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HORTICULTURAL STUDIES

RESEARCH PAPER

Phenolic, Organic Acid and Sugar Content of Garlic (*Allium sativum* L.) Genotypes Grown in Different Regions of Türkiye

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Abstract

In this study, the biochemical profiles of five different genotypes of garlic (*Allium sativum* L.) harvested in different regions of Türkiye were investigated in detail, focusing on phenolic compounds, organic acids and sugar components. The analyses were carried out using high-performance liquid chromatography (HPLC) and showed that there were significant biochemical differences between the genotypes. A total of 18 phenolic compounds, 12 organic acids and 3 sugar components were determined in the samples. With the phenolic compounds and the antioxidant activity, while Genotype 4 had the highest chlorogenic acid (174.99 mg kg^{-1}), Genotype 5 had the highest catechin hydrate (158.77 mg kg^{-1}), gallic acid (22.35 mg kg^{-1}) and *o*-coumaric acid (12.78 mg kg^{-1}). The profile of organic acids was also presented, where Genotype 2 was the richest genotype for citric acid (7374.66 mg kg^{-1}). Other significant organic acids, succinic (12747.34 mg kg^{-1}) and isobutyric acid (149.54 mg kg^{-1}) which were identified the highest in Genotype 5. As far as sugar components are concerned, sucrose levels showed a significant variation between the genotypes, where Genotype 5 had 3197.79 mg kg^{-1} and Genotype 4 had 1950.93 mg kg^{-1}. There were statistically significant differences between the genotypes in terms of phenolic compounds, organic acids and sugar components ($p<0.05$), which are indicate that biochemical differences between genotypes are important in terms of agricultural and nutritional value. These data can be utilized by garlic breeders and garlic producers by regions.

1. Introduction

Allium sativum L., commonly known as garlic, has been used traditionally for both culinary and medicinal purposes (Londhe et al., 2011). Garlic first served as a protective food by construction workers in ancient Egypt and later valued as a functional food that imparts beneficial health effects to humans (Rivlin, 2001). Since this plant contains numerous chemical components including phenolic compounds, organic acids, and sugars, it thus holds great nutritional value. The health-enhancing constituents of these vegetables comprise antioxidant, antimicrobial, and cardiovascular protective properties (Papu et al., 2014). Chemical composition and biological activities of garlic may depend on the different genotypes, environmental conditions, and growing techniques (Asif, 2015; Besirli et al., 2022). It is very important to study these biochemical differences related to the effects of garlic on health (Rahman, 2003). Phenolic compounds, such as those in garlic, are chemical substances found in abundance in plants and used widely as antioxidants. These agents protect cells against oxidative stress by neutralizing free radicals; through that action, they may prevent the development of chronic diseases such as cardiovascular diseases, cancer, and aging

(Santhosha et al., 2013; Kallel et al., 2014). Garlic is considered high in phenolic and organic acid content in local and traditional genotypes. These genotypes have also developed resistance to environmental stress factors, and the studies on the chemical constituents have provided important data on the nutritive and health benefits of garlic. In addition, conservation of local genotypes is very important for sustainable farming practices too. The antimicrobial and autoinflammatory features of phenolic compounds in garlic are also remarkable. Studies show that these phenolic compounds in garlic are effective against bacteria and fungi and they reduce inflammation (Wilson and Demmig-Adams, 2007). These components, therefore, enable the potential medical uses of garlic. Organic acids meanwhile, are important in the plant cell metabolism and delimit the taste and aroma profiles of garlic.

Ascorbic acid (vitamin C) and malic acid are chemicals that are derived from living work and have been shown to be very helpful during the high-protein digestion process and to down-regulate the stomach's acidity. The main ones are acids that are important for regulating the pH ranges and the elimination of toxins in the body (Khan and Iqbal, 2016; Ma et al., 2021). Garlic is a product that can be used in medical treatments, as it is one of the leading foods with polyphenolic, organic acids and sugars. Antioxidants such as the ones that defend cells from free radical damage and as a result, inhibit the process of aging are the components that prove the benefits of garlic against chronic diseases like cardiovascular diseases, cancers, and neurodegenerative diseases. Antimicrobial properties stand as the most powerful mechanism for preventing and treating infections. The beneficial effects of digestion are those that speed up digestion and regulate the pH of the stomach (Aversa et al., 2016).

Garlic production areas grown in Balıkesir, Kırklareli, Kütahya and Gaziantep provinces, which are the most common garlic production areas in our country, were taken into consideration. In addition to regional differences in our country, different garlic genotypes are grown in each region. However, it was noted that there is not enough data on the nutritional properties of these genotypes. According to the regions, Iranian genotypes in Balıkesir and Chinese genotypes in Kırklareli stand out as important international genotypes in terms of yield and quality. Black type garlic (Kütahya) and Araban genotypes (Gaziantep) have been grown locally for a long time. Purple type garlic is also produced in Aksaray and is a popular garlic genotype.

This research was conducted with the aim of comparing the character of these five different genotypes of garlic, *Allium sativum* L., regarding their phenolic compounds, organic acids, and sugars. Through high-performance liquid chromatography (HPLC) analysis, each genotype was examined for 18 phenolics, 12 organic acids, and 3 sugar compounds. This study deepens our understanding of the biochemical changes that occur across different garlic genotypes and their contributions to the health benefits of these varieties. By highlighting the biochemical distinctions among various garlic types, the research underscores the importance of phenolic, organic acid, and sugar components in both agriculture and nutrition. The key findings from this investigation illustrate the crucial role these elements play in the agricultural and nutritional sectors. Additionally, these insights lay the groundwork for further exploration into the unique biochemical characteristics of garlic genotypes native to Türkiye.

2. Material and Methods

2.1. Materials

The study was carried out to examine the diversity of five garlic genotypes produced in the same areas between 2021-2022 in different regions of Türkiye. The observed genotypes were Persian (Genotype 1, Balıkesir), Chinese (Genotype 2, Kırklareli), Purple (Genotype 3, Aksaray), Black Garlic (Genotype 4, Kütahya), and Araban (Genotype 5, Gaziantep). Each local genotype was selected to show regional diversity and analyses were carried out on these genotypes.

The garlic genotypes were sown between August and September and reaped between June and July. In the whole season of growing this garlic, the foliar fertilization as well as irrigation was the main factor that getting the highest yield at the end was maintained, and the optimal conditions that were obtained were due to proper fertilization and irrigation. The precise location and elevation of the collection sites for the garlic genotypes are as follows: Aksaray (38°31’37” N, 33°50’00” E; 944 m), Balıkesir (39°30’40” N, 27°50’53” E; 231 m), Gaziantep/Araban (37°04’05” N, 37°23’35” E; 850 m), Kütahya/Şaphane (38°58’43” N, 29°16’17” E; 781 m), and Kırklareli/Babaeski (41°34’33” N, 27°50’43” E; 314 m).

At harvest, the bulbs of the garlic genotypes grown under these conditions were taken to the laboratory under cold chain conditions for the determination of phenolic compounds, organic acids and sugars. Each analysis was carried out under optimal laboratory conditions and a total of 15 outputs, the set containing three samples for each garlic genotype, were checked.

2.2. Extraction method for phenolic compounds and total antioxidant activity

At harvest, garlic samples (bulbs) were first cleaned to remove surface dirt. Approximately 25 g of each cleaned garlic bulb was subjected to extraction by adding 100 mL of methanol in sealed round-bottomed flasks placed on magnetic stirrers at room temperature (Erol et al., 2024; Erol, 2024). The extraction process continued until the solvent was colorless and at least five times this process was repeated.

The resulting extracts were filtered through Whatman #1 filter paper and the filtrates were collected. The extracts were then concentrated by applying a Buchi R300 rotary evaporator at 60°C and 200 mm Hg. The residues that were still at the bottom of the flask were redissolved in the methanol in the different volumes of 200-300 µL, and the extracts were prepared for the analysis of total phenolic content and total antioxidant activity. This procedure has been optimized to efficiently extraction these plant-derived compounds from garlic samples.

2.3. Analysis of phenolic compounds by HPLC

The presence of phenolics in garlic extracts was established through ultra-high performance reversely-phase liquid chromatography (Agilent 1260 Infinity RP-HPLC, USA). The phenolic compounds were isolated utilizing C18 reversely-phase column (110 Å, 5 µm, 4.6 × 250 mm, ACE Generix). The injection volume was set at 10 µL and the mobile phases utilized were A (0.1% phosphoric acid-water solution) and B (100% acetonitrile). A gradient system was used, the temperature of the column oven was maintained at 30°C and measurements were made using a diode array detector (DAD).

Concentrations of chlorogenic acid, catechin hydrate, caffeic acid, 4-hydroxy benzoic acid, vanillin, rutin, trans-ferulic acid, hydroxycinnamic acid, naringin, *o*-coumaric acid, rosmarinic acid, salicylic acid, resveratrol, quercetin, trans-cinnamic acid, naringenin, chrysin and flavone components were determined by external standard method and characterized by retention times. The concentrations of phenolics were determined by the external standard method and characterized by retention times. The data obtained were reported in mg kg^{-1} wet weight (Uçan Türkmen et al., 2023; Uçan Türkmen et al,. 2024).

2.4. Antioxidant activity analysis method

The antioxidant capacity of the garlic extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical % inhibition method. The garlic extracts were first dissolved in methanol to prepare different concentrations for analysis. Meanwhile, 100 µL of the extracts dissolved in methanol were added to test tubes. 2.9 mL of the prepared 0.1 mM DPPH solution was added to each tube. The solutions were then kept in the dark for 30 min and the absorbance values were read against the solvent at a wavelength of 517 nm on a UV-Vis spectrophotometer (Shimadzu UV-1800). The % inhibition of DPPH radical was calculated from the absorbance values (Brand-Williams et al., 1995). Each sample was analyzed in triplicate.

2.5. Analysis of organic acids and sugars by HPLC

2.5.1. Extraction method

The analysis of organic acids and sugars in garlic samples was conducted using freshly harvested garlic bulbs, which were prepared for extraction. The samples were crushed and 2.5 g of samples were carefully placed in 50 mL Falcon tubes, which were then homogenized in 25 mL of a deionized water/methanol mixture (7/3, v/v) using a high-speed homogenizer (IKA model T18). The homogenized solution was incubated in a water bath at 40°C for 30 min. After this period, the centrifuge was run at 10,000 rpm for 10 min at 4°C to obtain the supernatant. This material was then filtered through a 0.45-micron syringe filter and stored at -20°C until analysis (Gallardo-Guerrero et al., 2010).

2.5.2. HPLC analysis conditions

Sugars and organic acids in garlic samples were analyzed by modifying the method of Korkmaz et al. (2020). Shimadzu Prominence Modular LC20A HPLC system was used for the analyses. For sugar analysis, a Rezex RCM-Monosaccharide Ca^{2+} (8%) LC Column (300 × 7.8 mm) was used. The column temperature for sucrose, glucose and fructose analysis was 80°C and isocratic mode, with a flow rate of 0.5 mL min^{-1}, using ultrapure water as mobile phase and completed in 15 min.

The results were calculated as mg g^{-1} fresh weight using calibration curves prepared with standard sugar solutions. All samples were analyzed in triplicate. Oxalic acid, citric acid, tartaric acid, malic acid, succinic acid, lactic acid, formic acid, acetic acid, fumaric acid, propionic acid, isobutyric acid and butyric acid analyses were conducted on the same HPLC system using a UV detector set at 210 nm, with the column temperature at 50°C, and a Rezex ROA-Organic Acid H^+ (8%) LC Column (300 × 7.8 mm) for organic acid separation. The analysis results were calculated using calibration curves generated from standard solutions and were expressed as mg kg^{-1} fresh weight.

2.6. Statistical analysis

Statistical analyses of the obtained data were performed using JMP 14 software. Each measurement was performed three times. Variance analysis and multiple comparisons were conducted using Tukey's HSD test. In the statistical analyses, results with a p-value below 0.05 for the variation parameters were considered statistically significant.

3. Results and Discussion

3.1. Phenolic compounds and antioxidant activity

In this study, the phenolic compound profiles and antioxidant activities of five different garlic genotypes were comprehensively evaluated. The amounts of chlorogenic acid varied widely among the genotypes, with the highest level found in Genotype 4 at 174.99 mg kg^{-1}, and the lowest in Genotype 2 at 34.86 mg kg^{-1}. Genotype 5 is the only genotype in which catechin hydrate was found and it had the highest level with 158.77 mg kg^{-1}. Caffeic acid was the highest in Genotype 5 with 0.95 mg kg^{-1} and the lowest in Genotype 1 with 0.36 mg kg^{-1}. In this study, important phenolic compounds such as gallic acid, o-coumaric acid and ferulic acid also differed among genotypes. Antioxidants were measured by DPPH% inhibition method and it was seen that antioxidant activity was linearly related to phenolic compounds. For example, DPPH% inhibition of Genotype 4 was determined as 53.99%, while that of Genotype 1 was 46.57% and that of Genotype 5 was 37.73%. Genotypes containing higher amounts of phenolic compounds exhibited higher antioxidant activity and these genotypes showed stronger antioxidant activity than the others.

Studies have established that the active compounds and, hence, antioxidant activity in garlic vary variable. For example, in the work of Fratianni et al. (2016), the content of chlorogenic acid in garlic was from 76.99 mg kg^{-1} to 93.95 mg kg^{-1}. In the work of Beato et al. (2011), the variability of caffeic acid was between 0.25 mg kg^{-1} and 13.74 mg kg^{-1}. The content of chlorogenic and caffeic acids in the studied genotypes was compared with amounts reported in the literature. According to Kim et al. (2013), the chlorogenic content in garlic ranged from 46.00 mg kg^{-1} to 134.00 mg kg^{-1} in different genotypes. The variation in the range of this value obtained by our study, and thus, it is very likely that because of the genotypes, there are differences in phenolic compounds, as in our case. The same findings were found by Šnirc et al. (2023), who mentioned that the amount of caffeic acid in garlic genotypes was between 11.43 mg kg^{-1} and 16.00 mg kg^{-1} on a dry weight basis, which is similar to this work.

