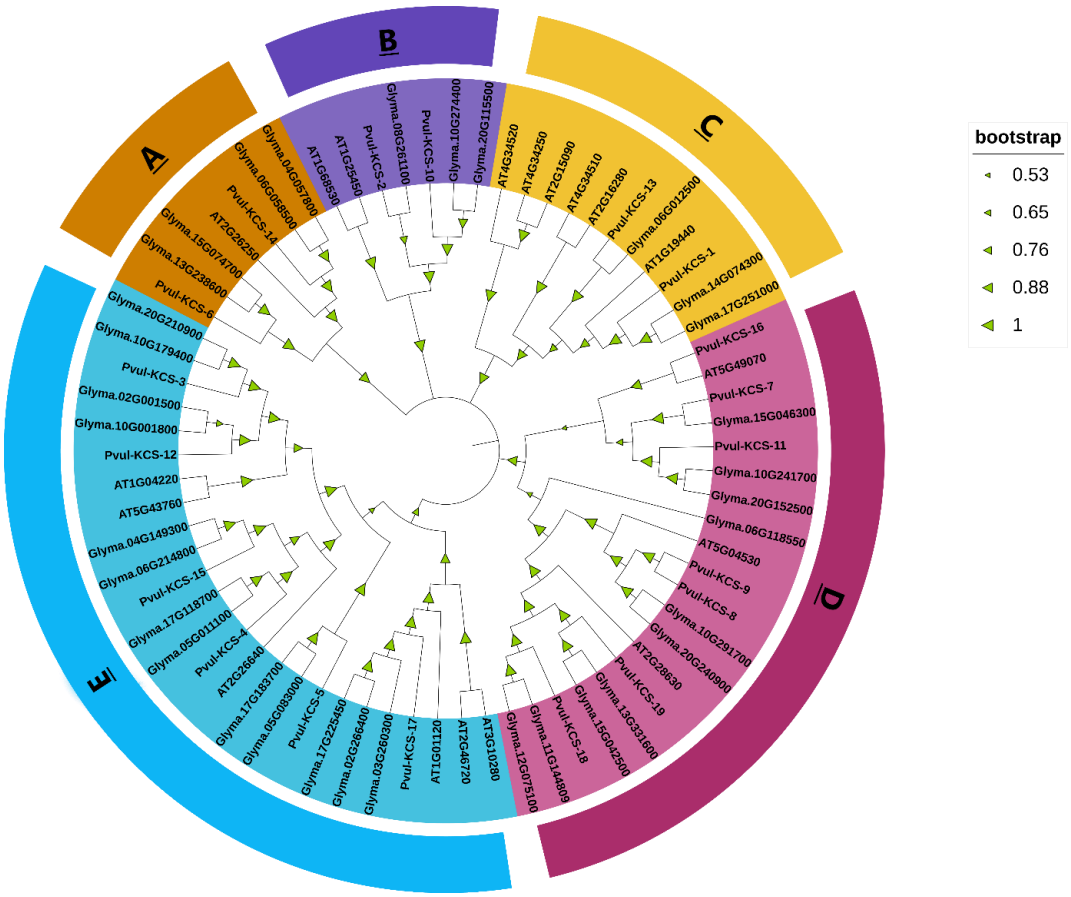

Figure 2. Phylogenetic tree constructed using three plant species' KCS proteins. *P. vulgaris* and two additional plant species' KCS full-length amino acid sequences were aligned with ClustalW, and a phylogenetic tree was created using the neighbor-joining (NJ) method with 1000 bootstraps using MEGA v11. Groups A, B, C, D, and E of the KCS subfamilies are denoted by the colours.

In their study, Zhang et al. (2022) observed that 25 *SbKCS* genes detected in the phylogenetic tree classification of *Sorghum bicolor* were divided into 5 groups with *A. thaliana*, *Oryza sativa*, *Zea mays* and *Brochypodium distachyon*.

Lian et al. (2020) observed that the 28 *MdKCS* genes identified in the phylogenetic tree classification of apple fruit (*Malus domestica*) were divided into 4 groups with *A. thaliana* and that there was a close relationship between different domains of KCS-related genes (KCS1-like, FAE1-like, FDH-like and CER6). In light of the information obtained as a result of the studies with KCS, it has been reached that *KCS* genes are divided into groups ranging from 3 to 8 in evolutionary terms and that these genes are mostly closely related to *A. thaliana* and *G. max* species.

In the conserved motif analysis of Pvul-KCS proteins using the MEME (v4.12.1) (Bailey et al., 2006) program, 10 conserved motifs were identified (Figure 3). It was discovered that the length of the identified motifs ranged from 21 to 50 amino acids. Pvul-KCS-5, -7, -8, -9, -16, -18 and -19 (9 motifs) had the least motifs, while the remaining Pvul-KCS's were equal and had the most motifs (10 motifs). Except for Motif 9, all motifs were detected in all KCS proteins. Also, the best matches corresponding to the motifs are given in Table 3.

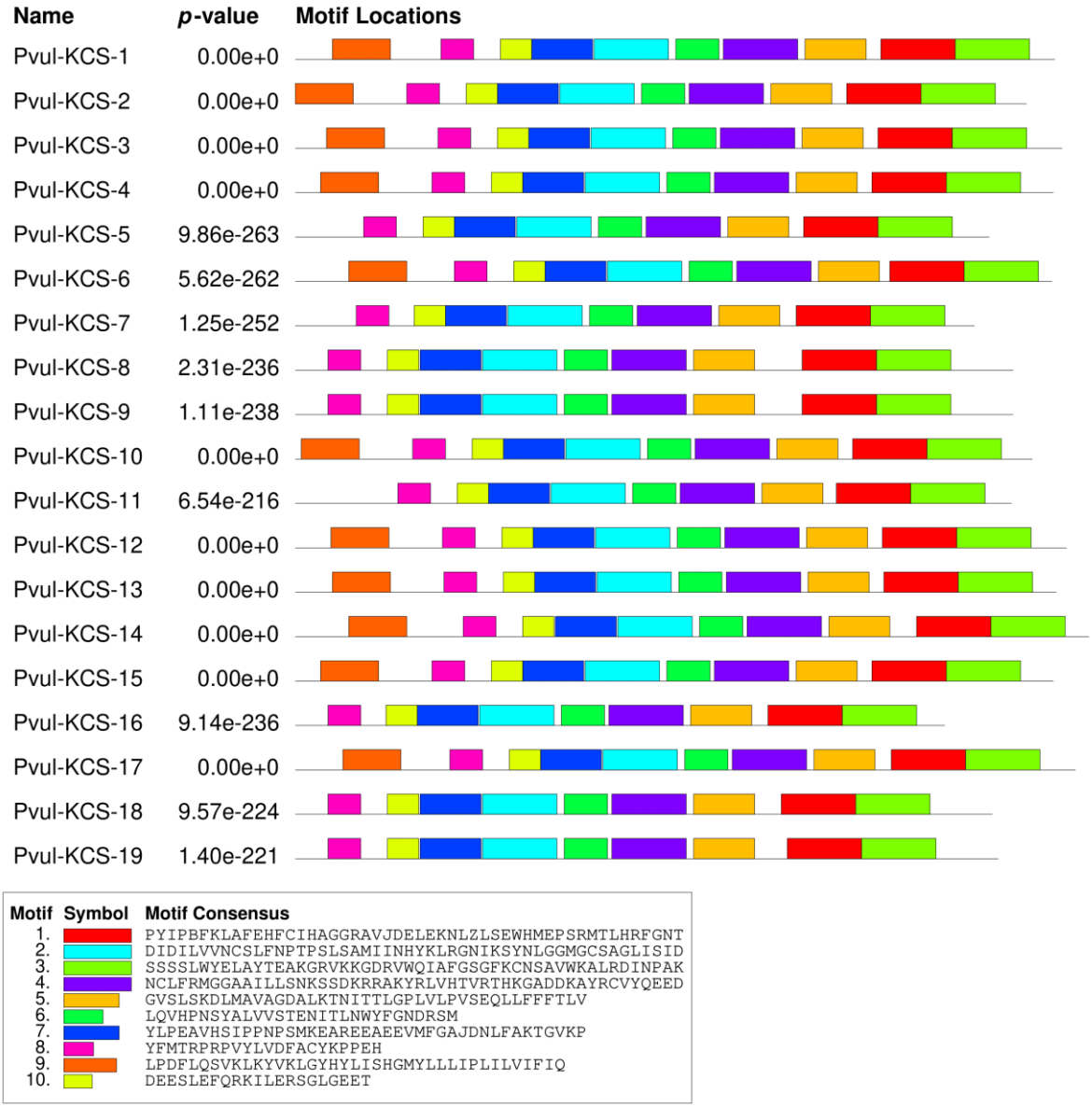

Figure 3. Predicted motif distribution in *Pvul-KCS* genes.

Zhang et al. (2022) predicted 10 motifs in *SbKCS* and identified the presence of five unique conserved motifs of these proteins; these five motifs were present throughout every single 25 SbKCS proteins, and they were placed in the same position throughout the protein sequences. This shows that practically all discovered *KCS* family genes exhibit a high degree of motif conservation.

Table 3. Predicted best possible matching information in *Pvul-KCS* genes.

MOTIF ID	WIDE	BEST POSSIBLE MATCH	Domain
MOTIF-1	50	PYIPDFKTAFEHFCIHAGGRAVIDELQKNLQLSEWHMEPSRMTLHRFGNT	Very-long-chain 3-ketoacyl-CoA synthase
MOTIF-2	50	DIDILVVNCSLFNPTPSLSAMIINHYKMRGNIKSYNLGGMGCSAGVISID	Very-long-chain 3-ketoacyl-CoA synthase
MOTIF-3	50	SSSSLWYELAYMEAKGRMKKGDRVWQIAFGSGFKCNSAVWKCMRDINPPK	NA
MOTIF-4	50	NCLFRMGGAAILLSNKPSDKRRAKYQLVHTVRTHKGADDKAYRCVYQEED	Very-long-chain 3-ketoacyl-CoA synthase
MOTIF-5	41	GVSLSKDLMAVAGDALKTNITTMGPLVLPMSEQLRFFFTLV	Very-long-chain 3-ketoacyl-CoA synthase
MOTIF-6	29	LQVHPNSYALVVSTENITPNWYQGNDRSM	NA
MOTIF-7	41	CPPEAVHYIPPNPTMKEAREEAEQVMFGAIDQLFAKTGVKP	Very-long-chain 3-ketoacyl-CoA synthase
MOTIF-8	22	YFMTRPRPVYLVDYACYKPPEH	NA
MOTIF-9	39	LPDFLQSVKLKYVKLGYHYLISHGMYLCLIPLIVVIFIQ	Very-long-chain 3-ketoacyl-CoA synthase
MOTIF-10	21	DEENLEFQRKILERSGLGEET	NA

NA: Not applicable.

Exon sizes (bp) and intron numbers were determined as a result of structural analyses performed on *Pvul-KCS* genes. As a result of the data obtained, *Pvul-KCS* was found to have 27 exons and 8 introns (Figure 4). The highest number of exons was 3 exons in *Pvul-KCS*-6 and *Pvul-KCS*-15 genes, while the other genes had 1 exon. However, region analysis data using the GSDS database showed that all *Pvul-KCS* members have symmetrical exons. Exons with symmetric splice sites at both ends are known as symmetric exons. Exon shuffling, recombination fusion, and protein domain exchange are probably facilitated by the abundance of phase 0 of symmetric exons (Gilbert, 1987; Patthy, 1987). The intron numbers in *Pvul-KCS* genes range from 0 to 2. It was observed that there were no intron regions in *Pvul-KCS* genes except *Pvul-KCS*-3, -5, -6, -7, -13, -15, and -17.

In their study, Zhang et al. (2022) demonstrated that the 25 *SbKCS* genes exhibited a range of intron counts, spanning from 0 to 1. Lian et al. (2020) showed that *KCS* genes within the same subgroup exhibited similar exon-intron distribution. In addition, it was observed that apple (*M. domestica*) *KCS* members have similar lengths and number of exon-introns as we determined in *P. vulgaris*.

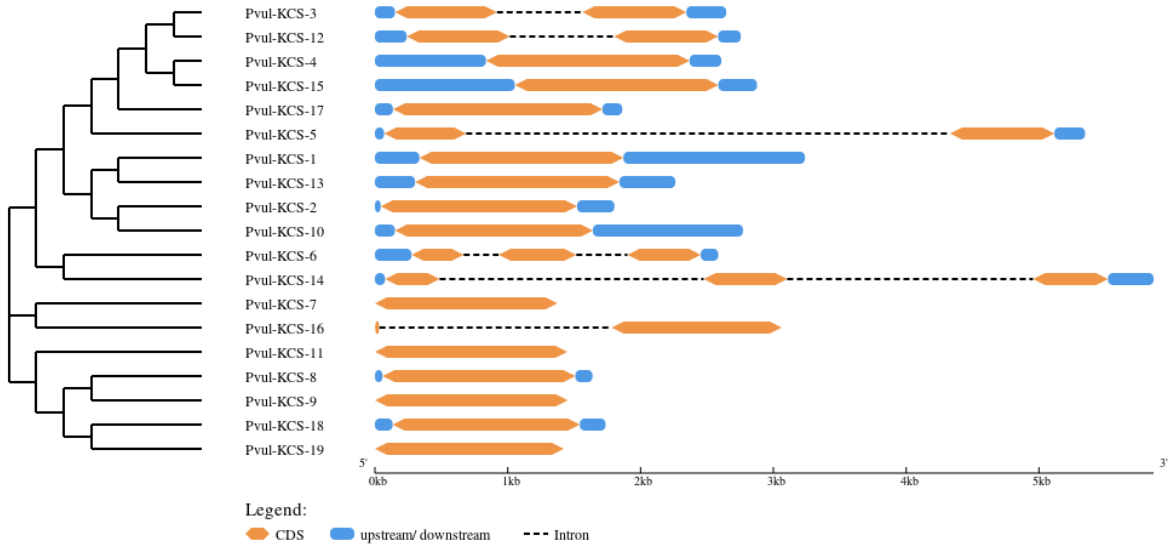

Figure 4. Exon and intron counts, lengths, and locations in *Pvul-KCS* genes.

3.4. Comparative Genomic Analysis

To better comprehend the *Pvul-KCS* gene family expansion and evolution in the *P. vulgaris* genome and the genomes of other species, synteny analysis was carried out. Tandem or segmental duplications of the *Pvul-KCS* gene were used to evaluate duplications. A comparative genomic analysis was conducted to identify homologous *KCS* genes in *Phaseolus vulgaris*, *Arabidopsis thaliana*, and *Glycine max*. The analysis revealed that some *KCS* genes were conserved among species, while others were unique to each species. Venn diagrams were used to visualize the shared and unique *KCS* genes among the three species (Figures 5 and 6).

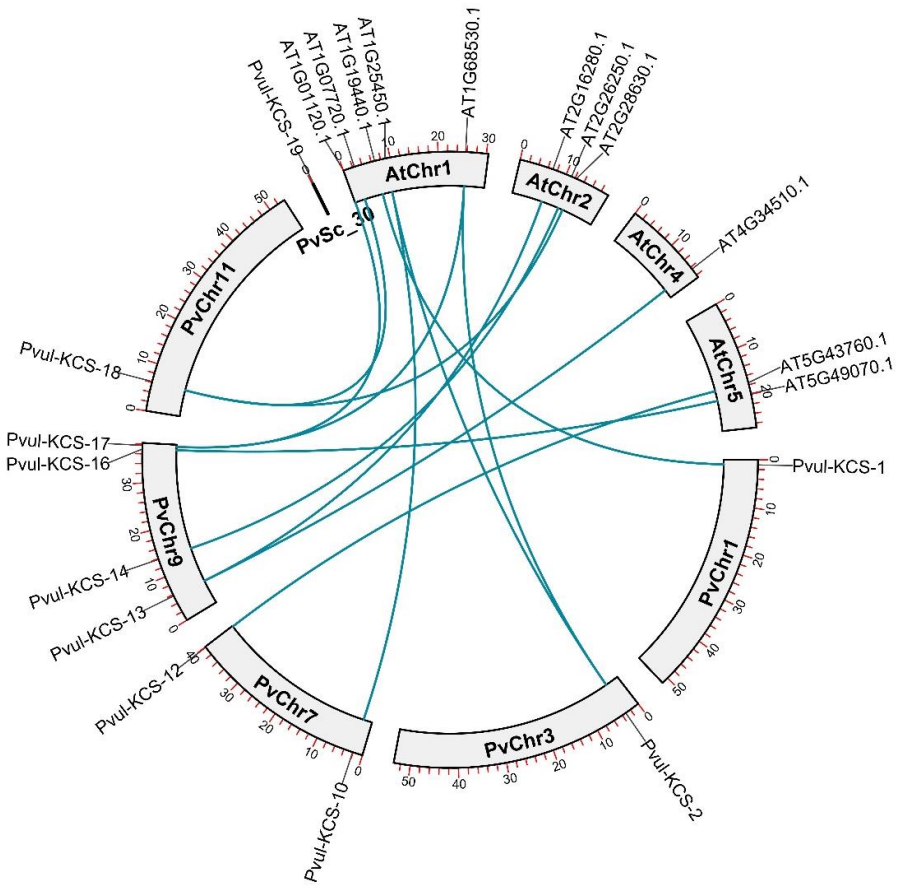

Figure 5. Synteny analysis of *P. vulgaris* and *A. thaliana* genes. *PvSc_30: scaffold_30 chromose.