Based on the antioxidant activity, the high catechin hydrate content in Genotype 5 indicates this genotype may be rich in antioxidants. A review of catechin hydrate's antioxidant and anti-inflammatory properties underline its health benefits (Pedro et al., 2020). The high chlorogenic acid content in Genotype 4 also means that this genotype may have significant antioxidant capacity.

We used principal component analysis (PCA) to try to understand the biochemical differences between the different genotypes (Figure 1). The PCA scores showed us how the first two principal components are related to each other, and together they account for 92.89% of the total variance (Component 1: 65.52%, Component 2: 27.37%). It means that the distinction between genotypes is to a great extent brought about by the phenolic content. Using catechin hydrate, 158.77 mg kg^{-1}, and chrysin, 36.44 mg kg^{-1}, as indicators, it can be inferred that such genotype as Genotype 5 is more liable to these two chemicals than any other genotypes. Moreover, Genotype 2 was found to be

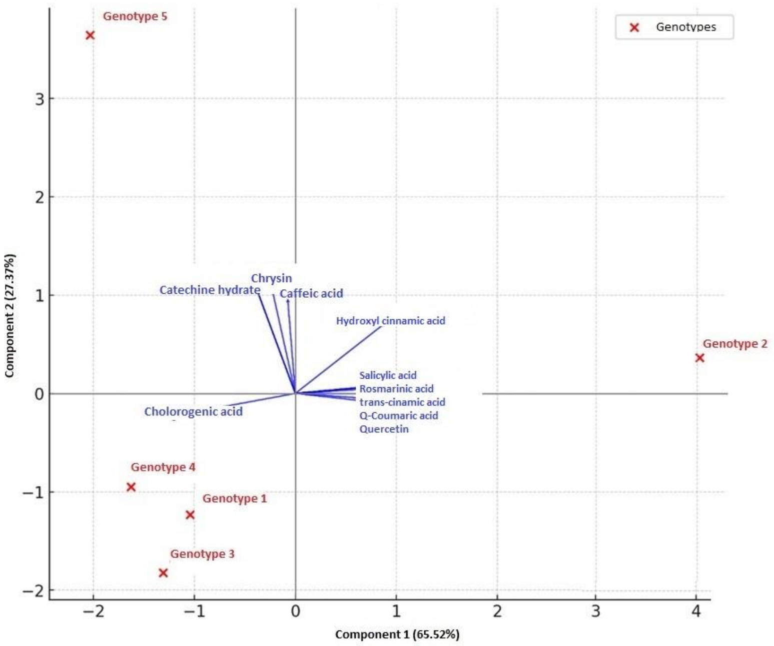

Figure 1. Principal component analysis (PCA) of phenolics.

the corelated to quercetin (4.14 mg kg^{-1}), salicylic acid (0.55 mg kg^{-1}), and rosmarinic acid (3.29 mg kg^{-1}). Genotypes 1, 3, and 4 showed the same phenolic profiles. It is evident from the literature that there is a considerable range of garlic phenolic compounds, which suggests that, genetic and environmental factors may play a role in influencing these profiles. This study therefore aims to highlight the potential health benefits of biochemical diversity among garlic genotypes, and to provide a valuable foundation for evaluating these genotypes as functional foods.

Interesting differences in the profile of phenolic compounds and in the antioxidant activities for different genotypes of garlic have been revealed within this study. The potential health benefits due to phenolic compounds present in these genotypes make them a great avenue for testing as functional foods. In addition, their phenolic profiles are of equal importance, since it forms the basis of breeding and cultivation within the garlic studies. This report should add to the literature that already exists on the biochemical diversity of garlic genotypes and its health impact.

3.2. Organic acid analysis

The aim of this study was to evaluate the organic acid profiles of five different garlic genotypes. The results indicate the potential for significant variations among the genotypes. The results clearly show that Genotype 5 had the highest levels of oxalic acid at 18.57 mg kg^{-1}, while Genotype 4 had the lowest levels (0.00 mg kg^{-1}). From the data available, it seems that Genotype 2 has the highest amount of citric acid, at 7374.66 mg kg^{-1}, while Genotype 4 has the lowest, at 2616.05 mg kg^{-1}. There appears to be considerable variation in tartaric acid levels, with the highest amount observed in Genotype 4 at 584.77 mg kg^{-1}. It is worth noting that some genotypes did not have any detectable levels. It would appear that malic acid is highest in Genotype 2 at 562.79 mg kg^{-1}, and that it is not detected in Genotypes 1 and 5. It would appear that succinic acid was present in the highest amount in Genotype 5 at 12747.34 mg kg^{-1}, although it was absent in Genotype 3. Similarly, acetic acid levels also varied among genotypes, with the highest level in Genotype 2 at 696.33 mg kg^{-1}, although it was not detected in some genotypes. Finally, formic acid was highest in Genotype 2 at 145.36 mg kg^{-1}.

The literature demonstrates that organic acids in garlic exhibit considerable variation. Şaşmaz et al. (2022), reported citric acid content in garlic ranging from 8.90 g kg^{-1} to 17.50 g kg^{-1} between genotypes. Citric acid levels in the present study were within this range, indicating that genetic and environmental factors may strongly determine the amount of citric acid in the genotype. Sangouni et al. (2021), reported variations in tartaric acid levels ranging from 50.00 mg kg^{-1} to 1200.00 mg kg^{-1} in garlic among genotypes studied. Tartaric acid values in the genotypes we studied were also within these above-mentioned ranges, representing a marked effect of genotypes on organic acid profiles in garlic. The great differences in concentrations between other organic acids, such as succinic acid and malic acid, are proof of the major functions they participate in during biochemical and physiological processes.

The principal component analysis (Figure 2) of organic acid profiles across all genotypes was determined. Two principal components accounted for 73.30% of the total variance: Component 1 = 52.53% and Component 2 = 20.77%. However, it is

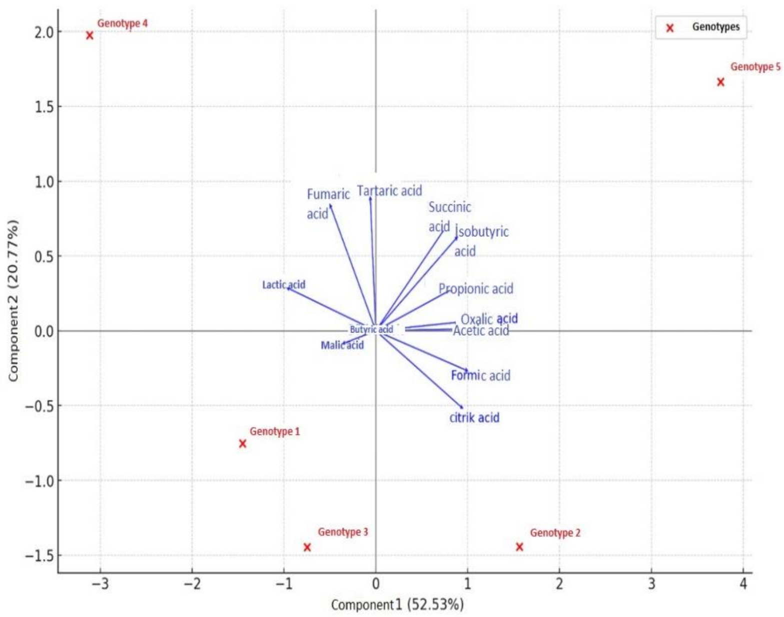

Figure 2. Principal component analysis (PCA) of organic acids.

essential to point out that Genotype 5 is separated from the others in the PCA plot since this genotype presents high succinic acid (12747.34 mg kg^{-1}) and isobutyric acid (149.54 mg kg^{-1}) content. This reveals that the genotype is rich in such compounds. Genotype 2 recorded high values for citric acid (7374.66 mg kg^{-1}) and acetic acid (696.33 mg kg^{-1}). Obviously, there was also an association with various other genotypes showing an association with certain organic acids, which reveals that this biochemical diversity might relate to organic acid profiles. These results imply that both genetic and environmental factors cause variation in the levels of organic acids between genotypes. It may thus be advantageous to include this variation in subsequent research on the breeding and growing of garlic. The present results restate that there is tremendous biochemical variation among different garlic genotypes concerning organic acids. The contents of organic acids make up very important parts of plant metabolism in connection with cellular respiration, energy production, and response to stresses. In this manner, differences express the genetic diversity and adaptability of the garlic plant toward environmental factors. Considering the potential health effects of organic acids provides an important basis for assessing these genotypes for nutritional and functional food purposes. The findings of the study, therefore, provide insights valuable for the biochemical diversity among genotypes of garlic and underline the health significance of such diversity.

3.3. Sugar analysis

A complete sugar profile of five garlic genotypes was intensively studied in the research. A sugar analysis was conducted that was confined to three central sugar components: fructose, glucose, and sucrose. Fructose and glucose were not identified in any of the genotypes, which, in turn, pointed to the substantial dissimilarities in the distribution of sugar components across the genotypes. Nevertheless, sucrose showed up in all genotypes, with quite significant variations between them. Particularly, Genotype 5 came out as the highest sucrose last at 3197.79 mg kg^{-1}, while Genotype 4 demonstrated the lowest at 1950.93 mg kg^{-1}.

Numerous studies in scientific literature have indicated that the amount of sugar components in different garlic plants can be quite divergent. Lisciani et al. (2017) are discussing the sugar content of garlic genotypes ranging from 100 mg kg^{-1} to 900 mg kg^{-1} of fructose and 300 mg kg^{-1} to 900 mg kg^{-1} of glucose. Our study had missed the fructose and glucose that the authors had mentioned to be the case in other studies. These results might be due to the fact that the levels of these components are influenced by environmental factors and genetic diversity. As for sucrose, the values are in line with literature that goes from 2420.00 mg kg^{-1} to 3080.00 mg kg^{-1} (Lisciani et al., 2017), and the value of 3197.79 mg kg^{-1} recorded in Genotype 5 in our study is close to the highest.

Our findings show that the garlic genotypes have notable biochemical differences in their sugar constituents. Sugars are the main source of energy for plants and are at the same time the key components that maintain the cellular energy balance (Patrick et al., 2013; Martins et al., 2016). One more thing, that the intake of the garlic genotypes can be an important tool for plant genetic uniqueness and its ability to adapt to the environment is quite clear from the differences in them. Furthermore, with the help of the potential health benefits of sugars, this is a significant groundwork not only for a healthy evaluation of these genotypes for food but also for their biological activity in functional food products.

The phenolic compounds, organic acids, antioxidant activity, and sugar values of the five different garlic genotypes were evaluated using a heatmap and clustering analysis (Figure 3). Genotype 1 and Genotype 4 were clustered among themselves by reason of their phenolic compound and organic acid profiles, respectively, especially displaying chlorogenic acid (88.35 mg kg^{-1} and 174.99 mg kg^{-1}) and lactic acid (1116.88 mg kg^{-1} and 1133.02 mg kg^{-1}) in great quantities. Genotype 5 was discovered to be quite unlike the rest, demonstrating high levels of succinic acid (1274.73 mg kg^{-1}) and no lactic acid, and also exhibiting high values for substances such as catechin hydrate (15.88 mg kg^{-1}) and chrysin (3.64 mg kg^{-1}). Genotype 2 has high levels of citric acid (737.47 mg kg^{-1}) and acetic acid (69.63 mg kg^{-1}), as well as quercetin (0.41 mg kg^{-1}) and rosmarinic acid (0.33 mg kg^{-1}) contents were its most striking components. In genotype 3, completely different phenolic compound and organic acid components were determined compared to other genotypes. These findings are important because they can serve as a guide for breeding well-adapted garlic varieties and subsequently releasing them for successful garlic cultivation.

4. Conclusion

In this study, the phenolic compounds, organic acids, and sugar components of five different garlic genotypes cultivated in various regions of Türkiye were examined. Encouragingly, the study found data showing biological differences between garlic populations. Notable variations were found in phenolic compounds and antioxidant activity. Genotype 4, for instance, was especially noticeable as it had the highest chlorogenic acid content, while Genotype 5, on the other hand, had the most catechin hydrate of any of the genotypes. With respect to the organic acid profiles, Genotype 2 was in the top position with its high citric acid content in

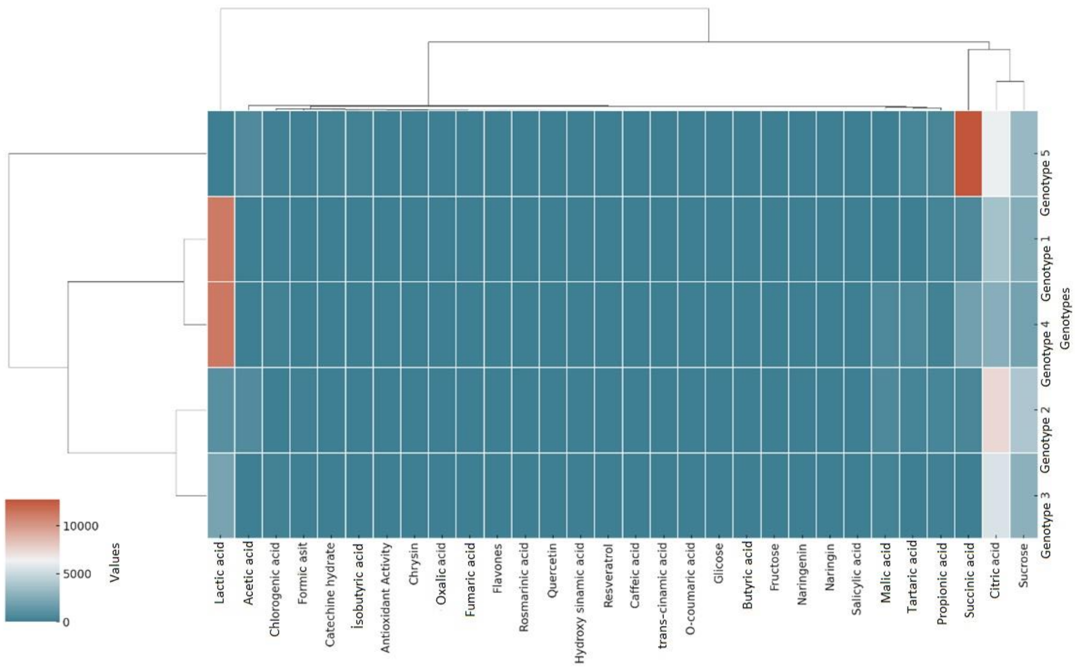

Figure 3. Heat map and cluster analysis of phenolics, organic acids, sugars and antioxidant activity contents.

the study. It was noted further that differences existed in the sucrose content of the genotypes. These measurements represent an important part of the study that would unveil the details on the nutritional characteristics and potentially derived health outcomes from the garlic and enable us to develop new varieties that are better adapted and more resistant. Among them is the identification of the biochemical diversity through phenolic compounds, organic acids, and sugar components, which is the necessary first stage in the process of understanding the genetic diversity attributes and adaptability to the environment of garlic.

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HORTICULTURAL STUDIES

RESEARCH PAPER

Investigation of the Usage Possibilities of Leonardite as a Growing Medium

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Abstract

Growing medium is very important as it affects both the germination of the seed and the growth of the seedling. The most used growing medium for seedling growth in the world is peat. Because peat deposits contain a significant amount of carbon, growing mediums that can be used as an alternative to peat are gaining importance in terms of climate change. In this study, the possibilities of using leonardite 100% and its mixtures with different materials as a seedling growing medium for melon were investigated. As a result of the study conducted under greenhouse conditions, it was observed that the germination rate decreased when 100% leonardite was used as a growing medium. The highest plant height, stem length, leaf number, plant fresh weight, and dry weight were obtained from the use of 100% peat material. The highest stem diameter was obtained from 50% peat + 25% vermiculite + 25% perlite, and the highest root length was obtained from 50% peat + 50% perlite. There was no difference between the growing medium in terms of fresh root weight and dry root weight. As a result, it has been determined that it is not suitable to use 100% leonardite as a growing medium. However, it was observed that growing mediums 33% leonardite + 33% vermiculite + 33% perlite, 50% leonardite + 50% vermiculite, and 25% leonardite + 25% peat + 25% vermiculite + 25% perlite could compete with seedling growing mediums without leonardite.

1. Introduction

The growing medium is important for seed germination. The growing medium used in seedling cultivation affects the growth of the underground and above-ground parts of the seedling as well as seed germination. The growing medium functions not only as a growing area but also as a source of nutrients for plant growth (Bhardwaj, 2014). Peat is a high-quality substrate used as a growing medium in horticulture. (Kern et al., 2017). The volume used annually as a growing medium is about half of the fuel peat (IPS, 2023). Sphagnum peat has been the most important growing medium component for decades (Schmilewski, 2008). Germany and Canada account for more than half of horticultural peat extraction. Other important peat producing countries are the Baltic countries, Finland, Ireland, and Sweden, as well as Chile and Argentina (IPS, 2023).

Peatlands are carbon-rich ecosystems that constitute the largest terrestrial carbon store. The protection of peatlands is recognized as a fundamental component based on nature in combating climate change on a global scale (Lourenco et al., 2023). Due to climate impacts, some European countries have developed national strategies to reduce peat use (Hirschler et al., 2022). Kern et al. (2017) reported that unsustainable peat extraction damaged peatland ecosystems, disappear in Central and Southern Europe. They also emphasized that degraded peatlands have become a source of greenhouse gases due to drainage and excavation. Authorities and many non-governmental organizations advocate reducing peat use (Michel, 2010). Rozas et al. (2023) reported that peat extraction has a global impact due to greenhouse gas emissions and has a significant impact on the local ecosystems from which it is obtained. Česonienė et al. (2023) reported that a large number of saplings are currently grown for blueberry fields and that it is important to conduct studies on the use of various organic and inorganic components in the lower layers to reduce the amount of excavated peat. Environmental concerns have increased the demand for alternative growing media to replace Sphagnum peat. However, growing medium formulations are still dependent on peat and alternatives are limited (Steiner and Harttung, 2014). In addition, the import of peat is an important cost element for countries that do not have sufficient peat resources. Heiskanen (2013) reported that as a result of increasing costs and environmental incentives, seedling growers are seeking more local growing medium components such as compost.

The use of different materials as growth media in seedling cultivation is an important subject due to both increasing costs and environmental concerns regarding the extraction of peat. Replacement of peat is possible using alternative growing medium components based on biomass (Hirschler et al., 2022). It is necessary to be careful when choosing an alternative growing medium for peat. Schmilewski (2008) stated that producers and users of growing medium will be exposed to a high risk if a significant amount of potentially unsuitable ingredients are included in the product.

There are some studies on different growing medium and their mixtures that can be used as an alternative to peat. Osman and Rady (2014) detected the highest rate of germination in a mixture of peat, vermiculite, and perlite in the ratios of 0-35-65, where humic acid was not applied to both eggplant and tomato transplants. Akbaşak and Koral (2014) obtained the lowest value in terms of all seedling characteristics, except for seedling germination rate, from the seedling growing medium consisting of 100% unground rice hull. Polat et al. (2017) used peat, peat+perlite (1:1), grape marc, and soil materials as growth medium for watermelon. Chrysargyris et al. (2019) mixed peat with different biochar materials as a growth medium for cabbage seedlings. Steiner and Harttung (2014) examined the growth of sunflowers in different growing mediums such as biochar, perlite, clay granules, Sphagnum peat and, peat mixed with biochar. Peng et al., (2018) researched the potential of using biochar as a container substrate component to replace peat moss to produce horticultural crops. Kern et al., (2017) reported that biochar can play an important role in replacing peat in the growing medium when biochar is available, meets quality needs, and is economically feasible to use. Fascella and Zizzo (2004) reported that perlite/coir dust caused more flowers and longer stems than pure perlite in open-cycle soilless rose cultivation in the greenhouse. El-Naggar and El-Nasharty (2009) reported that the use of different growing mediums in Amaryllis (*Hippeastrum vittatum*, Herb.) significantly affected most vegetative growth characteristics, flowering parameters, bulb productivity and, leaf chemical composition. In this study, maximum beneficial effect was obtained from the application of N, P and K (19:19:19) complete fertilizer at the rate of 5 g per plant grown on composted leaf medium or its mixture with sand (1:1 v/v) in terms of vegetative growth characteristics, flowering and bulb and bulblet production.

Rozas et al. (2023) examined growing medium mixtures based on the combination of compost, biochar and, peat. Growing media based on the combination of compost, biochar and peat maintained most of the *Lactuca sativa* L. (Oak Leaf variety) seedling traits obtained in the growing media based on only peat.

Leonardite is a stratified material as a result of the fragmentation, degradation, humification, oxidation, and metamorphosis of plant and animal remains deposited in lake environments and swamps in prehistoric times over millions of years under the influence of volcanism movements under pressure, temperature, and anaerobic conditions (Pekcan et al., 2018). Leonardite materials obtained from different regions may have different properties. Pekcan et al. (2018) determined that 28 leonardite samples obtained from different sources had different physical and chemical properties (pH, EC, organic matter, total humic+fulvic acid, carbon/nitrogen ratio, cation exchange capacity) and contents (macro plant nutrients and micro plant nutrients and heavy metals). The physical and chemical properties of leonardite affect its quality. Leonardite materials, which contain high amounts of organic matter, humic, and fulvic acid and and low amounts of $CaCO_3$ and moisture, can be used in agriculture. In addition, the heavy metal content of leonardite should be low. Leonardite is used in agriculture for purposes for improve soil properties, increasing plant nutrition status, yield and quality.

This study was planned to determine the possibilities of using leonardite 100% and its different mixtures with peat, vermiculite and perlite as a growing medium in melon seedling cultivation. Another purpose is determine the possibility of using leonardite as an alternative to peat as a growing medium in seedling cultivation in countries that have important leonardite deposits and can obtain leonardite at low cost.

2. Materials and Methods

2.1. Description of the study site

This study was carried out GAP International Agricultural Research and Training Center in Diyarbakır province of Türkiye in greenhouse conditions in 2023. The coordinates of the greenhouse where the study was carried out are 37°56'33.51" north meridian and 40°15'27.17" east longitude. During seedling growth in the greenhouse, the temperature was set at 25°C, and the humidity was set at 80%.

2.2. Materials

Melon (*Cucumis melo* L.) was used as plant material in this study. Some properties of leonardite was shown in Table 1.

The peat used in the study contains 25.86% organic matter and 1.29% $CaCO_3$. In addition, the pH value was determined as 5.33 and the EC value as 1.88 mmhos cm^{-1} of the peat used.

2.3. Experimental design

The study was carried out according to the randomized plot design with 3 replications. There were 5 plants in each replication.

2.4. Treatments

In this study, it was aimed to investigate the possibilities of using pure and different leonardite mixtures as seedling growing media and their comparison with different growing media. Seedling growing mediums examined in the study; 1: 100% leonardite; 2: 33% leonardite + 33% vermiculite + 33% perlite; 3: 100% peat; 4: 33% peat + 33% vermiculite + 33% perlite; 5: 50% peat + 50% perlite; 6: 50% leonardite + 50% perlite; 7: 50% leonardite + 25% vermiculite + 25% perlite; 8: 50% peat + 25% vermiculite + 25% perlite; 9: 50% peat + 50% vermiculite; 10: 50% leonardite + 50% vermiculite; and 11: 25% leonardite + 25% peat + 25% vermiculite + 25% perlite.

The growing media prepared in the determined proportions were filled in the seedling trays. Seeds were planted by hand on March 12, 2023. Seedling trays were watered after seed planting. Then, irrigation was continued by giving equal amounts of water to the root area every 2 days. Measurements were made on seedlings 45 days after seed planting. Germination rate, plant height, stem length, stem diameter, leaf number, plant fresh weight, plant dry weight, root length, fresh root weight, and dry root weight measurements were made in seedlings. Dry plantand root weights data were determined keeping fresh plants and roots at a temperature of 70°C for 24 hours in drying oven.

2.5. Statistical analysis

The data obtained from this study were evaluated by analysis of variance. Treatment means were compared by LSD Test at a 0.05.

3. Results and Discussion

This study, the parameters of germination rate, plant height, stem length, stem diameter, and actual leaf number, plant fresh weight, plant dry weight, root length, fresh root weight, and dry root weight were examined in seedling growing mediums.

It can be seen that growing mediums are important in terms of germination rate, plant height, stem length, stem diameter, and leaf number parameters (Table 2). Germination rate is very important in seedling cultivation. In this study, it is

Table 1. Some properties of leonardite used in the experiment.

Properties	Unit	Content
Organic matter	%	75.05
Humic+Fulvic acid	%	95.46
EC (1:10 distilled water)	mmhos cm^{-1}	1.94
pH (1:10 distilled water)	-	5.76
Moisture	%	35.00
$CaCO_3$	%	2.27
Total N	%	1.30
Total P	%	0.45
Total K	%	0.07
Total S	%	6.12
Total Na	%	0.90
Total Ca	mg kg^{-1}	14 569
Total Mg	mg kg^{-1}	2 825
Total B	mg kg^{-1}	37.00
Total Fe	mg kg^{-1}	9273.00
Total Cu	mg kg^{-1}	4.76
Total Ma	mg kg^{-1}	26.67
Total Cr	mg kg^{-1}	44.65
Total Mo	mg kg^{-1}	10.72

Table 2. Germination rate, plant height, stem length, stem diameter, and leaf number of seedling growing mediums.

Growing mediums	Germination rate (%)	Plant height (cm)	Stem length (cm)	Stem diameter (mm)	Leaf number (number)
1 (100% leonardite)	60.00 d	4.86 cd	2.36 d	2.43 d	3.05 bc
2 (33% leonardite + 33% vermiculite + 33% perlite)	100.00 a	5.66 ac	2.60 cd	2.58 bd	3.20 bc
3 (100% peat)	100.00 a	6.73 a	3.73 a	2.71 ad	3.93 a
4 (33% peat + 33% vermiculite + 33% perlite)	100.00 a	5.00 bd	2.26 d	2.60 bd	3.06 bc
5 (50% peat + 50% perlite)	93.00 ab	4.40 d	2.00 d	2.62 bd	3.06 bc
6 (50% leonardite + 50% perlite)	66.00 bc	4.91 bd	2.08 d	2.54 bd	3.16 bc
7 (50% leonardite + 25% vermiculite + 25% perlite)	66.00 bc	6.11 ab	3.41 ab	2.72 ad	3.28 bc
8 (50% peat + 25% vermiculite + 25% perlite)	66.00 bc	4.83 cd	2.27 d	3.05 a	2.85 c
9 (50% peat + 50% vermiculite)	100.00 a	4.86 cd	2.06 d	2.84 ab	3.00 bc
10 (50% leonardite + 50% vermiculite)	100.00 a	5.80 ac	3.06 bc	2.81 ac	3.40 b
11 (25% leonardite + 25% peat + 25% vermiculite + 25% perlite)	100.00 a	6.00 ac	3.00 bc	2.49 cd	3.33 bc
CV	19.46	13.01	13.35	7.49	9.34
LSD	28.66*	1.18**	0.60**	0.33*	0.52*

*: p<0.05, and **: p<0.01, CV: Coefficient of variation, LSD: Least significant difference.

Table 3. Plant fresh weight, plant dry weight, root length, fresh root weight, and dry root weight of seedling growing medium.