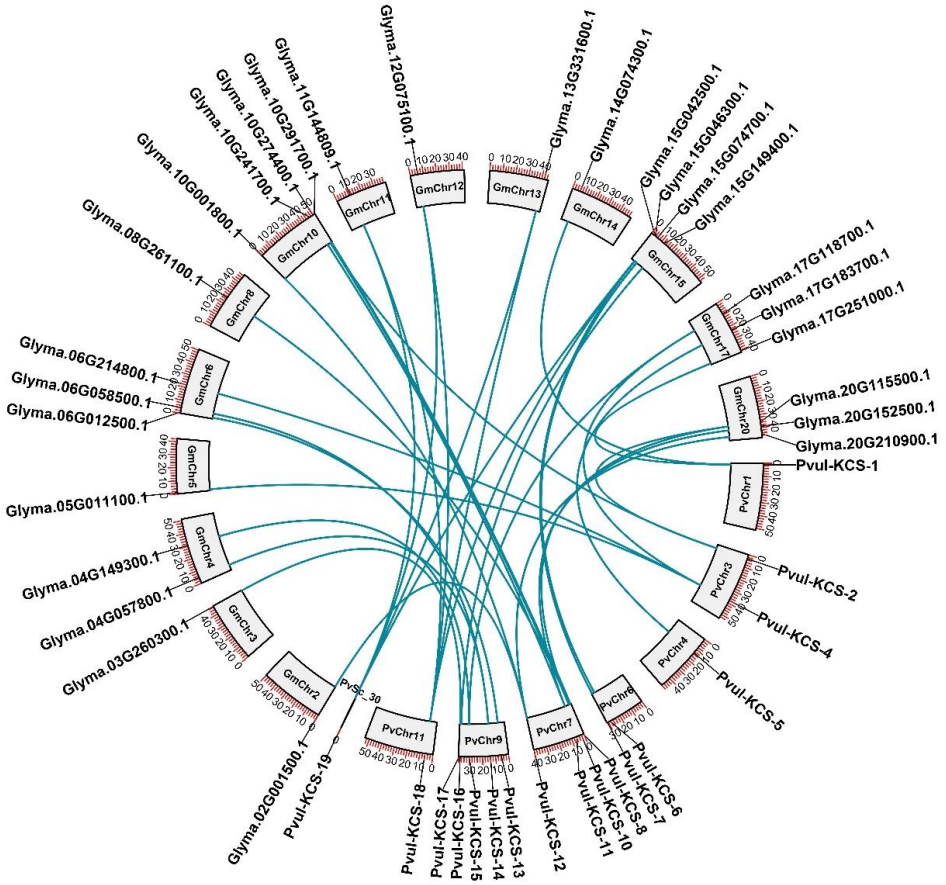

Figure 6. Synteny analysis of *P. vulgaris* and *G. max* genes. *PvSc_30: scaffold_30 chromose.

In order to demonstrate the evolutionary process of the *VvKCS* gene family, Zheng et al. (2023) created two comparative syntenic maps of grapevine linked to four representative plant species (*A. thaliana*, *M. domestica*, *O. sativa*, and *Musa acuminata*). A syntenic link between 14 *VvKCS* genes and those in *A. thaliana*, *M. domestica*, *O. sativa*, and *M. acuminata* was identified. Additionally, they discovered 25, and 15 orthologous pairs between *M. domestica* and *A. thaliana*, respectively.

3.5. Promoter Analysis of *Pvul-KCS* Genes

The sequences obtained from 2000 bp upstream of the 5' upstream regions of all *KCS* genes were analysed and it was determined that the promoter motifs in *KCS* genes play important roles in plant growth and development, adaptation to environmental conditions, molecular responses to abiotic and biotic stresses. The cis-acting elements located in the promoter regions of the *KCS* genes identified as a result of the data obtained from the PlantCARE database in the *P. vulgaris* genome were analysed and the detected cis-elements were made visually understandable using TBtools software (Figure 7). As a result of the data obtained, 82 cis-acting elements were detected in *Pvul-KCS* genes. It was determined that cis-acting elements such as MBS, ARE, W box, LTR, TC-rich repeats, which are associated with abiotic and biotic stresses, were localized in all *Pvul-KCS* genes.

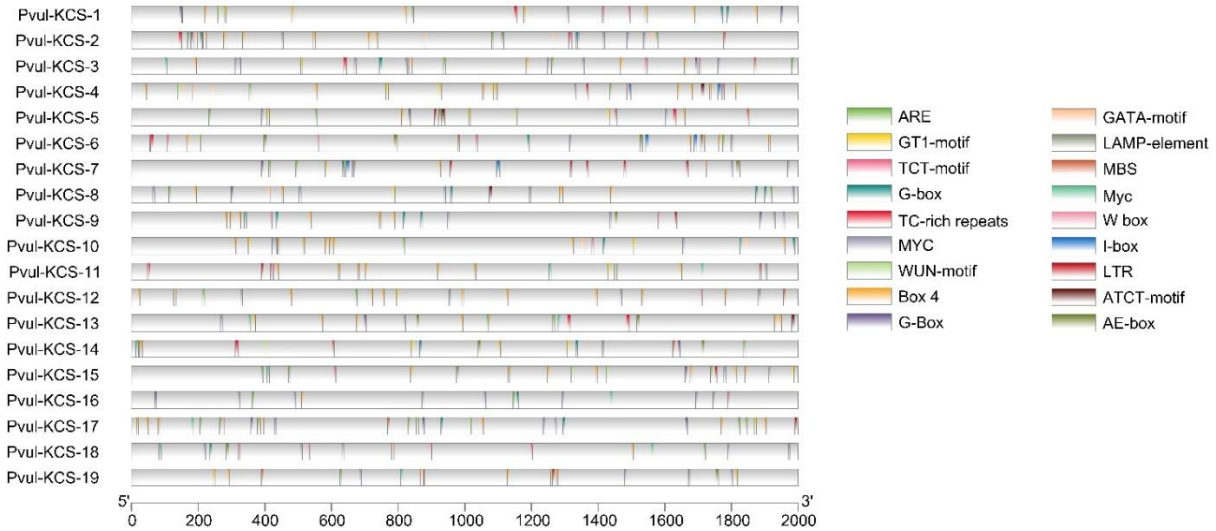

Figure 7. Promoter regions of *Pvul-KCS* genes. The promoter sequences (-2000 bp) of 19 *Pvul-KCS* genes were analysed with the help of the PlantCARE database. The scale indicates the upstream length along the translation codon. Different coloured boxes indicate different cis-acting elements.

In their study in *Passiflora edulis* fruit, Rizwan et al. (2022) found basically 4 different categories of cis-regulatory elements in PeKCS promoter regions. These are; 8 different cis-elements in plant growth and development, 10 different cis-elements in phytohormones, 15 different cis-elements in photosensitivity, and 7 different cis-elements in stress resistance.

3.6. Expression Profiling of *KCS* Genes in Response to Abiotic Stresses

To investigate the response of common bean *KCS* genes to abiotic stresses, RNA-Seq data from common bean leaf tissue subjected to drought and salt stress were analyzed. Differential gene expression analysis revealed that several *KCS* genes were differentially expressed under these stress conditions. This suggests that common bean *KCS* genes may play a role in the plant's response to abiotic stresses and could be potential targets for improving stress tolerance in common beans.

To determine the in-silico expression analysis of *Pvul-KCS* genes under salt and drought stress, the RNAseq data obtained from the SRA database were visualized (Figure 8). It was discovered that the expression levels of *Pvul-KCS* genes differed under salt and drought stress treatments based on the clustered heat map graph produced by CIMMiner with log2 transformation of RPKM values. While *Pvul-KCS*-9, *Pvul-KCS*-10, and *Pvul-KCS*-14 were the genes whose expression levels increased under salt stress, *Pvul-KCS*-13 and *Pvul-KCS*-15 were the genes whose expression levels increased under drought stress (Comparisons were made with the control group).

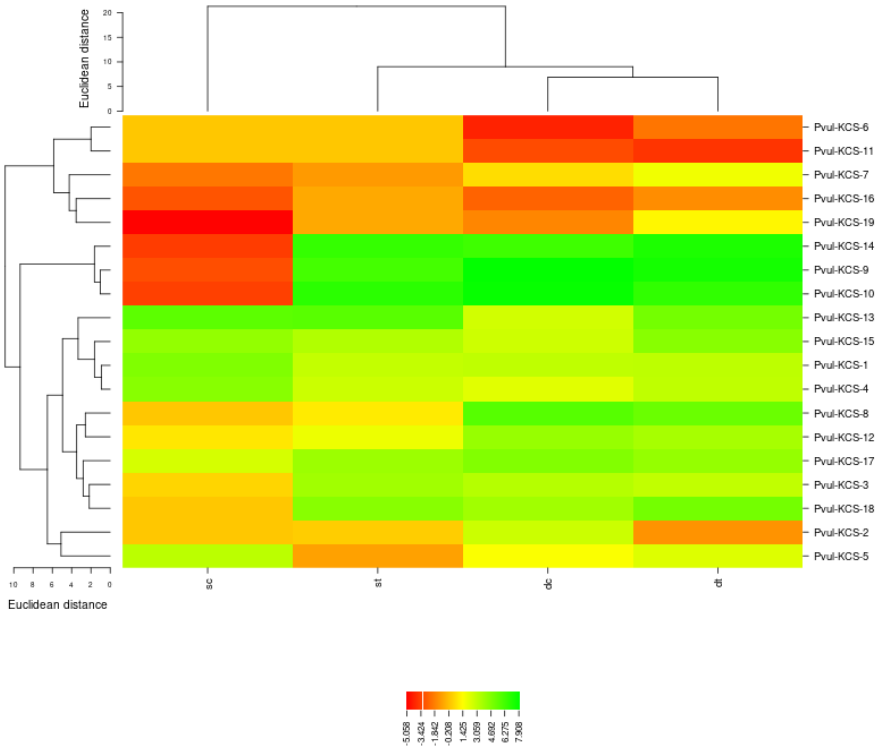

Figure 8. In silico expression analysis graph of *Pvul-KCS*.

Eight *MdKCS* genes in apple *MdKCS* genes showed an altered (down-up-down-regulation) trend under drought conditions in the study by Lian et al. (2020). Under drought-like conditions, *MdKCS12* and *MdKCS24* were observed to have up-regulated expression and *MdKCS6* expression was mostly down-regulated.

3.7. 3D Modelling of *Pvul-KCS* Genes and Their Intracellular Localization

With the help of Phyre2 database, blastp screening was performed with the data of KCS proteins obtained from the Protein Data Bank (PDB) and 3D homology modeling of KCS proteins was visualized with the help of these data (Figure 9).

In addition, the intracellular localization of Pvul-KCS proteins is shown in Figure 9. Using data from the WoLF PSORT database (Horton et al., 2007), all genes were predicted to be localized in regions such as plasma, vacuoles and endoplasmic reticulum.

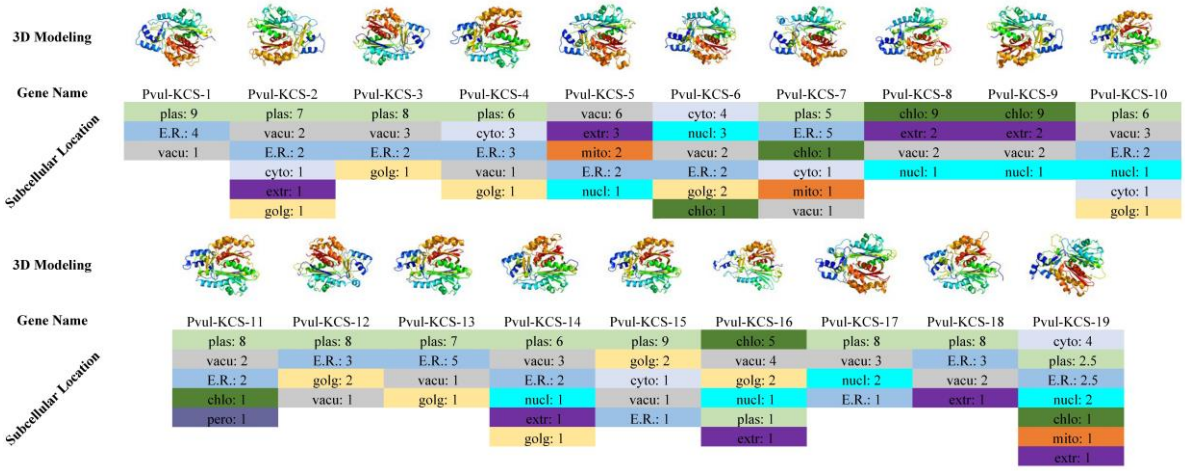

Figure 9. 3D structure modelling and intracellular localisation of Pvul-KCS proteins.

All 33 of the barley KCS proteins were found at the cell membrane in the study as a result of Tong et al. (2021), demonstrating that these proteins have highly conserved membrane protein catalytic activities.

Zhang et al. (2022) subcellular localization prediction analysis of Sorghum bicolor revealed that SbKCS proteins were primarily localized in the plasma membrane, followed by the mitochondria and chloroplast, indicating that SbKCS proteins may be expressed and function primarily in these organelles.

3.8. Protein-Protein Interactions of Pvul-KCS Proteins

Protein-protein interactions of Pvul-KCS proteins were visualized using Cytoscape software with the data obtained from the STRING database (Figure 10). Protein-protein interaction networks for particular gene families indicate the correlation between established members of the family (Piya et al., 2014). Rizwan et al. (2022) found that 31 PeKCS proteins demonstrated homology and interaction with established Arabidopsis KCS proteins in *P. edulis*. In their study, Rui et al. (2022) analysed the protein-protein interactions of *G. barbadense* KCS proteins, and found that over 90% of these proteins are involved in biosynthesis, elongation, and endoplasmic reticulum pathways related to fatty acids.

Figure 10. Protein-protein interactions (PPI) of identified KCS proteins.

4. Conclusion

In this study, a comprehensive analysis of the *KCS* gene family in common bean plants (*P. vulgaris* L.) was conducted. A total of 19 *KCS* genes were identified and characterized, providing insights into their molecular properties and subcellular localization. Phylogenetic analysis revealed the evolutionary relationships among KCS proteins in *P. vulgaris*, *A. thaliana*, and *G. max*. Additionally, the chromosomal location and gene duplication events of common bean *KCS* genes were investigated, shedding light on the expansion of this gene family in common beans.

The comparative genomic analysis showed that some KCS genes were conserved across common bean, Arabidopsis, and soycommon bean, while others were species-specific. This suggests that while certain *KCS* genes have essential functions shared among these plants, others may have evolved unique roles in each species. Understanding the conservation and divergence of *KCS* genes among different plant species can

provide valuable insights into their evolutionary history and functional significance.

Furthermore, the expression profiling of common bean *KCS* genes in response to abiotic stresses revealed differential gene expression patterns, implying potential roles in stress adaptation. These findings lay the foundation for future research aimed at elucidating the precise functions of specific *KCS* genes in stress tolerance mechanisms in common beans. Moreover, the information generated in this study can be leveraged for breeding programs focused on developing stress-tolerant common bean varieties, which are crucial for ensuring food security in regions susceptible to abiotic stress conditions.

Overall, this research contributes to our understanding of the *KCS* gene family in *P. vulgaris* and highlights their potential importance in stress responses, providing a basis for further functional studies and crop improvement efforts in common beans.

Acknowledgment

This study was presented at the VI international symposium "Biodiversity in Eurasia - SEAB 2023" and published in the Abstract Book as an abstract.

Conflict of Interest

The authors declare that they have no known financial conflicts of interest or close relationships that might have appeared to have an impact on the research presented in this study.

References

Araus, J. L., Slafer, G. A., Royo, C., & Serret, M. D. (2008). Breeding for yield potential and stress adaptation in cereals. *Critical Reviews in Plant Science*, *27*(6), 377-412. https://doi.org/10.1080/07352680802467736

Assefa, T., Mahama, A. A., Brown, A. V., Cannon, E. K., Rubyogo, J. C., Rao, I. M., & Cannon, S. B. (2019). A review of breeding objectives, genomic resources, and marker-assisted methods in common bean (*Phaseolus vulgaris* L.). *Molecular Breeding*, *39*(2), 20. https://doi.org/10.1007/s11032-018-0920-0

Aygören, A. S., Güneş, E., Muslu, S., Kasapoğlu, A. G., Yiğider, E., Aydın, M., & İlhan, E. (2023). Genome-wide analysis and characterization of SABATH gene family in Phaseolus vulgaris genotypes subject to melatonin under drought and salinity stresses. *Plant Molecular Biology Reporter*, *41*(2), 242-259. https://doi.org/10.1007/s11105-022-01363-5

Bailey, T. L., Williams, N., Misleh, C., & Li, W. W. (2006). MEME: Discovering and analyzing DNA and protein sequence motifs. *Nucleic Acids Research*, *34*(suppl_2), W369-W373. https://doi.org/10.1093/nar/gkl198

Bernard, A., & Joubès, J. (2013). Arabidopsis cuticular waxes: Advances in synthesis, export and regulation. *Progress in Lipid Research*, *52*(1), 110-129. https://doi.org/10.1016/j.plipres.2012.10.002

Blair, M. W. (2013). Mineral biofortification strategies for food staples: The example of common bean. *Journal of Agricultural and Food Chemistry*, *61*(35), 8287-8294. https://doi.org/10.1021/jf400774y

Broughton, W. J., Hernández, G., Blair, M., Beebe, S., Gepts, P., & Vanderleyden, J. (2003). Beans (*Phaseolus* spp.)–model food legumes. *Plant and Soil*, *252*, 55-128. https://doi.org/10.1023/A:1024146710611

Buruchara, R., Chirwa, R., Sperling, L., Mukankusi, C., Rubyogo, J. C., Mutonhi, R., & Abang, M. M. (2011). Development and delivery of bean varieties in Africa: The pan-Africa bean research alliance (PABRA) model. *African Crop Science Journal*, *19*(4), 227-245.

Chen, C., Chen, H., Zhang, Y., Thomas, H. R., Frank, M. H., He, Y., & Xia, R. (2020). TBtools: An integrative toolkit developed for interactive analyses of big biological data. *Molecular Plant*, *13*(8), 1194-1202. https://doi.org/10.1016/j.molp.2020.06.009

Dai, H., Zhang, Y., Jin, P., Song, F., Xu, W., Liu, A., Meng, Z., & Yang, T. (2021). Identification of KCS gene family and functional analysis of FAE-like genes from *Malania oleifera*. *Oil Crop Science*, *6*(1), 35-40. https://doi.org/10.1016/j.ocsci.2021.03.003

Ekinci, M., Ors, S., Yildirim, E., Dursun, A., Turan, M., Sahin, U., & Kul, R. (2020). Monitoring some antioxidant enzymes and physiological indices of chard exposed to nitric oxide under drought stress. *The Russian Journal of Plant Physiology*, *67*(4), 740-749.

Ghanevati, M., & Jaworski, J. G. (2001). Active-site residues of a plant membrane-bound fatty acid elongase β-ketoacyl-CoA synthase, FAE1 KCS. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1530*(1), 77-85. https://doi.org/10.1016/S1388-1981(00)00168-2

Gilbert, W. (1987). The exon theory of genes. *Cold Spring Harbor Symposia on Quantitative Biology*, *52*(0), 901-905. https://doi.org/10.1101/sqb.1987.052.01.098

Glick, B. R. (2012). Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica*, *2012*, 1-15. https://doi.org/10.6064/2012/963401

Gregorio Jorge, J., Villalobos-López, M. A., Chavarría-Alvarado, K. L., Ríos-Meléndez, S., López-Meyer, M., & Arroyo-Becerra, A. (2020). Genome-wide transcriptional changes triggered by water deficit on a drought-tolerant common bean cultivar. *BMC Plant Biology*, *20*, 525. https://doi.org/10.1186/s12870-020-02664-1

Hiz, M. C., Canher, B., Niron, H., & Turet, M. (2014). Transcriptome analysis of salt tolerant common bean (*Phaseolus vulgaris* L.) under saline conditions. *PloS One*, *9*(3), e92598. https://doi.org/10.1371/journal.pone.0092598

Horton, P., Park, K. J., Obayashi, T., Fujita, N., Harada, H., Adams-Collier, C. J., & Nakai, K. (2007). WoLF PSORT: Protein localization predictor. *Nucleic Acids Research*, *35*(suppl_2), W585-W587. https://doi.org/10.1093/nar/gkm259

Hu, B., Jin, J., Guo, A. Y., Zhang, H., Luo, J., & Gao, G. (2015). GSDS 2.0: An upgraded gene feature visualization server. *Bioinformatics*, *31*(8), 1296-1297. https://doi.org/10.1093/bioinformatics/btu817

İlhan, E. (2018). *Eucalyptus grandis* YABBY transkripsiyon faktörlerinin genom bazında analizi. *Türkiye Tarımsal Araştırmalar Dergisi*, *5*(2), 158-166. https://doi.org/10.19159/tutad.408654 (In Turkish)

İlhan, E., Kasapoğlu, A. G., Muslu, S., Aygören, A. S., & Aydin, M. (2023). Genome-wide analysis and characterization of *Eucalyptus grandis* TCP transcription factors. *Journal of Agricultural Sciences*, *29*(2), 413-426. https://doi.org/10.15832/ankutbd.1104949

Juretic, N., Hoen, D. R., Huynh, M. L., Harrison, P. M., & Bureau, T. E. (2005). The evolutionary fate of MULE-mediated duplications of host gene fragments in rice. *Genome Research*, *15*(9), 1292-1297. https://doi.org/10.1101/gr.4064205

Kasapoğlu, A. G., Ilhan, E., Kizilkaya, D., Hossein-Pour, A., & Haliloğlu, K. (2020). Sorgum [*Sorghum bicolor* (L.) Moench] genomunda BES1 transkripsiyon faktör ailesinin genom çaplı analizi. *Türkiye Tarımsal Araştırmalar Dergisi*, *7*(1), 85-95. https://doi.org/10.19159/tutad.671605 (In Turkish)

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., & Sternberg, M. J. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols*, *10*(6), 845-858. https://doi.org/10.1038/nprot.2015.053

Kunst, L., Taylor, D. C., & Underhill, E. W. (1992). Fatty acid elongation in developing seeds of *Arabidopsis thaliana*. *Plant Physiology and Biochemistry*, *30*(4), 425-434.

Lamesch, P., Berardini, T. Z., Li, D., Swarbreck, D., Wilks, C., Sasidharan, R., Muller, R., Dreher, K., Alexander, D. L., Garcia-Hernandez, M., Karthikeyan, A. S., Lee, C. H., Nelson, W. D., Ploetz, L., Singh, S., Wensel, A., & Huala, E. (2012). The Arabidopsis information resource (TAIR): Improved gene annotation and new tools. *Nucleic Acids Research*, *40*(D1), D1202-D1210. https://doi.org/10.1093/nar/gkr1090

Lescot, M., Déhais, P., Thijs, G., Marchal, K., Moreau, Y., Van de Peer, Y., Rouzé, P., & Rombauts, S. (2002). PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Research*, *30*(1), 325-327. https://doi.org/10.1093/nar/30.1.325

Letunic, I., & Bork, P. (2011). Interactive tree of life v2: Online annotation and display of phylogenetic trees made easy. *Nucleic Acids Research*, *39*(suppl_2), W475-W478. https://doi.org/10.1093/nar/gkr201

Lian, X. Y., Wang, X., Gao, H. N., Jiang, H., Mao, K., You, C. X., & Hao, Y. J. (2020). Genome wide analysis and functional identification of MdKCS genes in apple. *Plant Physiology and Biochemistry*, *151*, 299-312. https://doi.org/10.1016/j.plaphy.2020.03.034

Liu, E. K., Mei, X. R., Yan, C. R., Gong, D. Z., & Zhang, Y. Q. (2016). Effects of water stress on photosynthetic characteristics, dry matter translocation and WUE in two winter wheat genotypes. *Agricultural Water Management*, *167*, 75-85. https://doi.org/10.1016/j.agwat.2015.12.026

Lynch, M., & Conery, J. S. (2003). The evolutionary demography of duplicate genes. *Journal of Structural and Functional Genomics*, *3*, 35-44. https://doi.org/10.1023/A:1022696612931

Maggio, A., De Pascale, S., Ruggiero, C., & Barbieri, G. (2005). Physiological response of field-grown cabbage to salinity and drought stress. *European Journal of Agronomy*, *23*(1), 57-67. https://doi.org/10.1016/j.eja.2004.09.004

Muslu, S., Kasapoğlu, A. G., Güneş, E., Aygören, A. S., Yiğider, E., İlhan, E., & Aydın, M. (2023). Genome-wide analysis of glutathione S-transferase gene family in *P. vulgaris* under drought and salinity stress. *Plant Molecular Biology Reporter*. https://doi.org/10.1007/s11105-023-01400-x

Oner, B. M., Ilhan, E., Kasapoglu, A. G., Muslu, S., Aygoren, A. S., Ucar, S., & Aydin, M. (2022). Genome wide analysis and characterization of NPR-like gene family of *Phaseolus vulgaris* L. *Natural Products and Biotechnology*, *2*(1), 23-41.

Ors, S., Ekinci, M., Yildirim, E., & Sahin, U. (2016). Changes in gas exchange capacity and selected physiological properties of squash seedlings (*Cucurbita pepo* L.) under well-watered and drought stress conditions. *Archives of Agronomy and Soil Science*, *62*(12), 1700-1710. https://doi.org/10.1080/03650340.2016.1168517

Patthy, L. (1987). Intron-dependent evolution: Preferred types of exons and introns. *FEBS Letters*, *214*(1), 1-7. https://doi.org/10.1016/0014-5793(87)80002-9

Piya, S., Shrestha, S. K., Binder, B., Stewart Jr, C. N., & Hewezi, T. (2014). Protein-protein interaction and gene co-expression maps of ARFs and Aux/IAAs in Arabidopsis. *Frontiers in Plant Science*, *5*, 744. https://doi.org/10.3389/fpls.2014.00744

Quevillon, E., Silventoinen, V., Pillai, S., Harte, N., Mulder, N., Apweiler, R., & Lopez, R. (2005). InterProScan: Protein domains identifier. *Nucleic Acids Research*, *33*(suppl_2), W116-W120. https://doi.org/10.1093/nar/gki442

Rakhimzhanova, A., Kasapoğlu, A. G., Sapakova, A., İlhan, E., Zharmukhametova, R., Turan, M., Zekenova, L., Muslu, S., Kazhygeldiyeva, L., Aydın, M., & Çiltaş, A. (2023). Expression analysis and characterization of the CPP gene family of melatonin-treated common bean cultivars under different abiotic stresses. *South African Journal of Botany*, *160*, 282-294. https://doi.org/10.1016/j.sajb.2023.07.013

Rizwan, H. M., Shaozhong, F., Li, X., Bilal Arshad, M., Yousef, A. F., Chenglong, Y., & Chen, F. (2022). Genome-wide identification and expression profiling of KCS gene family in passion fruit (*Passiflora edulis*) under *Fusarium kyushuense* and drought stress conditions. *Frontiers in Plant Science*, *13*, 872263. https://doi.org/10.3389/fpls.2022.872263

Rui, C., Chen, X., Xu, N., Wang, J., Zhang, H., Li, S., & Ye, W. (2022). Identification and structure analysis of KCS family genes suggest their reponding to regulate fiber development in long-staple cotton under salt-alkaline stress. *Frontiers in Genetics*, *13*, 812449. https://doi.org/10.3389%2Ffgene.2022.812449

Sahin, U., Ekinci, M., Ors, S., Turan, M., Yildiz, S., & Yildirim, E. (2018). Effects of individual and combined effects of salinity and drought on physiological, nutritional and biochemical properties of cabbage (*Brassica oleracea* var. capitata). *Scientia Horticulturae*, *240*, 196-204. https://doi.org/10.1016/j.scienta.2018.06.016

Schmutz, J., McClean, P. E., Mamidi, S., Wu, G. A., Cannon, S. B., Grimwood, J., ... & Jackson, S. A. (2014). A reference genome for common bean and genome-wide analysis of dual domestications. *Nature Genetics*, *46*(7), 707-713. https://doi.org/10.1038/ng.3008

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*(10), 2731-2739. https://doi.org/10.1093/molbev/msr121

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, *25*(24), 4876-4882. https://doi.org/10.1093/nar/25.24.4876

Tong, T., Fang, Y. X., Zhang, Z., Zheng, J., Zhang, X., Li, J., & Zhang, X. (2021). Genome-wide identification and expression pattern analysis of the KCS gene family in barley. *Plant Growth Regulation*, *93*, 89-103. https://doi.org/10.1007/s10725-020-00668-3

Valliyodan, B., Cannon, S. B., Bayer, P. E., Shu, S., Brown, A. V., Ren, L., ... & Nguyen, H. T. (2019). Construction and comparison of three reference-quality genome assemblies for soybean. *The Plant Journal*, *100*(5), 1066-1082. https://doi.org/10.1111/tpj.14500

Verslues, P. E., Agarwal, M., Katiyar-Agarwal, S., Zhu, J., & Zhu, J. K. (2006). Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *The Plant Journal*, *45*(4), 523-539. https://doi.org/10.1111/j.1365-313X.2005.02593.x

Voorrips, R. (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity*, *93*(1), 77-78. https://doi.org/10.1093/jhered/93.1.77

Wang, Y., Tang, H., DeBarry, J. D., Tan, X., Li, J., Wang, X., Lee, T., Jin, H., Marler, B., Guo, H., Kissinger, J. C., & Paterson, A. H. (2012). MCScanX: A toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Research*, *40*(7), e49-e49. https://doi.org/10.1093/nar/gkr1293

Wang, X., Guan, Y., Zhang, D., Dong, X., Tian, L., & Qu, L. Q. (2017). A β-ketoacyl-CoA synthase is involved in rice leaf cuticular wax synthesis and requires a CER2-LIKE protein as a cofactor. *Plant Physiology*, *173*(2), 944-955. https://doi.org/10.1104/pp.16.01527

Weinstein, J. N., Myers, T. G., O'Connor, P. M., Friend, S. H., Fornace Jr, A. J., Kohn, K. W., & Paull, K. D. (1997). An information-intensive approach to the molecular pharmacology of cancer. *Science*, *275*(5298), 343-349. https://doi.org/10.1126/science.275.5298.343

Xiao, G. H., Wang, K., Huang, G., & Zhu, Y. X. (2016). Genome-scale analysis of the cotton KCS gene family revealed a binary mode of action for gibberellin A regulated fiber growth. *Journal of Integrative Plant Biology*, *58*(6), 577-589. https://doi.org/10.1111/jipb.12429

Xue, Y., Jiang, J., Yang, X., Jiang, H., Du, Y., Liu, X., Xie, R., & Chai, Y. (2020). Genome-wide mining and comparative analysis of fatty acid elongase gene family in *Brassica napus* and its progenitors. *Gene*, *747*, 144674. https://doi.org/10.1016/j.gene.2020.144674

Yang, Z., & Nielsen, R. (2000). Estimating synonymous and nonsynonymous substitution rates under realistic evolutionary models. *Molecular Biology and Eevolution*, *17*(1), 32-43. https://doi.org/10.1093/oxfordjournals.molbev.a026236

Yang, H., Mei, W., Wan, H., Xu, R., & Cheng, Y. (2021). Comprehensive analysis of KCS gene family in Citrinae reveals the involvement of CsKCS2 and CsKCS11 in fruit cuticular wax synthesis at ripening. *Plant Science*, *310*, 110972. https://doi.org/10.1016/j.plantsci.2021.110972

You, F. M., Li, P., Kumar, S., Ragupathy, R., Li, Z., Fu, Y. B., & Cloutier, S. (2014). Genome-wide identification and characterization of the gene families controlling fatty acid biosynthesis in flax (*Linum usitatissimum* L). *Journal of Proteomics & Bioinformatics*, *7*(10), 310-326. https://doi.org/10.4172/jpb.1000334

Zhang, A., Xu, J., Xu, X., Wu, J., Li, P., Wang, B., & Fang, H. (2022). Genome-wide identification and characterization of the KCS gene family in sorghum (*Sorghum bicolor* (L.) Moench). *PeerJ*, *10*, e14156. https://doi.org/10.7717/peerj.14156

Zheng, H., Liang, Y., Hong, B., Xu, Y., Ren, M., Wang, Y., & Tao, J. (2023). Genome-scale analysis of the grapevine KCS genes reveals its potential role in male sterility. *International Journal of Molecular Sciences*, *24*(7), 6510. https://doi.org/10.3390/ijms24076510

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

https://prensip.gen.tr

RESEARCH ARTICLE

A Study on the Germination of *Origanum acutidens* L. Seeds Subjected to Pre-Treatment of Gibberellic Acid and Colchicine

Halit Karagöz✉

Eastern Anatolia Agricultural Research Institute, Gezköy-Dadaşkent, Erzurum/Türkiye

ARTICLE INFO

Article History
Received: 06.10.2023
Accepted: 21.11.2023
First Published: 30.12.2023

Keywords
Colchicine
Endemic plant
O. acutidens
Seed germination
Thyme
Wild plant

ABSTRACT

In Türkiye, the general name for aromatic plant species belonging to the Lamiaceae family is "thyme". However, species containing thymol/carvacrol type essential oil are considered "thyme". *Origanum acutidens* is one of the thyme species that grows endemic in the Northeastern Anatolia Region of Türkiye. The low germination rate of its seeds is one of the factors limiting the studies conducted on this plant. This study was conducted to investigate the effects of different doses of colchicine and gibberellic acid on germination in *O. acutident* seeds. Seeds collected from the plant's natural habitat at the end of the flowering period were used as plant material. The experiment was planned as control (only distilled water) and treatments consisting of three different gibberellic acid (GA_3) [100 ppm (GA1), 200 ppm (GA2) and 300 ppm (GA3)] and four different colchicine doses [0.01 mM (C1), 0.02 mM (C2), 0.04 mM (C3) and 0.08 mM (C4)]. The applications were kept at 25±1 ºC for 12 hours. After the waiting period, all seeds were filtered and placed, 50 seeds each, in 9 cm diameter petri dishes between two layers of sterile filter paper sheets. The experiment was carried out in 4 replications. Some parameters of the germination (Germination rate (GR), Germination time (GT), Average germination time (AGT)) and early seedling period (Embryonal root length (ERL), Number of embryonal roots (NER), Root fresh weight (RFW), Root dry weight (RDW), Grass sheath length (GSH)) were measured and the results were statistically evaluated. In general, the highest values obtained from all evaluated germination (92.0% GR and 1.7 day AGT) and early seedling parameters (10.4 cm ERL, 4.6 NER, 0.095 g RFW, 0.028 g RDW and 3.6 cm GSL) were found to belong to the GA3 application. The lowest values obtained from the relevant parameters were obtained with the C4 application. In our study, it was observed that gibberellic acid applications significantly increased germination in this plant and positively increased the parameters related to germination. Based on the study results, we think that colchicine stimulates germination at certain rates, but causes death by having a toxic effect in increasing doses.

Please cite this paper as follows:

Karagöz, H. (2023). A study on the germination of *Origanum acutidens* L. seeds subjected to pre-treatment of gibberellic acid and colchicine. *Journal of Agricultural Production*, *4*(2), 152-158. https://doi.org/10.56430/japro.1372119

1. Introduction

Origanum known as oregano and marjoram (Davis, 1982; Baser, 2002) belongs to the Lamiaceae family. There are nearly 900 species of origanum in the world. Türkiye is also the gene center of this genus, and 22 species and 4 subspecies of Origanum. These species are of great economic importance (Kizil et al., 2009). Türkiye makes a significant contribution to the oregano trade, which is approximately 60 million dollars in the world, with its annual production of 15 thousand tons (Sari & Altunkaya, 2015). The use of *Origanum* species has been known for tens of thousands of years (Tepe et al., 2016). *Origanum* species are widely used in alcoholic beverages, as culinary herbs and to flavor food products (Aligiannis et al., 2001; Cetin et al., 2011).