Growing mediums	Plant fresh weight (g)	Plant dry weight (g)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)
1 (100% leonardite)	0.93 b	0.58 bc	10.37 bc	0.58	0.32
2 (33% leonardite + 33% vermiculite + 33% perlite)	0.86 bd	0.60 bc	13.50 ab	0.60	0.38
3 (100% peat)	1.26 a	0.84 a	10.40 bc	0.77	0.47
4 (33% peat + 33% vermiculite + 33% perlite)	0.73 bd	0.60 bc	11.45 bc	0.60	0.28
5 (50% peat + 50% perlite)	0.92 bc	0.55 b-d	15.30 a	0.55	0.35
6 (50% leonardite + 50% perlite)	0.87 bd	0.69 ab	12.05 bc	0.69	0.40
7 (50% leonardite + 25% vermiculite + 25% perlite)	0.95 b	0.60 bc	11.84 bc	0.60	0.34
8 (50% peat + 25% vermiculite + 25% perlite)	0.63 d	0.47 cd	10.26 c	0.47	0.23
9 (50% peat + 50% vermiculite)	0.66 cd	0.37 d	10.45 bc	0.43	0.30
10 (50% leonardite + 50% vermiculite)	0.90 bc	0.64 bc	10.60 bc	0.64	0.36
11 (25% leonardite + 25% peat + 25% vermiculite + 25% perlite)	0.96 b	0.63 bc	12.31 bc	0.63	0.27
CV	17.04	16.66	13.19	16.94	26.60
LSD	0.24**	0.16**	3.00*	n.s.	n.s.

ns: non-significant *: p<0.05, and **: p<0.01, CV: Coefficient of variation, LSD: Least significant difference.

seen that growing medium effect on the germination rate. The germination rate of pure leonardite was found to be lower than other growing medium. It was observed that the highest plant height was in growing medium number 3 (100% peat), and the lowest plant height was in growing medium number 5 (50% peat + 50% perlite). The highest stem length was obtained from growing medium number 3 (3.73 cm), and the lowest stem length was obtained from growing medium number 5 (2.00 cm). The proximate value to the growing medium number 3 in terms of stem length was obtained from the growing medium number 7 (50% leonardite + 25% vermiculite + 25% perlite). 100% leonardite was statistically in the same group as growing medium number 5. It has been observed that growing mediums also greatly affect stem diameter. While the stem diameter was the highest (3.05 mm) in the growing medium number 8 (50% peat + 25% vermiculite + 25% perlite), it was the lowest (2.43 mm) in the growing medium number 1 (100% leonardite). Growing mediums also affected the leaf number of the seedlings. The leaf number in growing medium number 3 (100% peat) was found to be highest (3.93) in other growing mediums. It was determined that the leaf number was lowest (2.85) in the growing medium number 8 (50% peat + 25% vermiculite + 25% perlite).

Although the germination rate is 60% when pure leonardite is used as a seedling growing medium, it has been determined that the germination rate is higher in growing mediums where leonardite is used as a mixture. All of the seeds germinated in growing mediums 2, 10 and, 11, in which leonardite was used as a mixture. Plant height was found to be higher in seedling growing mediums numbered 2, 6, 7, 10, and 11, in which leonardite was used as a mixture, compared to growing medium number 5 (50% peat + 50% perlite). Positive results were obtained in terms of the stem diameter of seedling growth mediums numbered 7 and 10, where leonardite was used as a mixture, and the stem length of seedling growing medium number 7. The highest leaf number after 100% peat was obtained from seedling growing medium number 10. Table 3 shows the plant fresh weight, plant dry weight, root length, fresh root weight, and dry root weight parameters of the seedling growing medium. When Table 3 is examined, it was seen that there were differences between the growing environments in terms of plant fresh weight, plant dry weight, and root length, but there is no difference in terms of fresh and dry root weights. The highest fresh weight (1.26 g) was obtained from 100% peat and the lowest (0.63 g) weight determined on 50% peat + 25% vermiculite + 25% perlite. Plant dry weight was found to be highest (0.84 g) in growing medium number 3 (100% peat) and lowest (0.37 g) in growing medium number 9 (50% peat + 50% vermiculite). It was determined that the root length was the highest (15.30 cm) in the growing medium number 5 (50% peat + 50% perlite). The growing medium with the lowest (10.26 cm) root length was growing medium number 8 (50% peat + 25% vermiculite + 25% perlite).

Although the seedling growing mediums in which leonardite was used as a mixture were found to be lower than 100% peat material in terms of plant fresh and dry weight, they were higher than the seedling growing mediums numbered 5, 8, and 9, in which leonardite was not used. Similarly, root length in all applications where leonardite was used as a mixture was statistically better or classified in the same group as growing medium 3, 4, 8, and 9, in which leonardite was not used.

It was determined that the peat and leonardite materials used in this study have similar properties in terms of organic matter, pH and electrical conductivity. However, it has been observed that pure peat material is a better growing medium than pure leonardite material in this study. The effects of growing medium on seedling germination and seedling growth have been investigated in different studies. Polat et al. (2017) in which different growing mediums in watermelon were examined, the highest actual number of leaves, stem length, stem diameter, plant fresh weight, plant dry weight, root fresh weight, and root dry weight were obtained from peat material. As a result of the study, the best results were obtained from peat and peat: perlite (1:1) mixture media. Yılmaz et al. (2018), the highest seedling height and root length were obtained from 100% peat application in tomato. As in these references, positive results were obtained from peat material in this study. 100% peat application was in the highest statistical group among seedling growing media in terms of germination rate, plant height, stem length, leaf number, plant fresh weight, and plant dry weight. The lowest values in terms of germination rate, stem length, and stem diameter were obtained from 100% leonardite medium. Compared to pure leonardite, higher values were obtained in terms of germination rate, plant height, stem length, stem diameter, leaf number, plant dry weight, and root length from the seedling growing media which leonardite was used as a mixture. In a study conducted by Pertuit et al. (2001) reported that adding 1/64 leonardite (v/v) to sand medium increased tomato root and shoot growth compared to plants produced with fertilizer only. Growth increased linearly from 0% to 25% with increasing leonardite levels, but 50% leonardite inhibited growth. Unal (2013) examined the effect of different organic media on the growth of vegetable seeds. As a result of the study, lower hypocotyl height, seedling height, and seedling root length were obtained from the peat-stable manure-prunings (2:1:1) mixture with leonardite added (5 g kg^{-1}) in tomato plants compared to other mixtures without leonardite. Erdal et al. (2024) examined the effects of different growing media (cocopeat, perlite, leonardite, vermicompost, and peat) and their mixtures on growth and yield in tomato plants and reported that vermicompost and its mixtures with peat were generally the most effective growth media on leaf and fruit nutrient concentrations.

As a result, it was understood that 100% peat material is a growing medium that positively affects the growth of seedlings. It has been determined that 100% leonardite material is not a suitable growing medium. However, better results were obtained from seedling growing mediums where leonardite was used as a mixture, compared to 100% leonardite. It was observed that all the seeds germinated in growing mediums 2, 10, and 11, in which leonardite was used as a mixture. In addition, it was determined that growing mediums 2, 10, and 11 could compete with seedling growing mediums without leonardite in terms of other parameters examined.

4. Conclusions

It was determined that growing mediums are very important in terms of germination rate and growth of seedlings in this study. Important results were obtained from this study that related the use of leonardite as a growing medium. 100% leonardite gave the lowest statistical group in terms of germination rate, plant height, stem length, leaf number, and root length parameters. For this reason, it is not recommended to use pure leonardite as a seedling growing medium. 100% germination rate, highest plant height, stem length, actual leaf number, plant fresh weight, and plant dry weight values were obtained in growing medium number 3, in which pure peat was used. However, higher values were obtained in terms of germination rate, plant height, and stem length, stem diameter, actual leaf number, plant dry weight, and root length in a seedling growing medium which leonardite was used as a mixture, compared to pure leonardite. In addition, seedling growing mediums using leonardite were able to compete with seedling growing mediums without using leonardite. As a result, it was determined by this study that the best seedling growing medium is 100% peat and it is not suitable to use 100% leonardite as a seedling growing medium. Although the results were not as good as the seedling growing medium using 100% peat material, it was determined that leonardite could be used in seedling growing medium mixtures as an alternative to the use of peat. Considering the 100% germination rate and other parameters, it was determined that growing mediums 33% leonardite + 33% vermiculite + 33% perlite, 50% leonardite + 50% vermiculite, and 25% leonardite + 25% peat + 25% vermiculite + 25% perlite could compete with seedling growing mediums without leonardite.

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HORTICULTURAL STUDIES

RESEARCH PAPER

Weed Species, Control Methods and Their Effects on Yield and Quality in Parsley Fields of İzmir Province of Türkiye

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Abstract

Parsley (*Petroselinum crispum* (Mill.) Nyman ex A.W. Hill) is an important vegetable due to its rich vitamin and mineral content, making it both a valuable health resource and a staple in culinary dishes. Weeds are a major challenge that limits the profitability of parsley cultivation. Twenty-four parsley field were surveyed in winter and summer during 2014 growing season. Additionally, the effects of physical (solarization) and mechanical (manual weeding) weed control on weed density, dry biomass, and yield and quality of parsley were determined with a two-year (2015-2016) field trial. As a result of survey studies, 45 different weed species belonging to 24 plant families were identified. *Urtica urens* had the highest plant density (12.6 plants m^{-2}) and *Stellaria media* was the most prevalent (68.3%) among dicotyledon weed species in winter. Among monocotyledon species, *Bromus tectorum* had the highest density (2.5 plants m^{-2}), while *Alopecurus myosuroides* was the most common (25.3%). In summer, *Portulaca oleracea* had both the highest density (14.1 plants m^{-2}) and prevalence (59.5%) among dicotyledons, while *Cyperus rotundus* exhibited similar dominance among monocotyledons (8.7 plants m^{-2}, 46.5%). As a result of field trial solarization (91.27%) and manual weeding (70.63%) were effective methods for weed control. Solarization proved to be promising for managing weeds in small, vacant areas, particularly in July. Effective weed control was shown to be important for improving the yield and quality of parsley.

1. Introduction

Parsley (*Petroselinum crispum* (Mill.) Nyman ex A.W. Hill), along with other raw vegetables, is an essential component of the human diet. It contributes not only to the visual and sensory qualities of meals with its vibrant color and distinct flavor but also provides substantial nutritional benefits (Dobričević et al., 2019). The antioxidants, vitamins, and minerals contained in parsley and other raw vegetables provide a range of health benefits, including the regulation of the digestive system, the neutralization of harmful substances, and the detoxification of the body (Eşiyok, 2012). The world's total minor vegetable production is approximately 298 million tons. China ranks first with around 170 million tons, followed by India in second place with 41 million tons, and Vietnam in third with 16 million tons. Türkiye ranks 30th in minor vegetable production, with approximately 647 thousand tons (FAOSTAT, 2022). Parsley production, which has an important place among minor vegetables in Türkiye, is approximately 127 thousand tons, and the Aegean region ranks 4th in production after the Mediterranean, Marmara and Black Sea Regions. Izmir is the province where the most parsley is grown in the region with a production of approximately 2000 metric tons (TÜİK, 2023).

Parsley, a typical Mediterranean plant, thrives in temperate and humid areas. In the mild climate of the Mediterranean and Aegean regions, parsley can be grown year-round (Eşiyok, 2012). Additionally, the ecological conditions in İzmir province support parsley production throughout the year, allowing for continuous agricultural activity in winter without leaving fields fallow. One of the most significant challenges in parsley cultivation is the weeds (Karkanis et al., 2012). Weeds, as with other cultivated plants, are a major factor negatively affecting yield and quality (Üstüner, 2022). When weeds are not adequately controlled, they cause substantial losses in both yield and quality. In parsley production, weed control is primarily conducted mechanically through hand weeding. However, this method is highly labour-intensive and costly, thereby increasing production costs (Simerjeet Kaur et al., 2017). In recent years, problems related to weed control in parsley production have been reported to the Plant Protection Research Institute in Bornova by producers, as well as by the Provincial and District Directorates of the Ministry of Agriculture and Forestry. Additionally, field studies have revealed similar issues. The lack of research on weeds in parsley production has contributed to the persistence of these problems. This study aims to identify the weed species, their density, and the control methods in parsley production areas in Izmir, as well as to assess the impact of weeds on yield and quality. The data obtained from this study are expected to provide solutions to the weed problem in parsley production areas and to guide future research in this field.

2. Material and Methods

2.1. Survey studies

Surveys were conducted in 2014 in the Kemalpaşa, Menemen, and Torbalı districts, where parsley cultivation is most concentrated within İzmir Province. Using a random sampling method, areas representing 2% of the total cultivation area were selected (Bora and Karaca, 1970). Care was taken to ensure that the samples accurately represented the region. Given that parsley production occurs year-round, both winter and summer weed species were identified separately. The identification of winter weed species was carried out in February-March, while summer weed species were detected during two periods, from July to September (Table 1).

During the surveys, weed density was determined based on field size. In fields with an area of 0.5 ha four sample points were established; in areas of 0.5-1.0 ha, six points; in areas of 1.0-2.0 ha, eight points; and in areas over 2.0 ha, twelve sample points were selected (Bora and Karaca, 1970). At each sample point, 1 m² frames were used to count the weed species. Field selection was made to ensure representative sampling across different field sizes.

In the weed counts, broad-leaved weeds were evaluated as whole plants, while the stems of narrow-leaved weeds were counted individually. Weed density and frequency were calculated based on the collected data. The prevalence of weed species was calculated using the formula by (Odum and Barrett, 1971).

$$PWS\ (R.S) = \frac{NM}{TNM} \times 100$$

where; PWS: The prevalence of weed species, NM: Number of measurements, TNM: Total number of measurements

The identification of weed species was conducted using the *Flora of Turkey* by (Davis, 1965), and the nomenclature was based on (Uluğ et al., 1993).

2.2. Weed control trials

Weed control trials were conducted in 2015 and 2016 in the Torbalı District of İzmir Province, where parsley production is concentrated. The weed species present in the trial fields were recorded and identified according to (Davis, 1965) and (Uluğ et al., 1993). The experiment was designed using a Randomized Block Design with four replications, and the plot size was 4.5 m² (Width: 3.0 m, Length: 1.5 m).

The weed control methods tested included mechanical (manual weeding) and physical (solarization) control. Prior to solarization, the soil was tilled and watered, and once the soil reached the appropriate moisture level, it was covered with a transparent plastic sheet of 50 µm thickness. The edges of the plastic cover were buried in furrows to secure it. The cover was removed after six weeks.

Table 1. The numbers and the areas of surveyed fields of parsley in the İzmir province in 2014.

District	Number of fields		Field area (da)	
	Winter* production	Summer** production	Winter production	Summer production
Menemen	4	11	0.7	4.8
Torbalı	1	3	0.4	17.5
Kemalpaşa	2	3	1.0	0.3
Total	24		24.7	

* in February-March, ** in July-September

Table 2. Practices, doses, application dates and forms in weed control experiments conducted in 2015 and 2016 in İzmir (Torbalı), parsley areas.

Applications	Dose	Application date		Methods of application
		2015	2016	
Solarization	-	28.08.2015 02.10.2015	20.07.2016 29.08.2016	The soil was treated, watered, covered with plastic after 5 weeks the cover was removed
Control	-	-	-	No application has been done
Manual weeding	2 times	01.09.2015 10.09.2015	29.09.2016 05.10.2016	Weeds taken by hand

Details of the applications and dosages used for weed control is provided in Table 2.

To determine the effectiveness of the treatments, weed species and their numbers were recorded by placing a 1 m² frame in each plot five times. Counts and observations were conducted 20 days after the treatments. The effectiveness of the applications was calculated using Abbott's formula:

$$\%\,Effect = \frac{NWCA - NWAA}{NWCA} \times 100$$

where; NWCA: Number of weeds in the control area, NWAA: Number of weeds in the application area.

For dry weight data collection, a 0.25 m² frame was placed in each plot four times. Parsley plants and weeds within each frame were cut, placed on paper bags, and labelled. The wet weights of these samples were measured in the laboratory, and they were then dried in an oven at 72°C for 48 hours. After drying, the samples were weighed to determine their dry weight.

2.3. Effect of weeds on yield and quality of parsley

During harvest, a 0.25 m² frame was placed in each plot four times. The parsley plants and weeds within each frame were cut, placed into separate bags, and labelled. The fresh weights of these samples were measured in the laboratory, and yield values per decare were subsequently calculated. During the harvest, approximately 700 g of parsley from each plot were sampled, placed in separate bags, and labelled for quality assessment. These samples were then evaluated in the laboratory for their physical properties, including decay status, colour, freshness, and other allure characteristics. The presence of weeds in the harvested crop was also assessed by counting and weighing any weeds present in the samples.

2.4. Statistical analysis

The data obtained were analysed using the SPSS statistical package. Variance analysis was performed, and the means were compared using Duncan's test at a 5% significance level. Additionally, interactions between the years were analysed, as the results provided include the average values for both years.

3. Results and Discussion

3.1. Survey studies

As a result of survey studies, 45 different weed species belonging to 24 families were identified, including one parasitic species, *Phelipanche ramosa* (L.) Pomel. Among the identified weeds, 8 species were monocotyledon, while the remaining were dicotyledons broad-leaved (Table 3). During the weed species counts in parsley fields, the following observations were made: The most prevalent winter dicotyledon weed was *Stellaria media*, followed by *Urtica urens*, *Capsella bursa-pastoris*, *Chenopodium album, Euphorbia microsphaera, Datura strumarium, Daucus carota, Hibiscus trionum*, and *Lactuca serriola*. The most prevalent winter monocotyledon weed was *Alopecurus myosuroides* followed by *Bromus tectorum*. The most prevalent summer dicotyledon weed was *Portulaca oleracea*, followed by *Lactuca serriola* L., Convolvulus arvensis, *Chenopodium album*, *Amaranthus albus*, and *Hibiscus trionum* L. The most prevalent summer monocotyledon weed was *Cyperus rotundus*. It was determined that *Stelleria media* (68.3%) was the most frequently encountered species, followed by *Urtica urens* (67,9%) and *Portulaca olearace* (59.5%) in winter and summer growing season (Table 3). Relevant literature indicates similarities and differences in weed species across different regions. For instance, (Karkanis et al., 2012) conducted a study in Greece and identified *Amaranthus retroflexus*, *Datura stramonium* L., and *Solanum nigrum* L. as problematic weed species. In a survey by (Telli & Üremiş, 2010) in parsley cultivation in Samandağ (Hatay), the most significant weed species were found to be *Orobanche aegyptiaca* Pers., *Orobanche ramosa* L., *Calendula arvensis* L., and *Cyperus rotundus*, respectively. Similarly, in Sweden, Norway, Finland, Germany, and France, frequent weed species included *Sinapis arvensis* L., *Polygonum persicaria* L., *Galium aparine* L., *Polygonum lapathifolium* L., *Myosotis arvensis* L., *Senecio vulgaris*, *Chenopodium album* L., and *Capsella bursa-pastoris*. Prevalence of *Papaver rhoeas* L., *Thlaspi arvense* L., *Silene noctiflora* L., *Poa annua*, *Chenopodium album*, and *Poa aviculare* reported in Israel (Brendstrup and Kloster, 1998; Rubin & Benjamin, 1983). Additionally, they reported *Melilotus sulcatus* L., *Malva nicaeensis* L., *Astragalus boeticus* L., and *Cyperus rotundus* among annual weeds. Most of the weed species

Table 3. The frequency (%) and the density (plant m^{-2}) of the weed species winter and summer season in İzmir, 2014.

Weed species	Winter season		Summer season	
	Frequency (%)	Density (plant m^{-2})	Frequency (%)	Density (plant m^{-2})
Alopecurus myosuroides	25.3	1.0	35.3	8.7
Amaranthus albus			27.3	2.0
Bromus tectorum	25.0	2.5		
Capsella burs-pastoris	45.2	6.5		
Chenopodium album	45.0	9.5	32.5	2.1
Convolvulus arvensis			36.8	2.4
Conyza canadensis	1.6	0.2		
Cyperus rotundus			46.5	8.7
Datura stramonium	26.7	2.7		
Daucus carota	25.0	3.0		
Echinochloa colonum			7.5	4.0
Echinochloa crus-galli			42.9	2.9
Elymus repens			34.8	8.2
Euphorbia microsphaera	40.0	3.2		
Heliotropium europaeum	10.7	2.7		
Hibiscus trionum	25.0	1.0	23.3	4.3
Lactuca serriola	25.0	2.0	36.9	2.0
Lolium perenne	6.8	1.9		
Malva neglecta	8.3	1.4		
Matricaria chamomilla	22.8	4.4		
Onopordum bracteatum	13.7	1.0		
Phelipanche ramose (L.)			0.0	0.0
Poa annua	2.3	0.3		
Portulaca oleracea			59.5	14.1
Raphanus raphanistrum	12.4	1.0		
Senecio vulgaris	25.0	1.3		
Sinapis arvensis	1.5	0.4		
Sisymbrium officinale	3.0	1.2		
Solanum nigrum			18.0	1.0
Sorghum halepense			5.3	1.5
Stellaria media	68.3	9.8		
Urtica urens	67.9	12.6		

Tablo 4. Weed species in the İzmir (Torbalı), in 2015 and 2016.

Years	Aplications	Species name
2015	Control	*Amaranthus retroflexus L., Hibiscus trionum* L., *Portulaca oleracea* L., *Cyperus rotundus* L., *Echinochloa crus-galli* L., *Sonchus oleraceus* L., *Solanum nigrum* L., *Matricaria chamomilla* L., *Lactuca serriola* L., *Silybum marianum* L., *Sorghum halepense* L.
	Manual weeding	*A. retroflexus, H. trionum , P. oleracea, C. rotundus*, *Echinochloa crus-galli* L., *S. oleraceus, S. nigrum*, *M. chamomilla*, *L. serriola, S. marianum, Alopecurus myosuroides* L., *Capsella bursa-pastoris* L., *Malva sylvestris* L., *Euphorbia helioscopia* L., *Eruca vesicaria*
	Solarization	*C. rotundus*, *Sorghum halepense* L.
2016	Control	*A. retroflexus, A.albus, C. album*, *C. canadensis*, *Convolvulus arvensis., H. trionum, P. oleracea* , *Poa anua*, *C. rotundus, E. crus-galli, Silybum marianum* L., *Sonchus oleraceus* L., *Solanum nigrum* L., *Matricaria chamomilla* L., *Lactuca serriola* L., *Silybum marianum* L., *Sorghum halepense* L., *Tribulus terrestris* L., *Erodium cicutarium* L., *Raphanus raphanistrum* L., *Conyza canadensis* L.
	Manual weeding	*A. retroflexus, A. Albus* L., *H. trionum, P. oleracea, C. rotundus*, *E. crus-galli*, *C. arvensis.*, *Raphanus raphanistrum* L., *Medicago* spp.
	Solarization	*C. rotundus*, *S. halepense*, *C. arvensis, P. oleracea*

detected in our parsley fields align with those identified in these studies, highlighting the commonality of certain problematic weeds across different regions.

3.2. Weed control trials

Different weed species that was found in the trial area are presented in the Table in 4 both years. The total weed density and the efficiency (%) of the applications are presented in Table 5. Table 5 reveals that in both 2015 and 2016, the number of weeds and the prevalence of common weed species were significantly reduced in areas treated with solarization compared to the control and manual weeding treatments. Solarization demonstrated the highest efficiency and was observed to be the most effective treatment. Solarization allows the soil temperature to be maintained above 40°C, effectively killing weed

Table 5. Effectiveness of the applications and total weed density in Izmir (Torbalı), Parsley, 2015 and 2016.

Applications	2015		2016		Average of 2015-2016	
	Total weed density (plant m^{-2})	Effectiveness (%)	Total weed density (plant m^{-2})	Effectiveness (%)	Total weed density (plant m^{-2})	Effectiveness (%)
Control	107.75	-	59.50	-	83.62	-
Manual weeding	39.75	63.11 b	13.00	78.15 ab	26.37	70.63 b
Solarization	4.31	96.00 a	8.00	86.55 a	6.15	91.27 a

** Different letters refer to different statistical groups (Duncan, P <0.05)

Table 6. Dry biomass of weeds and effectiveness of the obtained from the practice of combat tests in Izmir Province (Torbalı), Parsley, 2015 and 2016.

Applications	2015		2016		2015-2016	
	Weed dry biomass (g)	Effectiveness (%)	Weed dry biomass (gr)	Effectiveness (%)	Weed dry biomass (gr)	Effectiveness (%)
Control	31.32	-	38.84	-	35,05	-
Manual weeding	21.14	32.5 b	27.31 b	29.68 b	24.22 b	30.89 b
Solarization	11.57	63.0 a	21.04 a	45.82 a	16.30 a	53.49 a

** Different letters refer to different statistical groups (Duncan, P <0.05)

Table 7. Effects of the weed control treatment on yield (g ha^{-1}) of parsley.

Aplications	2015		2016	
	(g ha^{-1})	(bunch $pieces^{-1}$)	(g ha^{-1})	(bunch $pieces^{-1}$)
Control	5682 b	10.026 b	5003 b	10.006 b
Manual weeding	5917 a	10.795 a	5378 a	10.757 a
Solarization	5968 a	10.562 a	5331 a	10.663 a

** Different letters refer to different statistical groups (Duncan, P <0.05)

seeds and seedlings (Chase et al., 1999). In certain species, if the lethal temperature is not achieved, dormancy can be broken, leading to the emergence of a new wave of weed seedlings; this phenomenon can occur within the topsoil layer (Vidotto et al., 2013). When comparing the two-year results of the applications, solarization consistently provided the best efficiency (%), followed by manual weeding. Additionally, it was observed that perennial weeds, such as *Cyperus rotundus* L., *Sorghum halepense* L., and *Convolvulus arvensis*, as well as annual weeds like *Portulaca oleracea* L., were present in the solarization areas, particularly after irrigation. Because weed species have varying sensitivity to solarization: annual weeds are generally sensitive, but *Avena fatua* and *P. oleracea* show slight tolerance, while *Conyza canadensis* (L.) Cronq. is relatively tolerant. Perennial weeds, such as *Convolvulus arvensis* L., *Cyperus* spp., *C. dactylon, S. halepense*, and *Equisetum* spp., can range from relatively sensitive to tolerant (Pannacci et al., 2017).

Many minor crops, such as certain vegetables (e.g., cabbages, artichokes), seed crops, herbs, medicinal plants, and spices, can be managed using hoes for manual weed control. The effectiveness of hoe weeding depends on soil properties (such as moisture and texture), weather conditions after cultivation, and the characteristics of the tool (size, shape, and working depth). Rainy conditions following soil cultivation can reduce weeding effectiveness by 30-40% (Lichtenhahn et al., 2005). Due to their significant impact on the soil, manual weeding can control weeds from early stages (2-4 true leaf stage) to later stages when weeds are well-developed. However, in the case of perennial weeds, the effectiveness of hoeing may be reduced (Pannacci et al., 2017). The dry biomass of weeds is given in Table 6. Dry biomass of weeds was significantly reduced in both solarization, and manual weeding applications compared to the control. Solarization achieved the greatest reduction in dry weight, demonstrating the highest effectiveness, followed by manual weeding. Mechanical weed control methods are effective, fast, and leave no chemical residues on crop plants (Pannacci & Tei, 2014). For these reasons, mechanical methods are the primary means of directly suppressing weeds in organic and low-input cropping systems.

3.3. Effects of weeds on yield and quality of parsley

Table 7 presents the yield values per decare, calculated from data obtained from each plot during harvest. The results indicate that the efficiency of the applications was higher compared to the control, with all applications falling into the same statistical group (a) according to variance analysis. Additionally, during the surveys, parasitic weeds (*Phelipanche ramosa*) found in the parsley production areas of Menemen-Görece were associated with symptoms such as yellowing of parsley plants, growth retardation, and the formation of bare patches. Similarly, in a study conducted by (Üstüner, 2022), it was found that the presence of parasitic weed, dodder (*Cuscuta campestris* Yunck.) significantly reduced both the yield and quality of parsley. Field dodder had a 100% impact on the height development of parsley plants. It caused a 38.0% reduction in parsley yield and decreased the parsley's protein content by 8.31%, crude oil by 30.20%, calcium by 12.43%,

Table 8. Effects of the weed control treatment on quality of parsley in 2015 and 2016.

Aplications	2015			2016		
	Weed			Weed		
	Count (piece)	Weight (g)	Species	Count (piece)	Weight (g)	Species
Control	8.25	35.98	*C. arvensis* *P. olereceae* *E. crus-galli* *C. album* *C. rotundus* *M. oficinalis*	7.25	34.62	*C. arvensis* *P. olereceae* *E. crus-galli* *C. rotundus* *H. trionum* *S. nigrum*
Hand weedling	2.75	32.65	*C. album* *P. olereceae* *C. arvensis* *E. crus-galli*	1.75	23.41	*C. arvensis* *C. rotundus* *P. olereceae*
Solarization	2.00	21.23	*E. crus-galli* *C. arvensis*	1.52	21.56	*C. arvensis* *C. rotundus*

iron by 64.65%, and phosphorus by 14.22%, and sodium by 51.26%. Additionally, beyond the direct damage to parsley growth, dodder branches visually diminished the quality of parsley bundles by entangling the branches and leaves from the outside.