✉Correspondence
E-mail address: halit.karagoz@tarimorman.gov.tr

Origanum acutidens, an important member of the *Origanum* species, grows endemically in Türkiye, in the North East Anatolian region (Karagoz, 2018). *O. acutidens* is used as herbal tea by the people in the region where it grows (Tumen et al., 1995). It is known that this plant has antimicrobial and insecticidal effects, antitumor effects and antioxidant effects due to its high content of carvacrol (Karagoz & Parlakova Karagoz, 2019). In addition, it has the potential to become an ornamental plant with its magnificent appearance (Karagoz et al., 2022). It has very beautiful flowers (with white to pale yellow or pink corollas) that bloom between June and August (Ietswaart, 1980; Davis, 1982).

Applications such as the stratification of seeds, classifying them according to their size, soaking them before sowing, etching them with acids, treating them with growth regulators, sowing them in gel form after germination, keeping them in nutrients or osmotic solutions, and coating are among the pre-treatments before sowing (Yamaguchi & Kamiya, 2002; Karakurt et al., 2010). Gibberellic acid is widely used as a priming method and/or the pre-treatment. Because gibberellic acid is effective in eliminating dormancy in seed production (Yamaguchi & Kamiya, 2002; Karakurt et al., 2010; Yıldız et al., 2017). Colchicine ($C_{22}H_{25}NO_6$) is an alkaloid found in the seed or bulb of *Colchicum autumnale*, belonging to the Liliaceae family (Kwon et al., 2016). Colchicine is used in biological research to promote polyploidy and in cultivation and as a positive control in tubulin binding assays (Trease & Evans, 1983). It interferes with the poleward movement of chromosomes and microtubule formation during the middle stage of cell division and induces polyploidization (Hadlaczky et al., 1983). Additionally, researchers have produced studies demonstrating the germination stimulating effects of colchicine (Bond, 1942; Pande & Khetmalas, 2012; Abd El-Latif et al., 2018; Lv et al., 2021).

As a result of our preliminary studies, we determined that the germination rate of *O. acutidens* seeds was very low (2.5-8%). An important factor limiting studies on this plant is its low seed germination rate. It was carried out to evaluate the effects of gibberalic acid and colchicine applications at different doses on the germination characteristics of wild *O. acutidens* seeds.

2. Materials and Methods

Seeds forming the plant material of the study were collected from Askale district of Erzurum province of Türkiye, which is located approximately 2170 m altitude (40°1'19" N, 40°38'28" E) in October (2022), at the end of the flowering period of *O. acutidens*. For the identification of plant *O. acutidens* was used as a main source "Flora of Turkey and the East Aegean Islands" (Davis, 1982). It was identified by Prof. Dr. Ramazan Çakmakçı. Before setting up the germination experiment, the seeds were surface sterilized with 20% sodium hypochlorite for 10 minutes. After, the seeds were passed through distilled water seven times and left to dry in sterile conditions (Yıldız et al., 2017).

In the research, three different gibberellic acid (GA_3) (100 (*GA1*), 200 (*GA2*) and 300 (*GA3*) ppm) and four different colchicine doses (0.01 mM (*C1*), 0.02 mM (*C2*), 0.04 mM (*C3*) and 0.08 mM (*C4*) Colchicine) and distilled water (Control) were applied to *O. acutidens* seeds. Experiments were carried out in 4 replications using a total of 32 petri dishes.

The sterilized and dried seeds were kept in GA (100, 200 and 300 ppm), colchicine (0.01, 0.02, 0.04 and 0.08 mM) and distilled water (H_2O) in dark conditions at 25±1 °C for 12 hours until the moisture content reached 12-13% (Mangena, 2021). After the waiting period, all seeds were filtered and placed, 50 seeds each, in 9 cm diameter petri dishes between two layers of sterile filter paper sheets (ISTA, 1996). A germination experiment was conducted based on the germination protocol of the *Origanum vulgare* species (ISTA, 1996) and the germination experiment was completed on the 21st day.

At the end of the experiment, the germination rate (GR) was determined by counting the seeds that germinated at the same time every day. When the radicle reached 1-5 mm, the seed was considered germinated and removed from the petri dish. At the end of 21 days, the GR, GT and AGT parameters were calculated through the equations below (Gosh et al., 2014; Nasırcılar et al., 2020). In addition, embryonal root length (ERL) was determined by measuring embryonal roots with a millimetric ruler. Number of embryonal roots (NER) was determined as the total number of embryonal roots formed in a plant. Root fresh weight (RFW) was the fresh weight of embryonal roots separated from the seed. Root dry weight (RDW) was determined by drying the roots, whose fresh root weight was determined, at 72 °C for 72 hours. Grass sheath length (GSL) was obtained by measuring the part of the plant between the seed and the tip of the grass sheath where the leaf emerges, with a millimetric ruler.

Germination rate (GR): (Total number of seeds germinated /50) x 100 (1)

Germination time (GT): n1/t1 + n2/t2 + ……. + nn/tn (2)

In the formula, n1, n2… n = number of seeds germinated on day counted from the beginning of the test.

t1, t2….. denote the number of days in which germination occurs (Ellis & Roberts, 1981; Nasırcılar et al., 2020).

Average germination time (AGT): Σ Dn / Σn (3)

In the formula, D = days counted from the beginning of the test, n = number of seeds germinated on day D (Nasırcılar et al., 2020).

2.1. Statistical Analysis

Results obtained from the end of the experiment were evaluated according to analysis of variance in the SPSS

statistical program (Statistical Package for Social Sciences, Version 22.0). Supplementary statistics and significance levels of the analysis results are given in tables. Duncan (p=0.05 or 0.01) test was performed to determine the significance of the difference between applications. Also online heatmapper software was used for heatmapper of all treatments.

3. Results

In our study, the highest value for the germination rate (GR), which was determined by dividing the number of germinated seeds by the total number of seeds allowed to germinate, was determined in the GA3 application (92.0%). The lowest value for the GR was obtained from the C4 (4.0%) application. The highest germination time *(*GT) was determined in the GA3 (1.7) application, while the lowest GT was obtained from the C4 (0.07) application. While the maximum embryonal root length (ERL) was determined in GA3 (10.4 cm) application, the minimum ERL value was detected in C4 (4.6 cm) application. In terms of the number of embryonal roots (NER) parameter, the highest value was found in the GA3 (4.6) application (Table 1).

Table 1. Results of germination and early seedling development parameters of *O. acutidens* seeds.

Level	GR (%)**	GT**	ERL (cm)**	NER (Number)**	RFW (g)**	RDW (g)**	GSL (cm)**	AGT (day)*
Control	8.0f	0.16f	5.5d	3.5e	0.05e	0.016e	2.5f	13.0bc
C1	10.0ef	0.19ef	5.6d	3.6d	0.04f	0.011g	2.8d	13.0bc
C2	16.0d	0.31d	5.9d	3.7c	0.06d	0.019c	2.9c	13.1bc
C3	12.0e	0.24e	5.4d	3.6d	0.038f	0.012f	2.4g	12.5c
C4	**4.0g**	**0.07g**	**4.6e**	**3.1f**	**0.02g**	**0.006h**	**1.6h**	**14.5 a**
GA1	38.0c	0.75c	7.2c	3.7c	0.065c	0.018d	2.7e	12.8bc
GA2	72.0b	1.31b	9.4b	4.2b	0.075b	0.023b	3.2b	13.8ab
GA3	**92.0a**	**1.7a**	**10.4a**	**4.6a**	**0.095a**	**0.028a**	**3.6a**	13.7ab
Mean	31.5	0.59	6.75	3.75	0.060	0.020	2.71	13.31

According to the Duncan test, the averages shown with the same letter are not important in their group (p<0.01) and (*p<0.05).

In terms of highest NER, GA2 (4.2) and GA1 (3.7) applications followed the GA3 (4.6) application, respectively. The highest values in the RFW and RDW parameters, which are parallel to each other, were determined in the GA3 application (0.095 g and 0.028 g, respectively). The highest GSL value was determined in the GA3 (3.6 cm) application. The lowest GSL value was determined in C4 (1.6 cm) application. The highest AGT was found in C4 application and G2 and G3 applications were in the same statistical group with the C4 application. The lowest AGT value was in the C3 application (Table 1).

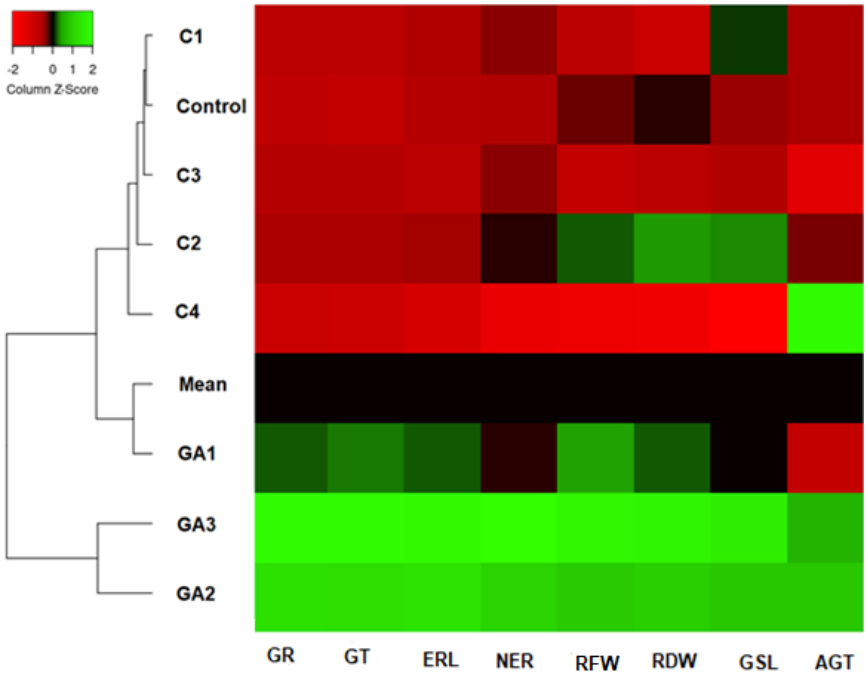

Figure 1. Heatmapper graph of applications, germination and early seedling development parameters.

Heat mapper analysis, which is an analysis method that classifies the averages of measured parameters by color, was created with our study data and the results are given in Figure 1. According to the heatmapper chart, red colors represent low values, green colors represent high values, and black color represents average values. According to this graph, while GA2 and GA3 applications have turned green and green tones in all parameters and it has been observed that these applications have the highest values. GA1 application received values close to the average and was in the same statistical group with the general mean. On the other hand, control and all colchicine applications received lower than average values, and it was observed that control and all colchicine applications were in the same statistical group (Figure 1).

When the correlation table showing the relationship between the germination and early seedling development parameters examined in the current study is evaluated, it can be stated that there is a positive relationship between all parameters except AGT (Table 2). There was a strong correlation (r=0.9995, p<0.01) between GT and GR (Table 2). There were highly significant (p<0.01) positive correlations with very strong values for the correlation of ERL with each of GR (r=0.9945) and GT (r=0.9946). There were highly significant (p<0.01) positive correlations with very strong values for the correlation of NER with each of GR (r=0.9411), GT (r=0.9416) and ERL (r=0.9575) (Table 2).

Table 2. Pearson's correlation coefficients among the germination and early seedling development parameters.

Traits	GR	GT	ERL	NER	RFW	RDW	GSL	AGT
GR	1							
GT	0.9995**	1						
ERL	0.9945**	0.9946**	1					
NER	0.9411**	0.9416**	0.9575**	1				
RFW	0.903**	0.9092**	0.9305**	0.943**	1			
RDW	0.8809**	0.8849**	0.91**	0.935**	0.9912**	1		
GSL	0.8011**	0.8047**	0.8485**	0.9387**	0.923**	0.9145**	1	
AGT	0.2744	0.2525	0.213	0.0328	-0.0131	-0.0101	-0.1727	1

*,** Significant at p<0.05 or 0.01, respectively.

Pearson's correlation analyses indicated that there were highly significant (p<0.01) positive correlations with very strong values for the correlation of RFW with each of GR (r=0.903), GT (r=0.9092), ERL (r=0.9305) and NER (r=0.943). RDW had highly significant positive correlations with GR (r=0.8809), GT (r=0.8849), ERL (r=0.91), NER (r=0.935) and RFW (r=0.9912). Also, there were highly significant (p<0.01) positive correlations with very strong values for the correlation of GSL with each of GR (r=0.8011), GT (r=0.8047), ERL (r=0.8485), NER (r=0.9387), RFW (r=0.923) and RDW (r=0.9145) (Table 2).

4. Discussion

In present study, it was determined that all applications except to C4 application were increased in values of all parameters except to AGT parameter. All doses of gibberellic acid applied in our study positively affected the germination and early seedling development characteristics of *O. acutidens* seeds. The highest values obtained for the parameters in the present study were generally determined at the highest gibberellic acid dose (GA3) application. Gibberellins are generally applied directly to the seeds and increase germination (Jacobsen et al., 2002). Also, gibberellins are used to eliminate dormancy. It promotes growth by increasing the plastids in the cell walls, converts carbohydrates into sugar and reduces the pressure on the cell wall. Thus, cell elongation occurs as water is taken into the cell (Arteca, 2013). İpek et al. (2008) reported that gibberellin application to seeds also promoted the production of some hydrolase enzymes, such as α-amylase. In another study, Yıldız et al. (2017) reported that the application of gibberellic acid increased the germination rate and germination-related parameters in *Dianthus barbatus* L. species under salt stress. In our study, it was observed that there was a significant increase in ERL, NER, RFW and RDW parameters with gibberellic acid application when compared to the control. The application of gibberellic acid positively affected peas (Okcu et al., 2005), pepper (Tepe et al., 2011), radish (Cavusoğlu & Kabar, 2007), carrots and spinach (Mufwanzala & Dikinya, 2010) seed germination. The aforementioned our results are consistent with the results of studies performed by Okcu et al. (2005), Cavusoğlu and Kabar (2007), Mufwanzala and Dikinya (2010), and Tepe et al. (2011).

In our study, colchicine applications have increased the parameters up to a certain point. According to some researchers tested different doses and durations of colchicine on the germinated seeds of different plants and increased the vegetative parameters by producing polyplioid plants (El-Nashar & Ammar, 2016; Sadat Noori et al., 2017; Khalili et al., 2020; Talei & Fotokian, 2020; Mo et al., 2020). The results we

obtained in our research, colchicine stimulates germination at certain rates, but colchicine causes death by having a toxic effect in increasing doses. Most seeds of bamboo (*Dendrocalamus brandisii)* could germinate normally after being inoculated in the basic Murashige and Skoog (1962) (MS) medium in culture tubes, supplemented with different concentrations of colchicine. It was observed that colchicine had no significant morphological effect on germination (Lv et al., 2021). Additionally, Lv et al. (2021) stated that some bamboo seeds showed abnormal germination phenomenon, particularly for high concentrations. Lv et al. (2021) determined that increasing colchicine dose increased the mortality rates, especially the highest mortality rate was 0.6%. At the same time as, they stated that seedling survival rates, germination rates and germination potential decreased constantly (Lv et al., 2021). It is known that the physiological level coupled with chromosomal damage causes a decrease in seed germination and an increase in mortality rate. Negativities caused by chemical mutagens may have caused deteriorations in the enzyme structure (Kulkarni, 2011). Previous studies (Bakry et al., 2007; Sourour et al., 2014) support the negative effect of high concentrations of colchicine on seed germination. The results of our study are parallel to Bakry et al. (2007), Grouh et al. (2011), Sourour et al. (2014), and Lv et al. (2021).

5. Conclusion

According to our findings, it was determined that GA2 and GA3 applications provided a significant increase in germination-related parameters. It was concluded that colchicine applications stimulated germination at a minimal level. It is thought that the application of gibberellic acid may be effective in solving the low germination problem in wild species such as *Origanum acutidents*. This study has identified some important new clues regarding the germination of *O. acutidents* seeds. In addition, it may be recommended to conduct detailed studies on both seed germination and colchicine doses and exposure times of *O. acutidens*.

Conflict of Interest

The author has no conflict of interest to declare.

References

Abd El-Latif, F. M., El-Gioushy, S. F., Islam, S. E., & Zakry, T. A. (2018). Impact of papaya seed soaking in different BA, colchicine and EMS solutions on germination, growth and chromosomal behaviour. *Asian Journal of Biotechnology and Genetic Engineering*, *1*(1), 1-17. https://doi.org/10.9734/AJBGE/2018/40538

Aligiannis, N., Kalpoutzakis, E., Mitaku, S., & Chinou, IB. (2001). Composition and antimicrobial activity of the essential oils of two origanum species. *Journal of Agricultural and Food Chemistry*, *49*(9), 4168-4170. https://doi.org/10.1021/jf001494m

Arteca, R. N. (2013). *Plant growth substances: Principles and applications*. Springer Science & Business Media.

Bakry, F., de la Reberdiere, N. P., Pichot, S., & Jenny, C. (2007). In liquid medium colchicine treatment induces non chimerical doubled-diploids in a wide range of mono- and interspecific diploid banana clones. *Fruits*, *62*(1), 3–12. https://doi.org/10.1051/fruits:2006043

Baser, K. H. C. (2002). The Turkish Origanum species. In S. Kintzios (Ed.), *Oregano: The genera Origanum and Lippia* (pp. 109-126). CRC Press.