Table 8 presents the data on the mixture of weeds and parsley in terms of both numbers and weights, as well as information on common weed species. It was found that weeds contaminated to the 0.25% to 1.65% of the parsley samples. Among the eight different weed species identified, *Portulaca oleracea*, *Convolvulus arvensis*, *Echinochloa crus-galli*, and *Cyperus rotundus* were the most prevalent. The physical properties of the samples were consistent across the different weed species. In Italy, both mechanical and cultural methods are employed alone or in combination to manage weeds in parsley cultivation (Campagna et al., 2012). In Greece, mechanical control during the early growth stages is deemed necessary to prevent yield loss (Karkanis et al., 2012). Additionally, (Shaddad et al., 2009) reported similar results where solarization of corn, parsley, and arugula plants for 6 weeks (August-September) led to a reduction in weed numbers and an increase in yield and quality 21 days after planting. However, more detailed information on the trial results should be provided.

4. Conclusion

This study investigated weed control methods in parsley cultivation in İzmir, focusing on the effectiveness of mechanical and physical approaches, specifically solarization and manual weeding. The results demonstrated that solarization was the most effective method, significantly reducing weed density and dry weight compared to the control and manual weeding treatments. The presence of parasitic weeds, particularly *Phelipanche ramosa*, was noted to adversely affect parsley growth, leading to yellowing, growth retardation, and the formation of bare patches. The consistency of results across different studies highlights the reliability of these methods. However, further detailed exploration of trial results and a more extensive comparison with previous research are necessary to fully understand the implications and optimize weed control strategies in parsley cultivation. Mechanical and physical weed control methods, when used within an Integrated Weed Management Strategy (IWMS), can also help reduce reliance on herbicides, prevent the selection of herbicide-resistant species, and maintain sustainable weed management for minor crops.

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RESEARCH PAPER

A Study on Clonal Propagation by Cutting of Native Boxwood Species *Buxus balearica*

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Abstract

The aim of the study was to determine the reproductive parameters of the endangered *Buxus balearica* species, which is naturally distributed in Türkiye, under in vivo conditions. The effect of 0, 2000, 4000, and 8000 ppm doses of indole acetic acid (IAA) and indole butyric acid (IBA) on the rooting of semi-wood cuttings of *B. balearica* was investigated. As a result, it was the 4000 ppm IAA application that increased the root length (5.4 cm) and root width (5.6 cm) the most compared to the control. In both hormone applications, 8000 ppm was the most effective application on root number (6 per plant) and root quality (3.1 and 3.0). In addition, the most effective application on shoot length (1.8 cm) and the number of leaves on the shoot (7.5 per shoot) was found to be 8000 ppm IBA application. Regarding the callus rate, 4000 ppm (21%) was the most effective application in both hormone applications. In terms of rooting rate, 8000 ppm IBA provided a 98.5% higher rooting rate compared to the control. Although hormone applications had different effects on root morphologies, IBA showed a better performance than IAA on rooting rate. Additionally, phytohormone applications had a positive effect on rooting. The data obtained can be used to propagate existing boxwoods in landscape areas and for the rehabilitation of disappearing forests.

1. Introduction

Buxaceae is a small family of mostly monoecious evergreen shrub or tree plants. It consists of six genera and distributed on all continents except Australia and Antarctica (Köhler, 2007). The genus *Buxus* can grow in a wide ecological range and even above 3000 m altitude. Europe, the Mediterranean basin and the Middle East, China, Japan, Korea, Malaysia and the Philippines, Africa, the Caribbean Islands, Mexico and South America, India, the northwestern Himalayas and the former Soviet Union regions are the regions where boxwood is most common. *B. balearica* is naturally distributed in the region between the Canary Islands and the Eastern Mediterranean (Larson, 1996; Decocq et al., 2004). It is found in the Balerian Islands and southern Spain, and a single population is found in Sardinia. It is also widespread in Morocco, Algeria, and Türkiye (Tutin et al., 1968). The distribution areas of *B. balearica* in Türkiye is distributed only in the provinces of Adana, Hatay and Antalya in the Mediterranean Region (Sarı et al., 2023).

Boxwoods are widely recognized for their versatility and aesthetic appeal in garden and landscape design. Historically, they were first used as ornamental hedge plants in Egyptian gardens around 4000 BC. Today, boxwoods serve various purposes, including single and mass plantings, hedges, potted plants, topiary, and cut greens (Larson, 1999; Batdorf, 1997; Batdorf, 2004; Van Trier et al., 2005; Sarı et al., 2023). Boxwood has significant economic value in the United States as a popular ornamental shrub, has significant economic value in the United States as a popular ornamental shrub. The economic impact of boxwood is significant, with annual sales exceeding 11 million plants and a market value estimated at approximately $126 to $140 million (Kramer et al., 2020; Dhakal et al., 2022). This makes boxwood one of the leading woody plants sold in the United States, often outpacing other popular species such as azaleas. Apart from ornamental plants, boxwoods were also used to make musical instruments, writing tablets, combs, carved ornaments, paintings and sculptures. Its branches with elegant and durable leaves were used in religious and festive ceremonies. For these reasons, it has been grown for centuries both in its natural distribution areas and outside its distribution area. Again, the high density of its wood and its ability to be shaped easily make it unique (Larson, 1996; Köhler, 2007; Van Trier and Hermans, 2007). In the 18th and 19th centuries, boxwood was used by engravers to print images. Boxwood also provides good sound projection as it is free from the veins produced by its growth rings due to its slow growth (Mitchell et al., 2018).

Again, boxwood is used in cosmetics to strengthen hair and in folk medicine to treat fever, rheumatism, arthritis, biliary tract infections, diarrhea and skin ulcers (Ait-Mohamed et al., 2011; Tuniyev, 2016). It has been reported that *B. balearica* is used against diabetes in Morocco (Benkhnigue et al., 2014). *Buxus* spp. is also used in cancer research (Ait-Mohamed et al., 2011). An alkaloid isolated from *B. sempervirens* has antimycobacterial properties that have been proven effective against *Mycobacterium tuberculosis* (Tosun et al., 2004).

While Türkiye's boxwood forests experienced their first intense destruction in the 19th century with the intensive export of boxwood (Mitchell et al., 2018), the real damage was caused by *Cydalima perspectalis* Walker (Lepidoptera: Crambidae, Spilomelinae). Sarı et al. (2022) found that the boxwood moth dried out approximately 85% of the boxwood forests in Türkiye. One of these pests, *C. perspectalis*, is a species of Asian origin and was first detected in Europe in Germany and the Netherlands in 2007 (Billen, 2007; Van der Straten and Muus, 2010). In Türkiye, its presence was first detected in parks and gardens in Istanbul in 2011, in Düzce and Artvin in 2015, and in Bartın in 2016 (Hizal et al., 2012; Öztürk et al., 2016; Göktürk, 2017; Kaygın and Taşdeler, 2018; Yıldız et al., 2018). Later, Ak et al. (2021), found that *Cydalima perspectalis* was detected in the Mediterranean Region of Türkiye. Considering the diversity of species associated with boxwoods, serious ecological impacts may occur as a result of the destruction of natural boxwood stands in limited areas due to pests. For this reason, the extinction of boxwoods may indirectly cause the disappearance of many living creatures (Mitchell et al., 2018). Considering the conditions that boxwoods are exposed to, it is important to investigate propagation methods to ensure the continuity of the species. Although boxwoods can be propagated by seed, boxwood seeds ripen between June and July, with a high percentage (about 80%) of the ripened seeds being removed by rodents and ants. Seeds germinate under boxwoods in March, but the germination rate is low. Most germinating plants die during the hot and dry summer months (Lazaro and Traveset, 2005; Köhler, 2007). In addition, the slow development process from seed requires the use of vegetative production techniques. The rooting of boxwood is slow and uneven. It has also been reported that structural obstacles and time have no effect on rooting (Langé, 2014; Güney et al., 2023). However, it is very important to choose the most appropriate root hormone concentration for successful plant production, which provides great benefits to farmers in the production of ornamental plants with cuttings (Kaushik and Shukla, 2020). Due to the extreme decrease in boxwood trees in nature, it has become important to determine the most effective propagation methods and techniques. Because determining the most effective method will save time and money both in the reproduction of natural species and in the production of producers. Therefore, by using phytohormones in optimal concentrations, root cuttings are formed earlier and in greater numbers, callus formation is stimulated, and shoot growth of the cuttings increases significantly (Hartman, 2002). Singh et al. (2018) reported that IBA, IAA, and NAA are still the most commonly used auxins in rooting root cuttings.

This study aimed to determine the effects of different doses of IAA and IBA on the clonal propagation of *B. balearica*, which is distributed in very small areas in Türkiye and is endangered. In addition, this study aims to make recommendations to producers for rapid and effective propagation of the plant based on the results obtained.

2. Material and Methods

2.1. Plant material

This study was conducted in the Black Sea Agricultural Research Institute greenhouse in 2022 and 2023. In areas where boxwood grows naturally, plants that have no disease or harmful effects were selected and cuttings were taken. Cuttings belonging to the *Buxus balearica* species were obtained from Habib-i Neccar Mountain Nature Reserve (36°12'34.00"N, 36°10'58.14"E, 180-200 m) in Hatay province (Figure 1).

2.2. Experimental design

For *B. balearica*, cuttings were taken from plants that were 0.5-1.0 m tall. These cuttings were taken

Figure 1. *B. balearica* location view.

Figure 2. Rooting medium and appearance of rooted cuttings.

from one-year-old upright branches located on the upper part of the plant. To prevent drying during transportation from the collection area to the production unit, 10-15 cm-long shoots were cut, moistened and placed in plastic bags. The cuttings brought to the institute were kept in cold storage for one day and then taken into the planting process.

The basal end of each cutting were dipped in 0, 2000, 4000, and 8000 ppm doses of Indole-3-butyric acid (IBA) and Indole-3-acetic acid (IAA) for 10 seconds. The cuttings were then planted in the perlite. The planted vials were placed on rooting tables with bottom heating and top fogging. The ambient air temperature was maintained at 20 ± 2°C, root table temperature at 25 ± 2°C, and air humidity level at 75 ± 2% (Figure 2).

2.3. Measurements

After a 110-day rooting period, the following variables were recorded: root length (cm), root width (mm), number of roots, root quality (0-5), callus rate (%), rooting ratio (%), shoot length (mm) and number of leaves (per shoot) were measured. Root length, root width and shoot length (mm) were measured with a digital caliper. Number of roots, callus rate, rooting ratio and number of leaves were determined by counting. Root quality was assessed using a modified version of the (0-5) scoring method developed by Çelik (1982) for grapevine cuttings. This method was adapted for boxwood cuttings, and the root system of each cutting was categorized into six seperate groups, with scores ranging from 0 to 5. Each cutting's root system was then evaluated numerically based on these categories. In this evaluation, 0: there is no rooting, 1: Poor rooting (those with number of roots between 1-2), 2: There is a medium level of rooting (those with a number of roots between 3-4), 3: Good rooting (those with number of roots between 5-6), 4: Rooting is very good (those with number of roots between 7-8), and 5: It states that rooting is perfect (those with 9-10 or more roots).

2.4. Evaluation of data

In the clonal propagation experiments established with cuttings obtained from the shoot, all experiments were set up according to the randomized parcel divided parcel design in the evaluation of the data, with 3 replications and 30 cuttings in each replication. The data obtained were subjected to statistical analysis in the SPSS 20.0 package program. All analyses were statistically calculated within 5% and 1% error limits, and differences between applications were compared with the Duncan test.

3. Results and Discussion

3.1. Clonal propagation

It has been determined that different hormones significantly affect the rooting of the *B. balearica* species. At the end of a 110-day rooting process, the planted cuttings were removed from the rooting medium. This study examined the effects of various doses of IBA and IAA on rooting during the propagation of *B. balearica* using half-wood cuttings. The first root formations were observed at 55 days after planting with a 4000 ppm IBA application and at 62 days with an 8000 ppm IBA application. Full rooting was achieved after 110 days.

3.2. Root length

In the study, the effect of hormone, dose and hormone × dose interaction on average root length was found to be significant. While the highest average root length of 54.4 mm was obtained from 4000 ppm IAA application; The lowest average root length was obtained from the control application of IBA with 12.6 mm. In the hormone × dose interaction, root length was found to be 1.7% higher in IAA at 4000 ppm, while it was 187% higher in IBA at 8000 ppm, compared to the control. In dose application, the highest results were obtained from 42.0 mm and 41.9 mm and 4000 and 8000 ppm applications, and there was no difference between these two applications. Although there was no difference between these two doses, 4000 ppm increased root length by 26.9% and 8000 ppm by 26.6% compared to the control. In hormone application, the highest root length was found in IAA medium with 47.5 mm. Root length was found to be 45.2% higher in IAA than in IBA (Table 1, Figure 3).

3.3. Root width

In the study, the effect of hormone, dose and hormone × dose interaction on the average root width was found to be significant. In the hormone x dose interaction, the highest average root width of 55.8 mm was obtained from 4000 ppm IAA application; The lowest average root width was obtained from the control application of IBA with 13.6 mm. Compared to the control, 4000 ppm in IAA provided 17.4% wider roots, while 8000 ppm in IBA provided 166% wider roots. In dose application, the highest results (38.7 and 38.3 mm) were obtained from 4000 and 8000 ppm applications, and there was no difference between these two applications. In terms of dose averages, 4000 ppm increased root width by 26.5% and 8000 ppm increased root width by 25.2% compared to the control. In hormone application, the highest root length was found in IAA application with 41.9 mm. A 43.2% higher root width was detected in IAA application compared to IBA (Table 2, Figure 3).

3.4. Number of roots

The effect of hormone, dose and hormone × dose interaction on root number was found to be significant. While the highest average root number was obtained from 8000 ppm application with 6.0 in both IAA and IBA hormone applications; The lowest average root number was obtained from IBA's control application with 2.8. In the hormone × dose interaction, 33% and 114.3% higher root numbers were detected in both IAA and IBA applications at 8000 ppm, respectively, compared to the control. A similar effect was observed in terms of dose averages, and the highest value was determined at 8000 ppm with the number of 6 roots. A similar effect emerged in terms of dose averages; at 8000 ppm, the number of roots was 76.5% higher than the control. In hormone application, the highest root number was found in the IAA application with 4.8. A 14.2% higher number of roots was detected in IAA application compared to IBA (Table 3, Figure 3).

3.5. Root quality

The effect of hormone, dose and hormone × dose interaction on root quality was found to be significant. The highest average root quality was obtained from 8000 ppm application as 3.1 and 3.0, respectively, in both IAA and IBA two hormone applications. In the hormone × dose interaction, both IAA and IBA applications showed a higher root quality of 41% and 50%, respectively, compared to the 8000 ppm control. In terms of dose averages, it was again detected with 3.1 points in 8000 ppm applications, while root quality was 47.6% better than the control at 8000 ppm. In hormone

Table 1. Effects of IAA and IBA doses on root length (mm).

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	53.5 ab	34.3 c	54.4 a	47.6 b	47.5 A
IBA	12.6 d	25.6 c	29.6 b	36.1 a	26.0 B
Dose average	33.1 C	30.0 C	42.0 A	41.9 A	
CV (%)	15				
Hormone (H)	**				
Dose (D)	*				
H × D	*				

There is a significant difference between the means with different letters (Duncan) within the error limits of P< 0.05 and P< 0.01.

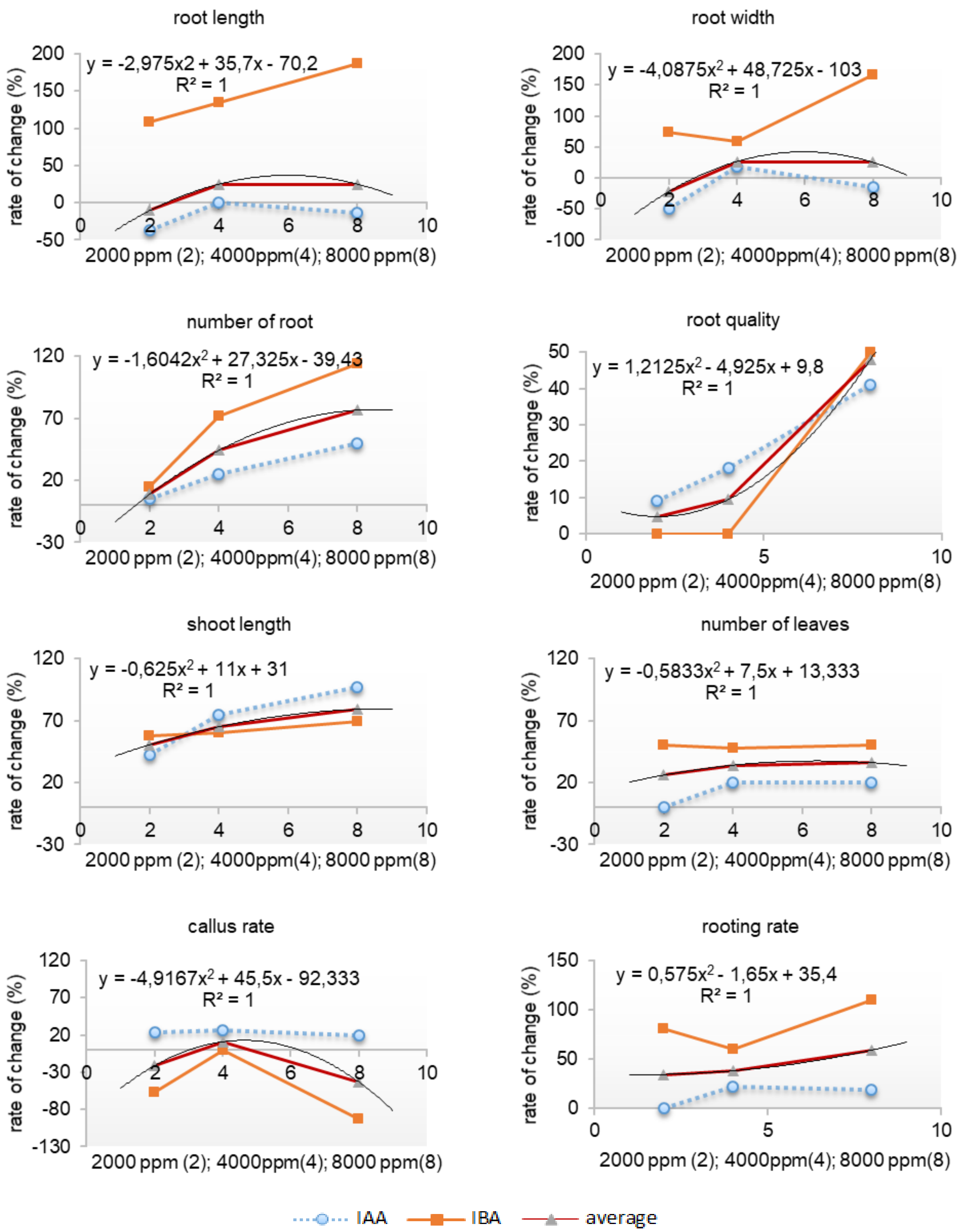

Figure 3. Change rates of rooting characteristics compared to control values after IBA and IAA applications.

Table 2. Effects of IAA and IBA doses on root width (mm).

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	47.5 ab	24.0 c	55.8 a	40.4 b	41.9 A
IBA	13.6 c	23.7 b	21.5 b	36.2 a	23.8 B
Dose average	30.6 B	23.9 C	38.7 A	38.3 A	
CV (%)	17				
Hormone (H)	**				
Dose (D)	*				
H × D	*				

There is a significant difference between the means with different letters (Duncan) within the error limits of P< 0.05 and P< 0.01.

Table 3. Effects of IAA and IBA doses on root number.

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	4.0 b	4.2 b	5.0 ab	6.0 a	4.8 A
IBA	2.8 c	3.2 c	4.8 b	6.0 a	4.2 B
Dose average	3.4 C	3.7 C	4.9 B	6.0 A	
CV (%)	13				
Hormone (H)	**				
Dose (D)	**				
H × D	**				

There is a significant difference between the means with different letters (Duncan) within the error limits of P< 0.05 and P< 0.01.

Table 4. Effects of IAA and IBA doses on root quality.

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	2.2 b	2.4 b	2.6 b	3.1 a	3.0 A
IBA	2.0 b	2.0 b	2.0 b	3.0 a	2.0 B
Dose average	2.1 B	2.2 B	2.3 B	3.1 A	
CV (%)	18				
Hormone (H)	*				
Dose (D)	*				
H × D	*				

There is a significant difference between the means with different letters (Duncan) within the error limits of P< 0.05 and P< 0.01.

Table 5. Effects of IAA and IBA doses on shoot length (mm).

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	7.2 c	10.2 bc	12.6 b	14.2 a	11.1 B
IBA	10.9 c	17.2 b	17.4 b	18.4 a	16.0 A
Dose average	9.1 C	13.7 BC	15.0 B	16.3 A	
CV (%)	16				
Hormone (H)	**				
Dose (D)	**				
H × D	**				

There is a significant difference between the means with different letters (Duncan) within the error limits of P< 0.05 and P< 0.01.

application, the highest root quality was found in the IAA application with 3.0 points. Root quality was found to be 12.5% better in IAA application compared to IBA (Table 4, Figure 3).

3.6. Shoot length

The effect of hormone, dose and hormone × dose interaction on shoot length was found to be significant. While the highest average shoot length of 18.4 mm was obtained from 8000 ppm IBA application; The lowest average shoot length was obtained from the control application of IAA with 7.2 mm. Although IAA and IBA applications provided different increases compared to the control in the hormone × dose interaction, higher shoot length was detected in both hormone applications at 8000 ppm, at the rates of 97.2% and 68.8%, respectively, compared to the control. In the dose application, it was detected in 16.3 mm and 8000 ppm applications, while a shoot length was found to be 79% higher than the control at 8000 ppm. In hormone application, the highest shoot length was found in IBA application with 16.0 mm. In IBA application, shoot length according to IAA was 44.1% higher than in the control (Table 5, Figure 3).

3.7. Number of leaves

The effect of hormone, dose and hormone × dose interaction on the number of leaves was found to be significant. Although IAA and IBA applications had different effects compared to the control in the hormone × dose interaction, 4000 ppm and 8000 ppm had the same effect in IAA, and 2000 ppm, 4000 ppm, and 8000 ppm had the same effect in IBA. The highest average number of leaves, 7.5, was detected in IBA's 2000 and 8000 ppm applications. In dosage application, it was determined that 4000 ppm (6.7 leaves per shoot) and 8000 ppm (6.8 leaves per shoot) applications had the same effect. Although there was no difference between 4000 ppm and 8000 ppm in terms of dose averages, 34% and 36% higher leaf numbers were obtained, respectively, compared to the control. In hormone application, the highest number of leaves was found in the IBA application with 6.9. The number of leaves was found to be 25.5% higher in IBA application compared to IAA (Table 6, Figure 3).

3.8. Callus rate

The effect of hormone, dose, and hormone × dose interaction on the callus rate was found to be significant. The highest average callus rate of 21.0% was observed with the 4000 ppm IAA and IBA applications, which showed similar effects. The lowest average callus rate of 1.5% was obtained from the 8000 ppm IBA application. In the hormone × dose interaction on the callus rate, IAA and IBA applications increased the callus rate by 25.7% and 10.5%, respectively, compared to the 4000 ppm

Table 6. Effects of IAA and IBA doses on number of leaves.

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	5.0 b	5.0 b	6.0 a	6.0 a	5.5 B
IBA	5.0 b	7.5 a	7.4 a	7.5 a	6.9 A
Dose average	5.0 B	6.3 AB	6.7 A	6.8 A	
CV (%)	18				
Hormone (H)	*				
Dose (D)	*				
H × D	*				

There is a significant difference between the means with different letters (Duncan) within the error limits of P< 0.05 and P< 0.01.

Table 7. Effects of IAA and IBA doses on callus rate (%).

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	16.7 b	20.7 ab	21.0 a	20.0 ab	19.6 A
IBA	19.0 a	9.0 b	21.0 a	1.5 c	12.6 B
Dose average	17.9 B	14.9 C	21.0 A	10.8 D	
CV (%)	13				
Hormone (H)	**				
Dose (D)	**				
H × D	*				

There is a significant difference between the means with different letters (Duncan) within the error limits of P< 0.05 and P< 0.01.

Table 8. Effects of IAA and IBA doses on rooting rate (%).

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	63.3 b	63.3 b	77.0 a	75.0 ab	69.7 B
IBA	47.0 d	85.0 b	75.0 c	98.5 a	76.8 A
Dose average	55.2 C	74.2 B	76.0 B	87.5 A	
CV (%)	15				
Hormone (H)	**				
Dose (D)	**				
H × D	*				

There is a significant difference between the means with different letters (Duncan) within the error limits of P< 0.05 and P< 0.01.

Figure 4. Root development by applications (a: Control, b: 2000 ppm, c: 4000 ppm, d: 8000 ppm).

control. In terms of dosage application, a callus rate of 21.0% was observed with the 4000 ppm application. Regarding dose averages, a 17.3% higher callus rate was found at 4000 ppm compared to the control. Among hormone applications, the highest callus rate was found with the IAA application at 19.6%. The callus rate was 55.6% higher in the IAA application than in the IBA application (Table 7, Figure 3).

3.9. Rooting rate

The effect of hormone, dose and hormone × dose interaction on the rooting rate was found to be significant. While the highest average rooting rate was detected in the 8000 ppm IBA application with 98.5%, the lowest average rooting rate was obtained from the control application of IBA with 47.0%. In the hormone × dose interaction, 4000 ppm in IAA increased the rooting rate by 21.6% compared to the control, while 8000 ppm in IBA increased it by 110%. In the dosage application, it was detected at 87.5% and 8000 ppm applications. At dose averages, 8000 ppm provided 58.5% more rooting than the control. In hormone application, the highest rooting rate was found in the IBA application with 76.8%. IBA application showed 10.2% more rooting than IAA (Table 8, Figures 3 and 4).

In this study, the effects of different doses of IBA and IAA on rooting in the propagation of *B. balearica* using half-wood cuttings were examined. The first root formations were observed at 55 days after planting with a 4000 ppm IBA application and at 62 days with an 8000 ppm IBA application. Full rooting occurred after 110 days. Vieira et al. (2018)

reported that approximately 116 days were needed for the rooting of semi-wood cuttings of *B. sempervirens*. In this study, where semi-wood cuttings were used, the full rooting time for both species was found to be similar to the duration reported by Vieira et al. (2018). Additionally, the period reported by Güney et al. (2023) was nearly twice the 57 days noted for *B. sempervirens*.

Three different doses of IAA and IBA were statistically effective on the rooting morphology of *B. balearica*. While IAA was the most effective application on root length, root width, number of roots, root quality and callus rate, IBA was more effective on shoot length, number of leaves and rooting rate. IAA application was more effective than IBA on root length, and almost twice as long roots were obtained in IAA. In hormone and dose interaction, 4000 ppm (5.4 cm) was the most effective application in IAA application, while 8000 ppm (3.6 cm) was the most effective application in IBA. However, in terms of dose averages, there was no statistical difference in the effect on root length between 4000 ppm and 8000 ppm applications. IAA and dose interaction on root length gave better results than other applications. A similar result was found for root width as for root length. Regarding the number of roots, 8000 ppm (6) IAA was the most effective application in all applications. Güney et al. (2023) in *B. sempervirens*, the highest values in terms of root length and number of roots were determined as 2.01 cm and 7.8, respectively, in 3000 ppm IBA application. Kaviani and Negahder (2017) in their study on *B. hyrcana* Pojark., they obtained a maximum root length of 7.3 cm and a number of roots of 8.7 with 1000 mg L^{-1} IBA and 1000 mg L^{-1} NAA. In the study conducted by Banko and Stefani (1986), as a result of IBA application, the number of roots and root length were as follows: 11.4 roots and 5.6 cm in *B. sempervirens*, 6.8 roots and 3.4 cm in *B. sempervirens* L. 'Suffruticosa', 8.6 roots and 9.8 cm in *B. microphylla* var. koreana Nakai, and 6.8 roots and 2.6 cm in *Buxus microphylla* var. japonica [(Mull. Arg.) Rehder & E.H. Wilson]. *Juniperus communis* 'Hibernica' the longest root length was 9.8 cm in the IBA 5000 ppm application, while the highest number of roots was determined as 8.1 roots in the IBA 3000 ppm application. Within the scope of this study, the longest roots of 23.2 cm were determined in the IAA 3000 ppm application in the *Juniperus chinensis* 'Stricta' variety (Güney et al., 2021). In fact, it is known that auxin stimulates root elongation (Davies, 2010). In addition, studies using green cuttings in cranberries have reported that the number of roots varies depending on the genetic structure (Balta et al., 2019) and that IBA application has a significant effect on the number of roots (Kalyoncu et al., 2008; Balta et al., 2019). As can be seen from previous studies, phytohormones have different effects on different species and varieties. While IBA was generally found to be more effective on root length and root number in studies conducted on boxwood, IAA was found to be more effective in this study. Additionally, unlike previous researchers, the effective dose in this study was found to be higher (8000 ppm). The reason for this difference was determined by Hartmann et al. (2011) and Azad and Matin (2015) reported that the effects on rooting in cuttings may vary depending on many internal (genetic structure, hormones, storage materials, etc.) and external (temperature, humidity, light, rooting mediums, cutting time) factors. These factors significantly affect the rooting rate, rooting quality and rooting properties of cuttings (Ağaoğlu et al. 2019).