Bond, L. (1942). Colchicine stimulation of seed germination in *Petunia axillaris*. *Journal of Heredity*, *33*(5), 200-201. https://doi.org/10.1093/oxfordjournals.jhered.a105167

Cavusoğlu, K., & Kabar, K. (2007). The effects of pretreatments of some plant growth regulators on germination and seedling growth of radish seeds under saline conditions. *Dumlupınar Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, *14*, 27–36.

Cetin, A., Arslanbas, U., Saraymen, B., Canoz, O., Ozturk, A., & Sagdic, O. (2011). Effects of grape seed extract and Origanum onit esessential oil on cisplatin-induced hepatotoxicity in rats. *International Journal of Hematology and Oncology*, *28*(4), 133-140. https://doi.org/10.4999/uhod.10060

Davis, P. H. (1982). *Flora of Turkey and east Aegean islands*. University Press.

Ellis, R. H., & Roberts, E. H. (1981). An investigation into the possible effects of ripeness and repeated threshing on barley seed longevity under six different storage environments. *Annals of Botany*, *48*(1), 93-96. https://doi.org/10.1093/oxfordjournals.aob.a086103

El-Nashar, Y. I., & Ammar, M. H. (2016). Mutagenic influences of colchicine on phenological and molecular diversity of *Calendula officinalis* L. *Genetics and Molecular Research*, *15*(2), 1-15. https://doi.org/10.4238/gmr.15027745

Gosh, P., Dash, P. K., Rituraj, S., & Mannan, M. A. (2014). Effect of salinity on germination, growth and yield of radish (*Raphanus sativus* L.) varieties. *International Journal of Biosciences*, *5*(1), 37-48. https://doi.org/10.12692/ijb/5.1.37-48

Grouh, M. S. H., Meftahizade, H., Lotfi, N., Rahimi, V., & Baniasadi, B. (2011). Doubling the chromosome number of *Salvia hains* using colchicine: Evaluation of morphological traits of recovered plants. *Journal of Medicinal Plants Research*, *5*(19), 4892–4898. https://doi.org/10.5897/JMPR.9000459

Hadlaczky, G., Bisztray, G., Praznovszky, T., & Dudits, D. (1983). Mass isolation of plant chromosomes and nuclei. *Planta*, *157*, 278–285. https://doi.org/10.1007/BF00405195

Ietswaart, J. H. (1980). *A taxonomic revision of the genus Origanum (Labiatae)*. The Hague: Leiden University Press.

İpek, A., Kaya, M. D., & Gürbüz, B., (2008). Çemen (*Trigonella foenum-graecum* L.) ve kimyon (*Cuminum cyminum* L.) tohumlarının çimlenmesi üzerine tohum yaşı ve GA3 uygulamalarının etkileri. *Tarım Bilimleri Dergisi*, *14*(1), 57-61. (In Turkish)

ISTA. (1996). *International rules for seed testing*. Seed Science and Technology.

Jacobsen, J. V., Pearce, D. W., Poole, A. T., Pharis, R. P., & Mander, L. N. (2002). Abscisic acid, phaseic acid and gibberellin contents associated with dormancy and germination in barley. *Physiologia Plantarum*, *115*(3), 428-441. https://doi.org/10.1034/j.1399-3054.2002.1150313.x

Karagoz, H. (2018). *Agronomic and molecular characterization of Origanum acutidens (hand.- mazz.) Ietswaart populations in northern Anatolia region* (Doctoral dissertation, Atatürk University).

Karagoz, H., & Parlakova Karagoz, F. (2019). Areas of utilization of *Origanum acutidens* (Hand. -Mazz) Ietswaart and Carvacrol. *International Journal of Current Research and Academic Review*, *7*(12), 46–55. https://doi.org/10.20546/ijcrar.2019.702.006

Karagoz, H., Hosseinpour, A., Parlakova Karagoz, F., Cakmakci, R., & Haliloglu, K. (2022). Dissection of genetic diversity and population structure in oregano (*Origanum acutidens* L.) genotypes based on agro-morphological properties and start codon targeted (SCoT) markers. *Biologia*, *77*(5), 1231-1247. https://doi.org/10.1007/s11756-021-00989-2

Karakurt, H., Aslantaş, R., & Eşitken, A. (2010). Tohum çimlenmesi ve bitki büyümesi üzerinde etkili olan çevresel faktörler ve bazı ön uygulamalar. *Uludağ Üniversitesi Ziraat Fakültesi Dergisi*, *24*(2), 115-128. (In Turkish)

Khalili, S., Niazian, M., Arab, M., & Norouzi, M. (2020). In vitro chromosome doubling of African daisy, *Gerbera jamesonii* Bolus cv. Mini Red. *Nucleus*, *63*(1), 59-65. https://doi.org/10.1007/s13237-019-00282-3

Kizil, S., Ipek, A., Arslan, N., & Khawar, K. M. (2009). Some agronomical characteristics and essential oil content of oregano (*Origanum onites* L.) as influenced by planting densities. *Journal of Essential Oil-Bearing Plants*, *12*(2), 172-180. https://doi.org/10.1080/0972060x.2009.10643707

Kulkarni, G. B. (2011). Effect of mutagen on pollen fertility and other parameters in horsegram (*Macrotyloma uniflorum* (Lam.) Verdc). *Bioscience Discovery*, *2*(1), 146-150.

Kwon, S. J., Seo, D. Y., Cho, G. Y., Lee, M. S., Moon, Y. J., & Boo, H. O. (2016). Effect of colchicine on chromosome doubling in *Codonopsis lanceolata*. *Korean Journal of Plant Resources*, *29*(3), 347–354. https://doi.org/10.7732/kjpr.2016.29.3.347

Lv, Z., Zhu, F., Jin, D., Wu, Y., & Wang, S. (2021). Seed germination and seedling growth of *Dendrocalumus brandisii* in vitro, and the inhibitory mechanism of colchicine. *Frontiers in Plant Science*, *12*, 784581. https://doi.org/10.3389/fpls.2021.784581

Mangena, P. (2021). Germination, morphological and physiological evaluation of seedlings pretreated with colchicine in soybean (*Glycine max* L). *Walailak Journal of Science and Technology (WJST)*, *18*(18), 9489. https://doi.org/10.48048/wjst.2021.9489

Mo, L., Chen, J., Lou, X., Xu, Q., Dong, R., Tong, Z., Huang, H., & Lin, E. (2020). Colchicine induced polyploidy in *Rhododendron fortunei* Lindl. *Plants*, *9*(4), 424. https://doi.org/10.3390%2Fplants9040424

Mufwanzala, N., & Dikinya, O. (2010). Impact of poultry manure and its associated salinity on the growth and yield of spinach (*Spinacea oleracea*) and carrot (*Daucus carota*). *International Journal of Agriculture & Biology*, *12*, 489–494.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, *15*(3), 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nasırcılar, A. G., Ulukapı, K., & Kurt, Z. (2020). Effects of salicylic acid on germination and vegetative growth properties of radish (*Raphanus sativus* L.) cultivars grown under drought stress conditions. *Turkish Journal of Agriculture-Food Science and Technology*, *8*(11), 2293-2299. https://doi.org/10.24925/turjaf.v8i11.2293-2299.3087

Okcu, G., Kaya, M. D., & Atak, M. (2005). Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum* L.). *Turkish Journal of Agriculture and Forestry*, *29*(4), 237-242.

Pande, S., & Khetmalas, M. (2012). Biological effect of sodium azide and colchicine on seed germination and callus induction in *Stevia rebaudiana*. *Asian Journal of Experimental Biological Sciences*, *3*(1), 93-98.

Sadat Noori, S. A., Norouzi, M., Karimzadeh, G., Shirkool, K., & Niazian, M. (2017). Effect of colchicine-induced polyploidy on morphological characteristics and essential oil composition of ajowan (*Trachyspermum ammi* L.). *Plant Cell, Tissue and Organ Culture*, *130*, 543-551. https://doi.org/10.1007/s11240-017-1245-0

Sari, A. O., & Altunkaya, M. (2015). Doğadan tarlaya: Kekik. *TÜRKTOB Dergisi*, *15*, 22-27. (In Turkish)

Sourour, A., Ameni, B., & Mejda, C. (2014). Efficient production of tetraploid barley (*Hordeum vulgare* L.) by colchicine treatment of diploid barley. *Journal of Experimental Biology and Agricultural Sciences*, *2*, 113-119.

Talei, D., & Fotokian, M. H. (2020). Improving growth indices and productivity of phytochemical compounds in lemon balm (*Melissa officinalis* L.) through induced polyploidy. *BioTechnologia*, *101*(3), 215-226. https://doi.org/10.5114/bta.2020.97880

Tepe, A., Kaya, H., Batmaz, G., Özkan, C. F., & Demirtaş, E. I. (2011). Effect of saline irrigation water applications on the yield of some pepper pure lines. *Derim Journal*, *28*(1), 1-11.

Tepe, B., Cakir, A., & Sihoglu Tepe, A. (2016). Medicinal uses, phytochemistry, and pharmacology of *Origanum onites* (L.): A review. *Chemistry & Biodiversity*, *13*(5), 504-520. https://doi.org/10.1002/cbdv.201500069

Trease, S. E., & Evans, D. (1983). *Colchicum seed and corn - Pharmacognosy*. Balliere Tindall.

Tumen, G., Baser, K. H. C., Kirimer, N., & Ozek, T. (1995). Essential oil of *Origanum saccatum* P. H. Davis. *Journal of Essential Oil Research*, *7*(2), 175-176. https://doi.org/10.1080/10412905.1995.9698493

Yamaguchi, S., & Kamiya, Y. (2002). Gibberellins and light-stimulated seed germination. *Journal of Plant Growth Regulation*, *20*(4), 369-376. https://doi.org/10.1007/s003440010035

Yıldız, S., Parlakova Karagoz, F., & Dursun, A. (2017). Giberellik asit ön uygulamasına tabi tutulmuş hüsnüyusuf (*Dianthus barbatus* L.) tohumlarının tuz stresinde çimlenmesi. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, *48*(1), 1-7. https://doi.org/10.17097/ataunizfd.320231 (In Turkish)

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

https://prensip.gen.tr

RESEARCH ARTICLE

Chemical Composition, Antibacterial and Antioxidant Activity of Essential Oils and Extracts of *Ferula orientalis*

Esin Dadasoglu[1]✉ • Nasibe Tekiner Aydın[2] • Aykut Oztekin[3]

[1]Atatürk University, Faculty of Agriculture, Department of Field Crops, Erzurum/Türkiye
[2]Artvin Çoruh University, Ali Nihat Gökyiğit Botanical Garden Application and Research Center, Artvin/Türkiye
[3]Ağrı İbrahim Çeçen University, Vocational School of Health Services, Department of Medical Services and Techniques, Ağrı/Türkiye

ARTICLE INFO

Article History
Received: 07.12.2023
Accepted: 25.12.2023
First Published: 30.12.2023

Keywords
Antibacterial activity
Antioxidant activity
Chryseobacterium indologenes
Essential oils
Ferula orientalis

ABSTRACT

This study aimed to determine the essential oil content, essential oil and extracts, which are known as *Ferula orientalis* and obtained from naturally grown plants in Narman (Erzurum, Türkiye) province, antioxidant effects and antimicrobial effects. *F. orientalis* essential oils were isolated by hydrodistillation and analyzed using gas chromatography-mass spectrometry to identify their components. The antimicrobial activity was measured by the disc diffusion methods and minimal inhibitory concentration (MIC) methods against *Chryseobacterium indologenes* which cause soft rot in certain vegetables and fruits. Total antioxidant and phenolic contents were analyzed by 2,2′-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging, 2,2'-azino-bis(3-ethylbenzothiazoline-6 sulphonic acid (ABTS), thiobarbituric acid reactive species (TBARS), β-carotene bleaching test (BCB) and Folin-Ciocalteu method. According to the results obtained; *F. orientalis* essential oil exhibited a high content of δ-3-Carene (40.38%) as major compound over 14 identified components by GC-MS analysis followed by γ-Terpinene (17.24%), (E)-β-Ocimene (10.51%), and β-Phellandrene (8.49%). The essential oil and extracts was evaluated for its antimicrobial activity against *C. indologenes* showed significant antibacterial activities with MIC values of 9-21 mm and 62.5 µg/mL, respectively, but extracts and antibiotics have no effect against *C. indologenes*. Hexane extract had the highest ABTS free radical scavenging activity with 14.2 (IC_{50} g/l), acetone extract had the highest DPPH capacity with 24.2 (IC_{50} g/l), and water extract had the highest amount of total phenolic compound with 15.13±3.82 mg GAE/g. In the TBARS test antioxidant activity increased as the amount of essential oil increased. The antioxidant capacity of *F. orientalis* essential oil exhibited reduction when evaluated by β-carotene bleaching assay. As a result, it is thought that *F. orientalis* essential oils and extracts can be used as an alternative natural antioxidant source for potential applications.

Please cite this paper as follows:

Dadasoglu, E., Tekiner Aydın, N., & Oztekin, A. (2023). Chemical composition, antibacterial and antioxidant activity of essential oils and extracts of *Ferula orientalis*. *Journal of Agricultural Production*, *4*(2), 159-168. https://doi.org/10.56430/japro.1401560

1. Introduction

Synthetic pesticides are widely used to control plant diseases and pests. Due to the negative effects of synthetic pesticides on the environment and human healths, organic agriculture has gained very crucial and the use of bioagents and natural chemicals as an alternative to synthetic pesticides for disease and pest control has come into use. Numerous studies have been carried out worldwide to control plant, food, and clinical saprophytic and pathogenic microorganisms using vegetable oils, some of their components, and plant extracts (Okeke et al., 2001; Sechi et al., 2001; Abu-Shanab et al., 2004; Adebolu & Oladimeji, 2005; Iroegbu & Nkere, 2005; Rojas et al., 2006; Maggi et al., 2016; Nguir et al., 2016). The number

✉Correspondence
E-mail address: edadasoglu@atauni.edu.tr

of studies on plant extracts and oils in our country has been increasing rapidly in recent years (Meral & Karabay, 2002; Yegen et al., 2002; Sahin et al., 2003, Adıgüzel et al., 2005; Tepe et al., 2006; Kotan et al., 2010; Kotan et al., 2014; Dadasoglu et al., 2015; Görmez et al., 2015; Dadasoglu et al., 2016).

Ferula L. is the largest genus in the Apiaceae family, with ~213 species (POWO, 2002). *Ferula* species are widespread in the temperate regions of the Euro-Asian continent, surrounded by the Canary Islands in the West, North Africa in the South, China and India in the East, and Central Europe in the North. *Ferula orientalis* L. grows on the rocky steps of the Eastern Anatolia region of Türkiye at 1600-2900 m altitude (Tubives, 2019). *Ferula* species are known for their medicinal and aromatic properties worldwide and they can be used as precious sources in drug development due to their promising bioactive components (Iranshahy & Iranshahi, 2011; Mahendra & Bisht, 2012; Mohammadhosseini et al., 2019). In recent years, the antioxidant activities, antimicrobial potentials of *Ferula* species were also studied (Kartal et al., 2007; Dadasoğlu et al., 2018; Topdas et al., 2020). Antioxidants prevent and limit the rate of oxidation through one or more mechanisms that involve inhibiting or reducing the effects of free radicals and oxidising compounds on oxidisable substrates. These compounds produced by plants have been the target of research into their antioxidant potential. Among these compounds, essential oils play an important role because many of their components can replace or be combined with synthetic substances. Consumer acceptance of these products has led to the widespread use of essential oils from various plants as raw materials in the food, pharmaceutical, and cosmetic industries (Miranda et al., 2014).

It is known that bacteria, as a disease agent, cause significant losses in the yield and quality of cultivated plants. They have a very wide host potential both in the world and in our country (Agrios, 2005). Among these, the bacteria that cause soft rot cause damage to many economically important plants (Perombelon & Kelman, 1980; Agrios, 2005). Soft rot diseases are caused by different numbers of bacteria belonging to the genera *Erwinia*, *Pseudomonas*, *Enterobacter*, *Chryseobacterium* and *Bacillus* (Dadaşoğlu, 2013). Pathogenic bacteria pose a major problem due to the proliferation of resistant microorganisms, in particular the contamination of various foods. Outbreaks of food poisoning are becoming increasingly common. Therefore, there is a need for compositions that can control antibiotic-resistant bacterial strains to reduce contamination and degradation of food products (Gomes et al., 2014).

The aim of this study was to chemically characterise the essential oil and extract of *F. orientalis*, a medicinal plant and to evaluate their antioxidant and antibacterial activities.

2. Materials and Methods

2.1. Plant Material and Plant Pathogenic Bacterial Strains

The aerial parts of *F. orientalis* were collected (10 kg) at the flowering stage in Erzurum, province of Türkiye in July-September 2017 and dried in the shade. The plant is conserved at Ataturk University, Faculty of Agriculture, Plant Protection Department, Plant Clinical Laboratory.

Eight bacterial strains (Table 1) were used, which were previously tested and are highly virulent. These strains had been identified as pathogens of different host plants and were stored at -80 °C in 15% glycerol and Luria Broth (LB) until used.

Table 1. Plant pathogenic bacterial strains used in the study.

Strains	MIS identification*	Host	SIM*	HR*
F-408	*Chryseobacterium indologenes*	Cucumber	0.84	+
F-491	*Chryseobacterium indologenes*	Cucumber	0.78	+
F-492	*Chryseobacterium indologenes*	Cucumber	0.82	+
F-502	*Chryseobacterium indologenes*	Tomato	0.35	+
F-544	*Chryseobacterium indologenes*	Cucumber	0.89	+
F-709	*Chryseobacterium indologenes*	Tomato	0.86	+
F-713	*Chryseobacterium indologenes*	Pepper	0.60	+
F-723	*Chryseobacterium indologenes*	Zucchini	0.79	+

*__MIS:__ Microbial identification system, __SIM:__ Similarity index, __HR:__ Hypersensitive response.

2.2. Preparation of the Essential Oil (EO) and Other Extracts

The dried aerial parts of *F. orientalis* plant samples (500 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 4 h. The EO was extracted with $CHCl_3$ and then dried over anhydrous Na_2SO_4 and stored under N_2 atmosphere at 20 ℃ in a sealed vial until required (Kotan et al., 2010).

The dried plant samples were powdered in a blender and then 50 g of each plant sample was extracted individually with

n-hexane, chloroform, acetone, and methanol at room temperature.

After filtration, the organic solvents were evaporated under reduced pressure and temperature. For the methanol extract of the plant sample, the concentrated methanol extract was dissolved individually in distilled water (60 ℃) and then filtered. The solutions were extracted three times with *n*-hexane to remove lipophilic compounds. The water solutions were then lyophilized in a Labconco 117 freeze dryer (Labconco Company, Kansas City, MO, USA) at 5 µg Hg and -50 °C. The extracts were stored at -20 °C until required (Kotan et al., 2010).

2.3. Gas Chromatography-Mass Spectrometry (GC-MS) Analyses

The oil composition was analysed by gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis was performed using a Thermo Finnigan Trace GC/Trace DSQ/A1300, (E.I. Quadrapole) equipped with an SGE-BPX5 MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). An electron ionisation system with an ionisation energy of 70 eV was used for GC-MS detection. The carrier gas was helium at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 220 and 290 ℃, respectively. The oven temperature was programmed from 50 to 150 °C at 3 ℃/min, then held isothermally for 10 min and finally increased to 250 °C at 10 ℃/min. Diluted samples (1/100 v/v, in methylene chloride) of 1 µl were injected manually in the splitless mode. The relative percentage of the oil constituents was expressed as percentages by peak area normalisation. The identification of individual compounds of essential oils was based on the comparison of their relative retention times with those of authentic samples on the SGE-BPX5 capillary column, and on the comparison of the mass spectra of their peaks with those of authentic samples and/or the spectra of the Wiley 7N and TRLIB libraries and published data (Jennings & Shibamoto, 1980; Adams, 2017).

2.4. Antibacterial Activity

The *in vitro* antimicrobial activities of the essential oil and extracts (methanol, ethanol, acetone, chloroform, n-hexane) of *F. orientalis* were evaluated by the disc diffusion method (Murray et al., 1995) with a slight modification using Tryptic Soy Agar (TSA, Merck, Germany) medium with the determination of inhibition zones (IZ). Essential oil and extracts were prepared by dissolition with 10% dimethyl sulfoxide (DMSO), and then were sterilised by filtration through 0.45 µm Millipore filters. Bacterial cultures were grown in Tryptic Soy Broth (TSB, Merck, Germany), and their suspension (100 µl) containing 1×10^8 colony-forming units/ml (cfu/ml) of bacteria spread was plated on TSA medium using a sterile swab. Disks (6 mm in diameter) containing 12.5 µl of essential oil and 10.0 mg/ml suspensions of the extracts were used and placed in the centre of the inoculated plates. The diameters of the inhibition zones around the discs were measured in millimetres (mm) after 24 and 48 hours of incubation at 25±2 °C. All studies were performed in triplicate. Standard antibiotic discs (6 mm in diameter) of penicillin and kanamycin (1 µg/disc) were used as positive controls for comparison. 10% DMSO solvent was also tested as a negative control.

2.5. Determination of Minimal Inhibition Concentration (MIC)

The minimum inhibitory concentrations of the extracts and essential oils were determined. Tenfold serial dilutions of the extracts and essential oils were prepared with 10% DMSO solution with an initial dilution of 1/1 v/v (concentration range from 500 µl/ml to 3.125 µl/ml). The concentrations of the bacterial strains grown in TSB were adjusted to $1x10^8$ cfu/ml and 100 µl of bacterial suspension was plated on TSA plates. Then, the blank discs (Oxoid) were directly impregnated with essential oil at different concentrations (12.5 µl and 1.25 mg of the extracts) by placing 6 pieces in each petri dish at equal intervals and were incubated at 25±2 °C for 48 h. The inhibition zone was checked and the lowest concentration of the essential oil showing a clear zone of inhibition was considered as the MIC. All tests were performed in triplicate.

2.6. Determining the Amount of Total Phenolic Compounds

The total amount of phenolic compounds in the extracts was measured according to the method (Singleton et al., 1999). Briefly, 1 ml of plant extract dissolved in ethanol (1 mg/ml) was mixed with 0.5 ml of Folin-Ciocalteu reagent (diluted 10 times in distilled water) in test tubes in triplicate. After 3 minutes, 3 ml of Na_2CO_3 (2% w/v) solution was added, and the test tubes were incubated for 2 hours in the dark with continuous shaking. After this time the absorbance of the samples was measured at a wavelength of 760 nm using a spectrophotometer. Gallic acid was used as a standard curve and distilled water was used as a blank.

2.7. Determination of Antioxidant Activity

Due to the complex reactive facets of phytochemicals, the antioxidant activities of plant extracts cannot be evaluated by only a single method, but at least two test methods have been recommended for the determination of antioxidant activity (Rodriguez et al., 1997). For this reason, the antioxidant capacity of *F. orientalis* essential oil and extracts was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6 sulphonic acid (ABTS), thiobarbituric acid reactive species (TBARS) and β-carotene bleaching (BCB) spectrophotometric methods.

2.7.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH)

50 µg/ml of plant extracts were added to 4 ml of DPPH solution (25 mg DPPH/l ethanol) in the test tube and incubated

at room temperature for 30 min. After incubation, the absorbance at 517 nm was measured against an ethanol blank. 4 ml of DPPH solution was used as a control (Shimada et al., 1992). The decreasing absorbance gives the remaining amount of DPPH in the solution and the free radical scavenging activity. The DPPH scavenging activity was calculated using the following equation:

DPPH scavending activity % = $[(A_0-A_1)/A_0]x100$ (1)

2.7.2. 2,2'-azino-bis(3-ethylbenzothiazoline-6 sulphonic acid (ABTS) radical scavenging activity

ABTS (Sigma-Aldrich, Canada) radical cation solution was prepared by reacting 7.0 mM ABTS stock solution with 2.45 mM (final concentration) potassium persulfate ($K_2S_2O_8$) was mixed in a 1:1 ratio and shaking continuously for 16 h at room temperature in the dark until a stable oxidation state was reached. The $ABTS^+$ radical solution was then diluted with ethanol to give an absorbance of 1.850±0.05 at 734 nm. This absorbance was used as the control absorbance. For the spectrophotometric assay, $ABTS^+$ (4 ml) and 50 µg of plant extract were thoroughly mixed and incubated at room temperature for 2 hours. After this time, the absorbance of the samples was recorded at 734 nm against phosphate buffer (PBS, pH = 7.4) as a blank (Wu et al., 2009). The decreasing absorbance gives the amount of $ABTS^+$. $ABTS^+$ was calculated using the following formula:

% ABTS radical scavenging activity= $[(A_0-A_1)/A_0]x100$ (2)

2.7.3. Thiobarbituric acid reactive species (TBARS) assay

A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the potential antioxidant capacity of the essential oil as a lipid-rich compound (Ruberto & Baratta, 2000). Briefly, 500 µl of 10% (w/v) essential oil and 0.1 ml of sample solutions (4.0, 20.0, and 40.0 g/L respectively), prepared immediately before use, to be tested in methanol, were made up to the 1.0 ml with distilled water. 0.05 ml of 2,20-azobis (2-amidinopropane) dihydrochloride (ABAP) solution (0.07 mol/L) in water was added to induce lipid peroxidation. 1.5 ml of 20% acetic acid (pH 3.5) and 0.8% (w/v) thiobarbituric acid were added in 1.1% (w/v) sodium dodecyl sulphate solution were added and the resulting mixture was vortexed and incubated at 95 °C for 60 min. The mixture was cooled, and 5 ml of butane-1-ol was added to each vial and centrifuged at 1200 g for 10 minutes. The supernatants were measured with a spectrophotometer at 532 nm.

The antioxidant index (AI%) was calculated from the following formula:

AI%=1-T/Cx100 (3)

(*C* is the absorbance value of oxidized control, and *T* is the absorbance of the sample)

2.7.4. β-carotene bleaching (BCB) assay

The antioxidant capacity of *F. orientalis* essential oil was determined using the β-carotene bleaching method with some modifications (Kulisic et al., 2004). β-Carotene (0.1 mg) was added to a boiling flask together with linoleic acid (20 mg) and Tween 40 (100 mg) was dissolved in chloroform. The solvent was evaporated, under vacuum at 50 °C in a rotary evaporator, 50 ml of oxygenated distilled water was added and the emulsion was allowed to stand for 1 minute in sonificator to form emulsion A. The emulsion was then mixed with 200 µl of the ethanolic stock solutions of each antioxidant (0.1; 0.5; 1.0; 2.0; 3.0 and 4.0 g/L respectively) in 5 ml of open-tope cuvettes. A control without antioxidants was prepared, consisting of 200 µl ethanol and 5 ml of emulsion A. In addition, a second emulsion (B) was prepared, consisting of 25 ml of oxygenated water, 10 mg of linoleic acid, and 50 mg of Tween 40. 5 ml of emulsion B and 200 µl of ethanol were used to zero the spectrophotometer. The absorbances of the samples were read immediately (t=0) and every 15 min interval for 120 min at 50 °C using a microplate reader at 470 nm. The percentage of bleaching of β-carotene was calculated according to by Mallet et al. (1994) using the following formula.

% Bleaching of β-Carotene= $(100-A_{A(t1)}-A_{C(t1)})/A_{C(t0)}-A_{C(t1)}x100$ (4)

(A: absorbance $_A$=antioxidant, $_C$=control, t1:120 min)

3. Results and Discussion

3.1. Composition of the Essential Oil

The constituents of the studied *F. orientalis* essential oil are shown in Table 2 and 14 bioactive compounds were identified by the GC-MS analysis. The highest percentage of compounds of *F. orientalis* essential oil was δ-3-Carene (40.38%), followed by γ-Terpinene (17.24%) and (E)-β-Ocimene (10.51%), respectively.

Several essential oils from *Ferula* species contain monoterpene hydrocarbons (α-pinene, β-pinene, myrcene, and limonene), oxygenated monoterpenes (linalool, α-terpineol, and neryl acetate), sesquiterpene hydrocarbons (α-caryophyllene, germacrene B, germacrene D, and δ-cadinene), oxygenated sesquiterpenes (caryophyllene oxide, α-cadinol, and guaiol), and sec-butyl disulphide derivatives (Sahebkar & Iranshahi, 2013).

Kartal et al. (2007) showed that *F. orientalis* EO from aerial parts contained high levels of β-phellandrene (23.6%), β-o-cymene (13.8%), α-pinene (12.5%), and α-phellandrene (11.5%). Ozkan et al. (2014) were determined, α-cadinol (10.45%), δ-cadinene (8.1%), germacrene D-4-ol (6.8%), and epi-α-muurolol (5.9%) as the major compounds of *F. orientalis* leaf and flower EOs. Topdas et al. (2020) found α-pinene (20.6%) to be the most abundant compound. These differences

may be due to various factors such as geographical origin, soil composition, climate, harvest time, and plant parts (Celiktas et al., 2007; Topdas et al., 2020).

Table 2. The main components of essential oil of *Ferula orientalis*.

RI[a*]	Components	*Ferula orientalis* (%)	Identification methods
932	α Pinene	2.44	GC, MS, RI
969	Sabinene	0.76	GC, MS, RI
988	Myrcene	1.10	GC, MS, RI
1008	δ-3-Carene	40.38	GC, MS, RI
1020	p-Cymene	3.66	GC, MS, RI
1025	β-Phellandrene	8.49	GC, MS, RI
1032	(Z)-β-Ocimene	1.00	GC, MS, RI
1044	(E)-β-Ocimene	10.51	GC, MS, RI
1054	γ-Terpinene	17.24	GC, MS, RI
1086	Terpilone	0.79	GC, MS, RI
1471	7-epi-1,2-dehydrosesquicineole	6.28	GC, MS, RI
1481	Widdra-2,4(14)-diene	2.42	GC, MS, RI
1559	Germacrene-β	1.13	GC, MS, RI
1666	14-hydroxy-(Z)-caryophyllene	2.45	GC, MS, RI

[*]**RI[a]:** retention indices in elution order from an HP-5 column.

3.2. Antibacterial Activity Assays

The antibacterial activities of *F. orientalis* essential oil and extracts against eight plant pathogenic bacteria were evaluated by recording IZ and MIC. The results are presented in Table 3.

Table 3. The antibacterial activities of essential oil, extracts of *Ferula orientalis* and standard antibiotics and MIC values of essential oil.

Strains	IZ * (mm)	MIC	M	A	C	H	P	K	DMSO
F-408	20	125	-	-	-	-	-	-	-
F-491	21	62.5	-	-	-	-	-	-	-
F-492	12	250	-	-	-	-	-	-	-
F-502	15	250	-	-	-	-	-	-	-
F-544	15	250	-	-	-	-	-	-	-
F-709	19	125	-	-	-	-	-	-	-
F-713	9	250	-	-	-	-	-	-	-
F-723	20	125	-	-	-	-	-	-	-

*IZ: Inhibition zones of essential oil (12.5 µl/ml), MIC: Minimal inhibition concentration, M: Methanol extract, A: Aceton extract, C: Chloroform extract, H: Hexan extract, P: Penicillin, K: Kanamycin, DMSO: Dimethilsulfoxoide.

Essential oil of *F. orientalis* showed antibacterial activity against all *C. indologenes* isolates with different ratios (9-21 mm) of inhibition zones compared to the positive standard antibiotics (penicillin and kanamycin). The highest inhibition zone was observed against strain F-491 (21 mm), followed by F-408 and F-723 (20 mm), respectively. The lowest inhibition zone was observed against isolate F-713 (9 mm). The degree of efficacy of essential oils with antimicrobial properties may vary between species belonging to the same genus. The four different extracts (n-hexane, chloroform, methanol, and acetone) of *F. orientalis* do not have any antibacterial activity against *C. indologenes* isolates. Similarly, the standard antibiotics penicillin and kanamycin, used as positive controls, and 10% DMSO, used as a negative control, were found to have no antibacterial activity.

Recent studies on the essential oils of species of the *Ferula* genus have shown that most of these plants have a wide range of biological, especially antimicrobial activities, which are generally related to the chemical composition of the oil (Pavlovic et al., 2012; Al-Ja'fari et al., 2013; Maggi et al., 2016;

Nguir et al., 2016; Topdas et al., 2020). It has been demonstrated that the essential oil of *F. orientalis* can be used in the control of *C. indologenes* (soft rot disease pathogen) by this study.

3.3. Determination of Minimal Inhibition Concentration (MIC)

The MIC values of the essential oil and extracts obtained from the *F. orientalis* plant in Petri dishes against *C. indologenes* isolates are given in Table 3. The MIC values were determined to be 62.5-250 µl/ml.

Copper compounds and antibiotics have been used to control phytopathogenic bacteria, but these applications have many disadvantages. Many studies have reported that phytopathogenic bacteria become resistant to many antibiotics over time. As a result, the use of antibiotics is banned in many countries. For example; there are a number of studies indicating that there are resistant strains of *Xanthomonas campestris* pathovars to kanamycin, ampicillin, penicillin, and streptomycin (Sahin & Miller, 1997; Schlesier et al., 2002; White et al., 2002). In this study, medium or high resistance to tested antibiotic was observed in pathogenic bacteria.

3.4. Determination of Antioxidant Activity

The DPPH method is applied widely used to measure the antioxidant activities of plant extracts. In the DPPH test, the antioxidants reduce the stable DPPH radical to the yellow coloured diphenylpricryhydrazine. In this study, acetone, methanol, water, chloroform, hexane extracts and essential oil obtained from *F. orientalis* were tested for their ability to scavenge free radicals (DPPH). The results are presented in Table 4. It can be seen that the acetone extract has the highest free radical scavenging capacity (DPPH) with 24.2 (IC_{50} mg/ml). The free radical-scavenging capacity of methanol, chloroform, hexane and water extracts and essential oil were found to be 23.3, 19.6, 17.2, 18.3, and 0.88 (ABTS IC_{50} (g/l)), respectively. The results indicated that the essential oil and extracts obtained from *F. orientalis* exhibited potential DPPH radical scavenging activity. The radical scavenging activity of the extracts and essential oil obtained from *F. orientalis* was determined by radical cation (ABTS) according to the reported procedure (Wu et al., 2009). In our study, the scavenging ability of the essential oil and extracts on the ABTS free radical is shown in Table 4. In this reaction system, the ABTS scavenging activities of methanol, acetone, chloroform, hexane, water extracts and essential oil were determineted to be 8.1, 12.4, 9.7, 14.2, 6.2 and 0.67 (ABTS IC_{50} (g/l)), respectively. The results showed that the acetone extract had the highest ABTS free radical scavenging activity with 12.4 (ABTS IC_{50} (g/l)).

3.5. Determining the Amount of Total Phenolic Compounds

The amounts of total phenolic compounds in the extracts were determined according to the Folin-Ciocalteu procedure (Singleton et al., 1999). The results (Table 4) show that the total phenolic compounds in *F. orientalis* extracts ranged from 4.21 (mg GAE/g) to 15.13 and the highest amount of phenolic compounds was found in the water extract. In our study, we determined the antioxidant capacity and total phenolic content of extracts obtained from *F. orientalis* extracts, which have not been studied before. The results obtained are similar when the antioxidant capacities of the extracts are determined by different tests. The results of the investigation show that the higher the concentration of total phenolic compounds the lower the amount of residual DPPH and $ABTS^{+}$ radical cation, the higher the free radical scavenging activity.

Table 4. The antioxidant capacity and total phenolic compounds of *Ferula orientalis*.

Antioxidants	ABTS IC_{50} (g/l)	DPPH IC_{50} (g/l)	Total Phenolic Compounds (mg GAE/g)
F. orientalis water	6.2	18.3	15.13±3.82
F. orientalis Acetone	12.4	24.2	6.37±0.98
F. orientalis methanol	8.1	23.3	9.62±1.23
F. orientalis hexane	14.2	17.2	4.21±0.88
F. orientalis chloroform	9.7	19.6	6.12±0.83
F. orientalis essantial oil	0.67	0.88	-
Bütilhidroksitoluen (BHT)	$6x10^{-2}$	$9x10^{-2}$	-
α-tocopherol	$2.8x10^{-3}$	$4.7x10^{-3}$	-
Ascorbic acid	$1.6x10^{-3}$	$1.4x10^{-3}$	-

3.6. Thiobarbituric Acid Reactive Species (TBARS) Assay

The ability of *F. orientalis* essential oil to act as a radical scavenger was investigated in conjunction with α-tocopherol and BHT. As shown in Table 5, the antioxidant capacity of *F. orientalis* essential oil, BHT and α-tocopherol increased respectively.

Table 5. Antioxidant activity of *Ferula orientalis* essential oil, α-Tocopherol and BHT measured by TBARS method.

Antioxidants	Antioxidant Index (AI%)		
	100 ppm	500 ppm	1000 ppm
F. orientalis essential oil	12.3± 3.2	18.4±4.4	27.8±6.2
BHT	26.1±2.2	30.4±3.3	38.3±7.6
α-tocopherol	62.8±5.7	68.9±9.8	73.1±7.8

3.7. Determination of Antioxidant Activity with the β-Carotene Bleaching (BCB) Test

BCB method based on the measurement of β-carotenoids yellow colour due to the reaction with radicals was formed by oxidation of linoleic acid in an emulsion. The bleaching rate of β-carotene decreases in the presence of antioxidants. Figure 1 shows the decrease in absorbance of β-carotene in the presence of the *F. orientalis* essential oil. The antioxidant effects of BHT and *F. orientalis* essential oil were compared in Figure 2. It can be seen that the bleaching of β-carotene is prevented in the presence of essential oil and BHT. It was observed that the essential oil is effective even when compared to a very strong antioxidant BHT (Figure 2).

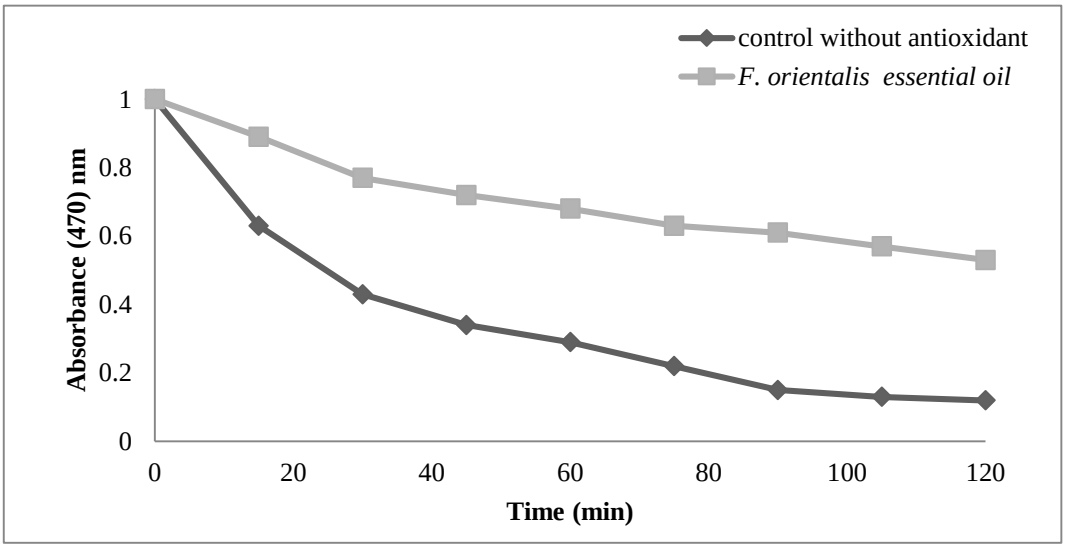

Figure 1. β-Carotene bleaching activity of *Ferula orientalis* essential oil.

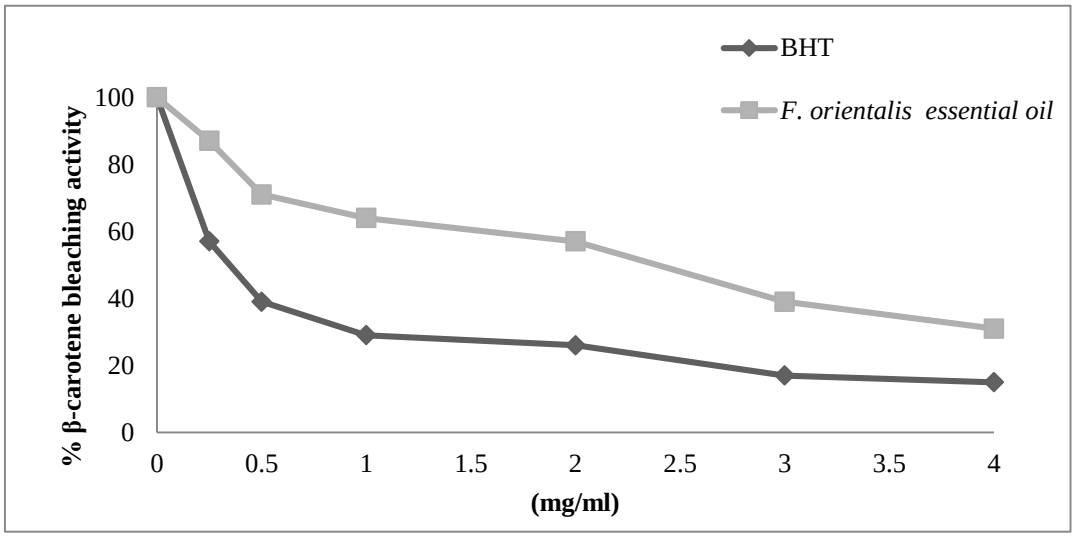

Figure 2. Antioxidant activity of the *Ferula orientalis* essential oil with the β-Carotene bleaching (BCB) test.

As a result, the most important feature of this study is that it is the first study in which the essential oil and extracts obtained from the *F. orientalis* plant, naturally grown in Türkiye and other regions of the world, have been used against the eight soft

rot *C. indologenes* isolates. The successful results obtained from the essential oil of the plant used in the study are also the first results obtained for this group of pathogens. The fact that the antibiotics used in the study had no effect and that the essential oil was lethal to all strains has increased the importance of the work. This is evidence that the pathogens have become more resistant to antibiotics over time.

5. Conclusion and Recommendations

The results obtained from this study include conclusions and recommendations to organic farming, and sustainable agricultural systems that are increasingly important in recent years. In addition, the concentrations that are effective from the results obtained will be tested in practical applications, and if the expected results are obtained, the targeting and marketing of products intended for use in the control of these pathogens will be targeted.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Abu-Shanab, B., Adwan, G., Abu-Shanab, D., Jarrar, N., & Adwan, K. (2004). Antibacterial activities of some plant extracts utilized in popular medicine in Palestine. *Turkish Journal of Biology*, *28*(2), 99-102.

Adams, R. P. (2017). *Identification of essential oil components by gas chromatography/mass spectrometry*. Gruver.

Adebolu, T. T., & Oladimeji, A. S. (2005). Antimicrobial activity of leaf exracts of *Ocimum gratissimum* on selected diarrhoea causing bacrteria in southwestern Nigeria. *African Journal of Biotechnology*, *4*(7), 682-684. https://doi.org/10.5897/AJB2005.000-3126

Adıgüzel, A., Güllüce, M., Şengul, M., Öğütçü, H., Sahin, F., & Karaman, I. (2005). Antimicrobial effect of *Ocimum basilicum* (Labiatae) exract. *Turkish Journal of Biology*, *29*, 155-160.

Agrios, G. N., (2005). *Plant pathology*. Department of Plant Pathology, University of Florida, Elsevier Academic Press.

Al-Ja'fari, A. H., Vila, R., & Freixa, B. (2013). Antifungal compounds from the rhizome and roots of *Ferula hermonis*. *Phytotherapy Research*, *27*(6), 911-915. https://doi.org/10.1002/ptr.4806

Celiktas, O. Y., Kocabas, E. E. H., Bedir, E., Sukan, F. V., Özek, T., & Baser, K. H. C. (2007). Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. *Food Chemistry*, *100*, 553-559. https://doi.org/10.1016/j.foodchem.2005.10.011

Dadaşoğlu, F. (2013). *Artvin, Erzincan, Erzurum, Iğdır illerinde bazı meyve ve sebzelerde yumuşak çürüklüğe sebep olan bakterilerin izolasyonu klasik ve moleküler yöntemler ile karakterizasyonu* (Doctoral dissertation, Atatürk University). (In Turkish)

Dadasoglu, F., Kotan, R., Cakır, A., Cakmakcı, R., Kordalı, S., & Ozer, H. (2015). Antibacterial activities of essential oils, extracts and some of their major components of *Artemisia* spp. against seed-borne plant pathogenic bacteria. *Fresenius Environmental Bulletin*, *24*, 2715-2724.

Dadasoglu, F., Kotan, R., Cakır, A., Karagoz, K., Dikbas, N., & Ozer, H. (2016). Use of essential oils and extracts from *Satureja* and *Origanum* species as seed disinfectants against *Xanthomonas axonopodis* pv. *vesicatoria* (Doidge) Dye. *Fresenius Environmental Bulletin*, *25*(1A), 5989-5998.

Dadasoglu, E., Oztekin, A., & Dadasoglu, F. (2018). Antibacterial and antioxidant activity of essential oil and extracts of *Ferula communis* and determination of chemical composition of its essential oil. *Fresenius Environmental Bulletin*, *27*(6), 4186-4191.

Gomes, M. S., Cardoso, M. G., Soares, M. J., Batista, L. R., Machado, S. M. F., Andrade, M. A., Azeredo, C. M. O., Resende, J. M. V., & Rodrigues, L. M. A. (2014). Use of essential oils of the genus citrus as biocidal agents. *American Journal of Plant Sciences*, *5*, 299-305. https://doi.org/10.4236/ajps.2014.53041

Görmez, A., Bozarı, S., Yanmış, D., Güllüce, M., Şahin, F., & Ağar, G. (2015). Chemical composition and antibacterial activity of essential oils of two species of Lamiaceae against phytopathogenic bacteria. *Polish Journal of Microbiology*, *64*(2), 121-127.

Iranshahy, M., & Iranshahi, M. (2011). Traditional uses, phytochemistry and pharmacology of asafoetida (*Ferula assa-foetida oleo-gumresin*)-A review. *Journal of Ethnopharmacology*, *134*, 1-10. https://doi.org/10.1016/j.jep.2010.11.067

Iroegbu, C. U., & Nkere, C. K. (2005). Evalution of the antibacterial properties of *Picralima nitida* stembark extracts. *International Journal of Molecular Medicine and Advance Sciences*, *1*(2), 182-189.

Jennings, W., & Shibamoto, J. (1980). *Qualitative analysis of flavor and fragrance volatiles by capillary gas chromatography*. Academic Press.

Kartal, N., Sokmen, M., Tepe, B., Daferera, D., Polissiou, M., & Sokmen, A. (2007). Investigation of the antioxidant properties of *Ferula orientalis* L. using a suitable extraction procedure. *Food Chemistry*, *100*(2), 584-589. https://doi.org/10.1016/j.foodchem.2005.09.084

Kotan, R., Cakir, A., Dadasoglu, F., Aydin, T., Cakmakci, R., Ozer, H., Kordali, S., Mete, E., & Dikbas, N. (2010). Antibacterial activities of essential oils and extracts of Turkish *Achillea*, *Satureja* and *Thymus* species against

plant pathogenic bacteria. *Journal of the Science of Food and Agriculture*, *90*, 145-160. https://doi.org/10.1002/jsfa.3799

Kotan, R., Cakir, A., Ozer, H., Kordali, S., Cakmakci, R., Dadasoglu, F., Dikbas, N., Aydin, T., & Kazaz, C. (2014). Antibacterial effects of *Origanum onites* against phytopathogenic bacteria: Possible use of the extracts from protection of disease caused by some phytopathogenic bacteria. *Horticultural Science*, *172*, 210-220. https://doi.org/10.1016/j.scienta.2014.03.016

Kulisic, T., Radonicb, A., Katalinicc, V., & Milos, M. (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry*, *85*(4), 633-640. https://doi.org/10.1016/j.foodchem.2003.07.024

Maggi, F., Papa, F., Dall'Acqua, S., & Nicoletti, M. (2016). Chemical analysis of essential oils from different parts of *Ferula orientalis* L. growing in central Italy. *Natural Product Research*, *30*, 806-813. https://doi.org/10.1080/14786419.2015.1071365

Mahendra, P., & Bisht, S. (2012). *Ferula asafoetida*: Traditional uses and pharmacological activity. *Pharmacognosy Reviews*, *6*(12), 141. https://doi.org/10.4103%2F0973-7847.99948

Mallett, J. F., Cerrati, C., Ucciani, E., Gamisana, J., & Gruber, M. (1994). Antioxidant activity of plant leaves in relation to their α-tocopherol content. *Food Chemistry*, *49*, 61-65. https://doi.org/10.1016/0308-8146(94)90233-X

Meral, G., & Karabay, N. U. (2002). *In vitro* antibacterial activities of three *Hypericum* species from west Anatolia. *Electronic Journal of Biotechnology*, *19*, 6-10.

Miranda, C. A. S. F., Cardoso, M. G., Machado, S. M. F., Gomes, M. S., Andrade, J., & Teixeira, M. L. (2014). Correlacao entre composicao quimica e eficacia antioxidante de oleos essenciais de plantas condimentares por. *E-xacta*, *7*(1), 65-74. https://doi.org/10.18674/exacta.v7i1.1223 (In Portuguese)

Mohammadhosseini, M., Venditti, A., Sarker, S. D., Nahar, L., & Akbarzadeh, A. (2019). The genus *Ferula*: Ethnobotany, phytochemistry and bioactivities-A review. *Industrial Crops Production*, *129*, 350-394. https://doi.org/10.1016/j.indcrop.2018.12.012

Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C., & Yolke, R. H. (1995). *Manual of clinical microbiology*. Amer Society For Microbiology.

Nguir, A., Mabrouk, H., Douki, W., Ismail, M. B., Jannet, H. B., Flamini, G., & Hamza, M. A. (2016). Chemical composition and bioactivities of the essential oil from different organs of *Ferula orientalis* L. growing in Tunisia. *Medicinal Chemistry Research*, *25*, 515-525. https://doi.org/10.1007/s00044-016-1506-1

Okeke, M. I., Iroegbu, C. U., Eze, A. S., Okoli, A. S., & Esimone, C. O. (2001). Evaluation of exract of root of *Landolphia owerrience* for antibacterial activity. *Journal of Ethnopharmacology*, *78*, 119-127. https://doi.org/10.1016/S0378-8741(01)00307-5

Ozkan, H., Yanmıs, D., Karadayı, M., Bal, T., Barıs, O., & Gulluce, M. (2014). Determination of genotoxic and antigenotoxic properties of essential oil from *Ferula orientalis* L. using *Ames*/*Salmonella* and *E. coli* WP2 bacterial test systems. *Toxicology and Industrial Health*, *30*(8), 714-723. https://doi.org/10.1177/0748233712462479

Pavlovic, I., Petrovic, S., Radenkovic, M., Milenkovic, M., Couladis, M., Brankovic, S., Drobac, M. P., & Niketic, M. (2012). Composition, antimicrobial, antiradical and spasmolytic activity of *Ferula heuffelii* Griseb. ex Heuffel (Apiaceae) essential oil. *Food Chemistry*, *130*(2), 310-315. https://doi.org/10.1016/j.foodchem.2011.07.043

Perombelon, M. C., & Kelman, A. (1980). Ecology of the soft rot erwinias. *Annual Review of Phytopathology*, *18*(1), 361-387. https://doi.org/10.1146/annurev.py.18.090180.002045

POWO (Plants of the World Online). (2002). *Ferula L.* Retrieved Aug 30, 2022, from https://powo.science.kew.org/taxon/30105171-2#publications

Rodriguez, H., Agular, L., & Lao, M. (1997). Variations in xanthan production by antibiotic-resistant mutants of *Xanthomonas campestris*. *Applied Microbiology and Biotechnology*, *48*, 626-629. https://doi.org/10.1007/s002530051106

Rojas, J. J., Veronica, J. O., Ocampo, S. A., & Munoz, J. F. (2006). Screening for antimicrobial activity of ten medicinal plant used in Colombian folkloric medicine: A possible alternative in THA treatment of non-nosocomial infections. *BMC Complementary Medicine and Therapies*, *6*, 2. https://doi.org/10.1186/1472-6882-6-2

Ruberto, G., & Baratta, M. T. (2000). Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chemistry*, *69*(2), 167-174. https://doi.org/10.1016/S0308-8146(99)00247-2

Sahebkar, A., & Iranshahi, M. (2013). Volatile constituents of the genus *Ferula* (Apiaceae): A review. *Journal of Essential Oil Bearing Plants*, *14*(5), 504-531. https://doi.org/10.1080/0972060X.2011.10643969

Sahin, F., & Miller, S. A. (1997). Identification of the bacterial leaf spot pathogen of lettuce, *Xanthomonas campestris* pv. *vitians*, in Ohio, and assessment of cultivar resistance and seed treatment. *Plant Disease*, *81*,1443-1446. https://doi.org/10.1094/PDIS.1997.81.12.1443

Sahin, F., Karaman, I., Gulluce, M., Ogutcu, H., Sengul, M., Adıguzel, A., Öztürk, S., & Kotan, R. (2003). Evalation of antimicrobial actives of *Satureja hortensis* L. *Journal of Ethnopharmacology*, *87*, 61-65. https://doi.org/10.1016/S0378-8741(03)00110-7

Schlesier, K., Harwat, M., Bohm, V., & Bitsch, R. (2002). Assessment of antioxidant activity by using different *in vitro* methods. *Free Radical Research*, *36*, 177e187. https://doi.org/10.1080/10715760290006411

Sechi, L. A., Lezcano, I., Nunez, N., Espim, M., Duprè, I., Pinna, A., Molicotti, P., Fadda, G., & Zanetti, S. (2001). Antibacterial activity of ozonized sunflower oil (oleozon). *Journal of Applied Microbiology*, *90*, 279-284. https://doi.org/10.1046/j.1365-2672.2001.01235.x

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*, *40*, 945-948. https://doi.org/10.1021/jf00018a005

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, *299*, 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

Tepe, B., Sokmen, M., Akpulat, A. H., & Sokmen, A. (2006). Screening of THA antioxidant potentials of six *Salvia* species from Turkey. *Food Chemistry*, *95*, 200-204. https://doi.org/10.1016/j.foodchem.2004.12.031

Topdas, E. F., Sengul, M., Taghizadehghalehjoughi, A., & Hacımuftuoglu, A. (2020). Neuroprotective potential and antioxidant activity of various solvent extracts and essential oil of *Ferula orientalis* L. *Journal of Essential Oil Bearing Plants*, *23*(1), 121-138. https://doi.org/10.1080/0972060X.2020.1729247

Tubives. (2019). *Turkish Plants Data Service*. Retrieved Jun 18, 2019, from http://194.27.225.161/yasin/tubives/index.php?sayfa=1&tax_id=4347

White, D. G., Zhao, S., Simjee, S., Wagner, D. D., & Mcdermott, P. F. (2002). Antimicrobial resistance of food-borne pathogens. *Microbes and Infection*, *4*, 405-412. https://doi.org/10.1016/S1286-4579(02)01554-X

Wu, L., Chang, L., Chen, S., Fan, N., & Ho, J. A. (2009). Antioxidant activity and melanogenesis inhibitory effect of the acetonic extract of *Osmanthus fragrans*: A potential natural and functional food flavor additive. *Food Science and Technology*, *42*, 1513-1519. https://doi.org/10.1016/j.lwt.2009.04.004

Yegen, O., Basım, H., Laux, P., & Zeller, W. (2002). Biocontrol of *Erwinia amylovora* with a natural product. *Iobc Wprs Bulletin*, *25*(10), 147-150.

AIMS & SCOPE

Journal of Agricultural Production aims to create an environment for researchers to introduce, share, read, and discuss recent scientific progress and adopts the policy of providing open access to the readers who may be interested in recent scientific developments. *Journal of Agricultural Production* publishes papers in the categories of research articles, review articles, short communications, and letters to the Editor.

Research areas include but not limited to:

- Agriculture
- Aquaculture
- Fisheries
- Ecology
- Environmental sciences
- Horticulture
- Crop protection
- Bio systems
- Landscape
- Agriculture and aquaculture economics
- Agriculture and aquaculture biotechnology
- Soil science and plant nutrition
- Husbandry
- Basic sciences

And closely related life sciences, as well as engineering topics.

AUTHOR GUIDELINES

Manuscripts must be submitted to the journal in electronic version through online submission system at https://dergipark.org.tr/en/pub/japro following the Author Guidelines of the journal.

Types of Paper

Research articles, review articles, short communications, and letters to the Editor.

- Research articles: original full-length research papers which have not been published previously and should not exceed 7500 words or 25 manuscript pages (including tables and figures).
- Review articles: on topical subjects and up to 10000 words or 25 manuscript pages (including tables and figures).
- Short communications: describing work that may be of a preliminary nature or a case report; preferably no more than 3000 words or 10 manuscript pages (including tables and figures).
- Letters to the Editor: should be included on matters of topical interest and not exceeding 2000 words or 10 manuscript pages (including tables and figures).

Preparation of Manuscripts

Papers must be written in English. Prepare your text using a word-processing software and save in ".doc" or ".docx" formats. Manuscripts must be structured in the following order:

- ***Title Page File***
  - Title (Concise and informative. Avoid abbreviations and formulae)
  - Author names and affiliation addresses (Full names should be given, no abbreviations. The corresponding author should be identified with an asterisk. Each affiliation address should include institution, faculty/school, department, city, and country)
  - ORCID numbers for all authors.
  - Corresponding author's e-mail, telephone number, and address
  - Acknowledgements (if applicable. Keep these to the absolute minimum)
  - Compliance with Ethical Standards
    - ❖ Conflict of Interest Statement
    - ❖ Statement on the Welfare of Animals (if applicable)
    - ❖ Statement of Human Rights (if applicable)
- ***Main File***
  - Title
  - Abstract (Should be between 100 and 350 words. References and abbreviations should be avoided)
  - Keywords (Minimum 3, Maximum 6 keywords)
  - Introduction
  - Materials and Methods
  - Results
  - Discussion (Can be combined with Results section if appropriate)
  - Conclusion
  - References
  - Table(s) with caption(s) (on appropriate location in the text)
  - Figure(s) with caption(s) (on appropriate location in the text)
  - and appendices (if any)

Manuscript Formatting

Use a 12-point Times New Roman font, including the references, table headings and figure captions, double-spaced and with 25 mm margins on all sides of A4 size paper throughout the manuscript. The text should be in single-column format.

- Each page must be numbered with Arabic numerals, and lines must be continuously numbered from the start to the end of the manuscript.
- Use italics for emphasis.
- Use only SI (international system) units.
- Use "dot" for decimal points.
- Use italics for species name.

References

Journal of Agricultural Production uses APA style (7th edition). Accordingly, authors must format their references as per the guidelines below. Please ensure that each reference cited in the text is also presented in the reference list. Authors should always supply DOI or URL of the work cited if available.

In-text citation (Narrative):

…The results of Bliss (2022) support…
…Sönmez and Taştan (2020) indicated that…
…According to the method of Öztürk et al. (2021)…

In-text citation (In parenthesis):

…It was found to be isometric (Öztürk, 2018)…
…is highly susceptible to diseases (Doma & Craig, 2019)…
…have been studied (Kale et al., 2020)…

Two or more works in the same parenthesis:

…extremely toxic for the environment (Sönmez, 2018, 2019; Öztürk et al., 2020a; Kadak & Taştan, 2021)…

Citation in the reference list:

References should be listed first alphabetically and then further sorted chronologically at the end of the article. The citation of all references should conform to the following examples:

Article:

Lastname, N., Lastname, M., & Lastname, O. (Year). Title of the work. *Title of the Journal*, *Volume*(Issue), Page numbers. DOI

Tort, S. (1998). Stress and immune modulation in fish. *Developmental & Comparative Immunology*, *35*(12), 1366-1375. https://doi.org/10.1016/j.dci.2011.07.002

Kasumyan, A. O., & Døving, K. B. (2003). Taste preferences in fishes. *Fish and Fisheries*, *4*(4), 289-347. https://doi.org/10.1046/j.1467-2979.2003.00121.x

Özçelik, H., Taştan, Y., Terzi, E., & Sönmez, A. Y. (2020). Use of onion (*Allium cepa*) and garlic (*Allium sativum*) wastes for the prevention of fungal disease (*Saprolegnia parasitica*) on eggs of rainbow trout (*Oncorhynchus mykiss*). *Journal of Fish Diseases*, *43*(10), 1325-1330. https://doi.org/10.1111/jfd.13229

Article by DOI (early access):

Salem, M. O. A., Salem, T. A., Yürüten Özdemir, K., Sönmez, A. Y., Bilen, S., & Güney, K. (2021). Antioxidant enzyme activities and immune responses in rainbow trout (*Onchorhynchus mykiss*) juveniles fed diets supplemented with dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*) extracts. *Fish Physiology and Biochemistry*. https://doi.org/10.1007/s10695-021-00962-5

Book:

Lastname, N., Lastname, M., & Lastname, O. (Year). *Title of the work*. Publisher.

Oidtmann, K., Xao, Q., & Lloyd, A. S. (2018). *The food need by the year 2050*. Elsevier.

Book Chapter:

Lastname, N., Lastname, M., & Lastname, O. (Year). Title of the chapter. In N. N. Lastname, A. Lastname & B. Lastname (Eds.), *Title of the book* (pp. Page numbers). Publisher.

Pickering, A. D. (1993). Growth and stress in fish production. In G. A. E. Gall & H. Chen (Eds.), *Genetics in Aquaculture* (pp. 51-63). Elsevier. https://doi.org/10.1016/b978-0-444-81527-9.50010-5

Dissertation or Thesis:

Lastname, N. (Year). *Title of dissertation/thesis* (Doctoral dissertation/Master's thesis, Name of Institution).

Sönmez, A. Y. (2011). *Karasu ırmağında ağır metal kirliliğinin belirlenmesi ve bulanık mantıkla değerlendirilmesi* (Doctoral dissertation, Atatürk University).

Taştan, Y. (2018). *Tatlısu kerevitindeki (Astacus leptodactylus) siyah solungaç hastalığı etkeni mantar Fusarium oxysporum'un PCR yöntemi ile teşhisi* (Master's thesis, Akdeniz University).

Conference Proceedings:

Lastname, N., Lastname, M., & Lastname, O. (Year). *Title of the work*. Title of the Conference. City.

Ken, A., & Kumar, S. (2020). *A new statistical model for fuzzy logic evaluation*. 3rd International Congress on Statistics. İstanbul.

Institution Publication:

Institution name. (Year). *Title of the work*. URL

FAO. (2020). *Fishery and aquaculture statistics 2018*. http://www.fao.org/3/cb1213t/CB1213T.pdf

Internet Source:

Lastname, N. (Year). *Title of the work*. Retrieved May 15, 2020, from URL

Perreault, L. (2019). *The future of agriculture: Polyculture*. Retrieved January 12, 2020, from https://www.agriculture.com

Table(s)

Tables, numbered in Arabic, should be in separate pages with a short descriptive title at the top. Place footnotes to tables below the table body and indicate them with superscript lowercase letters (or asterisks for significance values and other statistical data).

Figure(s)

All illustrations should be labelled as 'Figure' and numbered in consecutive Arabic numbers, Figure 1, Figure 2 etc. in the text. If panels of a figure are labelled (a, b, etc.) use the same case when referring to these panels in the text. Figures are recommended to be in electronic formats such as PNG, JPEG, TIFF (min. 300 dpi). All figures or tables should be presented in the body of the text.

Online Manuscript Submission

Authors are requested to submit manuscripts via the journal's online submission system at https://dergipark.org.tr/en/pub/japro

Submission Checklist

- Author Guidelines of the journal has been read and adhered

- **Title Page File**
  - Title
  - Full names, e-mails, and affiliation addresses of all authors
  - ORCID numbers of all authors
  - Corresponding author's e-mail, telephone number, and address
  - Ethical statements

- **Main File**
  - Continuous page numbers
  - Continuous line numbers
  - Blinded document (no personal information is present)
  - Title
  - Abstract (100-350 words)
  - Keywords (3-6 keywords)
  - All figures and tables are numbered and cited in order in text
  - Completeness and accuracy of the references have been checked
  - References have been edited as per the Journal guidelines

Publication Frequency

The journal includes original scientific articles on a variety of different subjects in English and is being published twice a year in June and December.

Price Policy

Journal of Agricultural Production requests article submission fee. This fee is collected from all articles sent to the journal regardless of the acceptance/rejection decision.

Authors will receive an email regarding the payment upon submission. Authors should make the payment after this email. The evaluation process will be initiated once the payment is completed.

Submission Fee: 50$ (For Turkish Authors: 1000 TL)

Editorial Processing Charge: Free

Article Processing Charge (or any other post-acceptance fee): Free.

The articles are open access and freely available to all readers.

PUBLICATION ETHICS

Details about Publication Ethics can be found at https://dergipark.org.tr/en/pub/japro/policy

ETHICAL GUIDELINES FOR THE USE OF ANIMALS IN RESEARCH

Details can be found at
https://dergipark.org.tr/en/pub/japro/policy

OPEN ACCESS POLICY

Details can be found at
https://dergipark.org.tr/en/pub/japro/page/13659

REVIEW PROCESS

Double-Blind Review and Evaluation Process

Double-Blind Review is a method applied for publishing scientific publications with the highest quality. This method forms the basis of an objective evaluation of scientific studies and is preferred by many scientific journals.

The views of referees have a decisive place in the publication quality of a Journal. *Journal of Agricultural Production* uses the double-blind review method, which means that both the reviewer and author identities are concealed from the reviewers, and vice versa, throughout the review process, in the evaluation process of all studies. For this reason, the authors are asked to blind their manuscripts before submitting. All the studies submitted to *Journal of Agricultural Production* are evaluated by double-blind review method according to the following steps.

1. Initial Evaluation Process

The studies submitted to *Journal of Agricultural Production* are first evaluated by the editor. At this stage, studies that are not in line with the aim and scope of the journal, are weak in terms of language and narrative rules in English, contain scientifically critical mistakes, are not original worthy, and cannot meet publication policies are rejected. Authors of rejected studies will be notified within one month at the latest from the date of submission. Eligible studies are sent to the field editor to which the study is relevant for pre-evaluation.

2. Pre-Evaluation Process

In the pre-evaluation process, the field editors examine the studies, introduction and literature, methods, findings, results, evaluation and discussion sections in detail in terms of journal publication policies, scope and authenticity of study. Study which is not suitable as a result of this examination is returned to the author with the field editor's evaluation report within four weeks at the latest. The studies which are suitable for the journal are passed to the referee process.

3. Referee Process

The studies are sent to the referees according to their content and the expertise of the referees. The field editor examining the study may propose at least two referees from the pool of *Journal of Agricultural Production* Advisory Board or referee pool according to their field of expertise or may propose a new referee appropriate to the field of study. The editors evaluate the referee's suggestions coming from the field editor and the studies are submitted to the referees. Referees are obliged to guarantee that they will not share any process or document about the study they are evaluating.

4. Referee Evaluation Process

The period given to the referee for the evaluation process is 15 days. Proposals for corrections from referees or editors must be completed by the authors within 1 month according to the "correction instruction". Referees can decide on the suitability of the study by reviewing the corrections and may also request multiple corrections if necessary.

Referee Reports

Referee evaluations are based in general on the originality of the studies, the method used, and the conformity with the ethical rules, the consistent presentation of the findings and results, and the examination of the literature.

This review is based on the following elements:

1. Introduction and Literature: The evaluation report contains the presentation and purpose of the problem addressed in the study, the importance of the topic, the scope of the relevant literature, the

timeliness and the originality of the study.

2. Methodology: The evaluation report includes information on the suitability of the method used, the choice and characteristics of the research group, validity and reliability, as well as on the data collection and analysis process.

3. Findings: The evaluation report includes opinions on the presentation of the findings obtained in the frame of the method, the correctness of the analysis methods, the aims of the research and the consistency of the findings, the presentation of the required tables, figures and images and the conceptual evaluation of the tests used.

4. Evaluation and discussion: The evaluation report includes the opinion on the subject based on findings, relevance to research questions and hypotheses, generalizability and applicability.

5. Conclusion and suggestions: The evaluation report contains the opinion on the contributions to the literature, future studies and recommendations for the applications in the area.

6. Style and narration: The evaluation report includes compatibility of the title with the content, appropriate use of English in the study, refers and references in accordance with the language of the study and APA rules.

7. Overall evaluation: The evaluation report contains opinion on the authenticity of the study as a whole, its contribution to the educational literature and the applications in the area. The journal considers that scientists should avoid research which kills or damages any species of animal which, using IUCN criteria, is regarded as threatened or is listed as such in a Red Data Book appropriate for the geographic area concerned. In accordance with this view, papers based on such research will not be accepted by the Journal, unless the work had clear conservation objectives.

Plagiarism Detection

In agreement with publishing policies of *Journal of Agricultural Production*, plagiarism check is required for each study that has undergone the "Review Process". The Turnitin plagiarism checker software is used for plagiarism detection.

Proofs

Proof documents will be sent to the corresponding authors via e-mail. Proofs should be checked immediately and responses should be returned back within 15 working days. It is the responsibility of the authors to check carefully the proofs. No changes will be allowed after this stage.

DISCLAIMER

The Publisher and Editors cannot be held responsible for errors or any consequences arising from the use of information contained in this journal; the views and opinions expressed do not necessarily reflect those of the Publisher and Editors.

Editor or members of the editorial board are not responsible for the author's opinions and manuscript contents. Authors are responsible for the ethical originality of and possible errors in their manuscripts.

This journal is available online at https://dergipark.org.tr/en/pub/japro

Kindly visit website to search the articles and register for table of contents e-mail alerts.

LICENCE

INDEXING

Journal of Agricultural Production is currently indexed by

- TRDİZİN
- EBSCO
- CABI
- ROAD-Directory of Open Access Scholarly Resources
- Google Scholar
- Crossref

PUBLISHER

Gökhan ARSLAN
Atatürk University
agriprojournal@gmail.com
Yakutiye mah. Atatürk Üni. Su Ürünleri Fakültesi,
Merkez/Erzurum, TÜRKİYE

PUBLISHER SUPPORT

Prensip Publishing and Consultancy Ind. Trade. Co. Ltd.
Beyçelebi mah. Eski Ankara cad. 41/3 No:10,
Merkez/Kastamonu, 37200, TÜRKİYE
https://prensip.gen.tr
info@prensip.gen.tr

EXPRESS

JOURNAL OF AGRICULTURAL PRODUCTION