In the study, applications affected shoot length. In the hormone × dose interaction, the highest average shoot length of 1.8 cm was obtained from 8000 ppm IBA application; The lowest average shoot length was obtained from the control application of IAA with 0.7 cm. 8000 ppm was the most effective dose in both hormone applications. Yeshiwas et al. (2015) in their study on rose varieties, the shoot length was found to be 14.4 cm with 1000 ppm IBA application. Similarly, Akhtar et al. (2015) found the maximum shoot length to be 10.67 cm in the application of 450 ppm IBA on *Rosa centifolia*. In our study, IBA application increased shoot length. However, a lower shoot length was detected compared to previous studies on different species. The reason for this can be explained as boxwoods showing a low growth rate.

Applications also affected the number of leaves. Different results were obtained for the number of leaves in terms of hormone and dose interaction in both applications. The highest average number of leaves, 7.5 per plant, was observed in the 2000 ppm and 8000 ppm IBA applications. In terms of dose averages, the highest results were obtained at 4000 ppm (6.7 leaves per plant) and 8000 ppm (6.8 leaves per plant), with both doses showing similar effects. In the study conducted by Muhammad Shahab (2021) on the ornamental plant *Clerodendrum splendens*, it was observed that the maximum number of leaves per plant (14.0) was observed in cuttings treated with 20% IBA, followed by 10% IBA (12.3) and 0% IBA (12.0). The minimum number of leaves per plant (10.6) was determined for cuttings treated with 30% IBA. In their study on *Citrus medica* L., Al-Zebari and Al-Brifkany (2015) found that the 1000 ppm IBA application resulted in the highest number of leaves per shoot (12.72), while the control application produced the lowest value (8.36). In the 2000 ppm application, a lower number of leaves (11.42) was observed compared to the 1000 ppm application. The results of the researchers showed that IBA applications were effective in increasing the number of leaves on the shoot. In this study, IBA applications increased the number of leaves on the shoot compared to the control.

Rooting quality is an important feature that affects the seedling quality of cuttings in which root formation occurs (Yıldız et al., 2009). Significant differences were detected among the examined boxwood species and types in terms of rooting quality. Accordingly, root quality ranged from 2.0 to 3.1. The IAA application resulted in better root quality than the IBA application. The highest root quality was observed at a dose of 8000 ppm in both hormone applications. In terms of dose averages, 8000 ppm application increased root quality by 47.6% compared to the control. IAA (3) showed a 50% higher root quality than IBA (2). Compared to the control, rooting was high in all cultivars, including 'Winter Beauty,' 'Globosa,' and 'Rotundifolia,' meaning that most cuttings formed more than three roots (Salaš et al., 2012). Balta et al. (2019) found that rooting quality in cranberries increased with hormone application and varied significantly depending on the genotype. In our study, it was determined that the rooting quality of boxwoods varies according to species and variety, and improves as the dose of the applied hormone increases. Additionally, it was observed that boxwoods predominantly exhibit a medium level of rooting quality.

When it came to the interaction between hormone and dose on callus rate and dose averages, 4000 ppm was the most successful application in both cases. IAA and IBA treatments raised the callus rate by 25.7% and 10.5%, respectively, in the hormone and dosage interaction on the callus rate when compared to the 4000 ppm control. In terms of dose averages, a 17.3% higher callus rate was found at 4000 ppm compared to the control. The callus rate was 55.6% higher in the IAA application compared to the IBA application. In the study conducted on *Juniperus chinensis* 'Stricta', the highest callus formation (73.3%) was observed with the 3000 ppm IAA application (Güney et al., 2021). The results obtained in this study revealed that the callus rate also differs between species and varieties. In fact, the callus formation rate is an important factor that affects the rooting rate in cuttings (Kalyoncu et al., 2008). Propagation studies conducted with cranberries have reported that the callus rate varies significantly depending on the genotype (Yavuz, 2015; Balta et al., 2019).

In terms of hormone and dose interaction on rooting rate, 4000 ppm was the most effective dose in the IAA application, while 8000 ppm was the most effective dose in the IBA application. In terms of dose averages, 8000 ppm was the most effective dose in both applications. The highest average rooting rate was observed in the 8000 ppm IBA application, with 98.5%, while the lowest average rooting rate was obtained from the IBA control application, with 47%. It has also been reported that structural obstacles and time have no effect on rooting (Langé, 2014; Güney et al., 2023). However, in our study, it was determined that the rooting rate generally increased with the increase in hormone dose (control: 55.2%, 2000 ppm: 74.2%, 4000 ppm: 76.0% and 8000 ppm: 87.5%). In the study by Banko and Stefani (1986), rooting rates were found to be 92.5% in *B. sempervirens,* 51.3% in *B. sempervirens* L. 'Suffruticosa', 80.4% in *B. microphylla var. koreana Nakai*, and 72.8% in *Buxus microphylla var. japonica* [(Mull. Arg.) Rehder & E.H. Wilson]. Again, in the study conducted by Wang (1989), the rooting rate varied between 4% and 68%. In another study, for the control group, *B. sempervirens* semi-hardwood cuttings treated with IBA at 1500 mg L^{-1}, 3000 mg L^{-1}, and 6000 mg L^{-1} showed the highest rooting percentage of 97.5% (Vieira et al., 2018). Güney et al. (2023) again observed that on *B. sempervirens*, the highest rooting percentage (100%) was observed in GM-1 with IBA 3000, IBA 5000, IAA 3000, NAA 3000 and NAA 5000 ppm applications and in GM-2. Specified in IBA 3000 and IAA 3000 ppm applications. Unlike boxwoods, Güney et al. (2021) on junipers, the highest rooting percentage *for J. chinensis* 'Stricta' was obtained with 66.67% in the IAA 5000 ppm treatment. The highest rooting percentage for *J. chinensis* 'Stricta Variegata' was obtained as 60% at 3000 ppm application of IBA. This study clearly showed that two different hormones and different doses of these hormones may be effective in two different juniper varieties. Similarly, a study conducted with soft cuttings of *Berberis thunbergii* 'Atropurpurea Nana' showed that NAA (3000 ppm) and IAA (8000 ppm) hormones were more effective in promoting root formation and development in cuttings compared to the IBA hormone (Pulatkan et al., 2018). When the studies in the literature are generally evaluated, it is seen that IBA hormone doses are more successful in rooting cuttings (Pulatkan et al., 2018). However, as reported by Kaushik and Shukla (2020), rooting rates differed according to species and types. In this study, similar to the findings of the researchers, the effects varied between hormones and their doses. Specifically, the highest rooting rate was obtained from the 8000 ppm IBA application, while 4000 ppm was found to be the most effective dose in the IAA application, despite having a lower rooting rate compared to the 8000 ppm IBA application. Furthermore, Walter (1997), in research conducted on *Buxus*, found that high doses of IBA, such as 12000 ppm (1.2%), were the most effective application. These results indicate that there is no fixed hormone dose for rooting *Buxus* species and varieties.

4. Conclusion

In the study, it was determined that IBA and IAA applications had different effects on root morphology. While IAA was the most effective application on root length, root width, number of roots, root quality and callus rate, IBA was more effective on shoot length, number of leaves and rooting rate. As for the hormone dose, it was found that 8000 ppm, IBA application was more effective on the rooting and root morphology of boxwoods. In terms of root quality, boxwoods showed a medium level rooting quality. It is known that there are no internal or external inhibitors on the rooting of boxwoods. In other words, shrubs can be rooted without phytohormone application. However, it has been revealed by previous studies that phytohormone applications increase rooting and is supported by the results of this study. In addition, this study determined that rooting and root morphology development of boxwoods may differ depending on the applied hormone and dose. Approximately 85% of boxwood areas in Türkiye have disappeared due to the effect of the boxwood moth. Rehabilitating these destroyed areas will be possible by rapidly and effectively multiplying the remaining boxwood genetic resources. In addition, the results of this study, in which the best production conditions are investigated, provide guidance to producers in ensuring the sustainable use of boxwood and especially its use as ornamental plants.

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HORTICULTURAL STUDIES

RESEARCH PAPER

Effectiveness of Four Rootstocks against Fusarium wilt, Yield and Quality in Cucumber

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Abstract

Fusarium crown root rot caused by *Fusarium solani* is one of the most important diseases that limiting cucumber cultivation all around the world. There is more than one way to deal with this disease, but sometimes these practices may be insufficient. For this reason, the use of resistant varieties and rootstocks gains importance in the control of soil-borne diseases. The objectives of this study are to determine the resistance of breeding materials to Fusarium and to evaluate their rootstock performance and to reveal their effects on fruit yield and quality. To determine the Fusarium resistance level, 48 breeding materials were tested and four moderate resistant materials were grafted onto the hybrid Gordion variety. The effects of grafted plants on fruit yield and vegetative growth were evaluated. Early yield was also significantly higher in grafted plants than in the ungrafted control. Strongtosa had the highest fruit per plant, followed by RS 841 and 13×18 hybrid rootstock. Although higher yields were generally obtained in grafted plants compared to the control group. Additinally fruit length, fruit diameter, fruit shape index, fruit firmness and panel test were evaluated in grafted plants. These materials used as rootstocks increased plant growth and yield.

1. Introduction

Cucumber is the most grown and economically important crop species of the Cucurbitaceae family. Several fungal soilborne diseases and several fungal pathogens threaten cucurbits all around the world. The *Fusarium wilt* is considered one of the major pathogens which constrains cucumber yield and its quality (Shi et al., 2016).

Fusarium wilt of cucumber is a serious vascular disease in the worldwide. One of them is Fusarium crown root rot caused by *F. solani* considered one of the most destroying diseases in cucurbits around the world (Champaco et al., 1993, Hamdi et al., 2019). *Fusarium solani* f.sp. *cucurbitae* exhibits host specificity within the cucurbitaceae family and is able to infect crops, such as melon, watermelon and cucumber under field conditions (Perez Hernandez et al., 2020). This pathogen was responsible for cortical roots at the stem base and at the upper portion of the tap roots causing yellowing and wilting of leaves, soft circular lesions developed for fruits in contact with soil (Hamdi et al., 2019). Fusarium wilt disease is a constant problem because it survives in the soil for years. Cultural measures, solarization, soil fumigation, biological control and fungicides are generally used to control this disease, but sometimes these practices may be insufficient.

Grafting is among the most ancient agricultural techniques having been practise since 2000 BC (Kyriacou et al., 2020). Many agricultural practices have been changed due to several environmental stresses, including the agronomic, breeding and genetic programs. Grafting is an important agronomic technique that could save the costs and time of breeding programs (Bayoumi et al., 2021). Nowadays vegetable grafting is widely used in Cucurbitaceous and Solanaceous crops (Kyriacou et al., 2016, 2017). Grafted vegetables onto tolerant or resistant rootstocks can cope with different purposes including increasing the vigor of plants and enhancing the water and nutrients uptake (Sallaku et al., 2019). One of the purpose of grafting is controlling soil-borne pathogens (Lee et al., 2010; Louws et al., 2010) resistant rootstocks is one of the most common alternative methods for the control of soil-borne pathogens. It has been executed over the past few decades and recently seems to be the most successful control means (Reyad et al., 2021).

The use of grafted seedlings provides resistance to soil-borne pathogens including Fusarium. Additionally grafted plants that are tolerant to abiotic stress factors, increase plant power, yield and quality (Jabnoun-Khiareddine et al., 2019, Bayoumi et al., 2021). There are many species compatible with cucumber in grafting. They are *Cucurbita maxima*, *Cucurbita moschata*, *Cucurbita ficifolia*, squash interspesific hybrid (*Cucurbita maxima* x *Cucurbita moschata*), *Lagenaria siceraria*, wax gourd, burr cucumber, luffa and melon (King et. al., 2010, Wang et al., 2004). The most widely used of these is the squash interspecific hybrid (*C. maxima* × *C. moschata*) (Davis et al., 2008; Velkov and Pevicharova, 2016; Balkaya et al., 2018; Guan et al., 2020; Kamel and Taher, 2021). *Cucurbita* spp. rootstocks also provide grafted plants tolerance to Fusarium wilt (Guan et al., 2020). Commercial rootstocks used for watermelon are also used for cucumber. However, there is a need for the widespread use of local rootstocks for cucumber and their adoption by producers.

From this point of view, the aim of this study was to evaluate the materials derived from the crosses between *C. maxima* and *C. moschata* and derived from *C. moschata* against *F. solani* f. sp. *cucurbitae*, evaluate the rootstock performance of the tolerant materials, evaluate plant growth, fruit quality and yield of commercial hybrid Gordion F1 grafted onto Fusarium tolerant materials.

2. Material and Methods

2.1. Material

The material of the study consisted of 48 genotypes with rootstock potential developed from *C. maxima* and *C. moschata* species within the scope of “Breeding of Rootstock in Cucumber” (Project number: TAGEM/BBAD/10/A01/P01/14). In the project the root structures and graft compatibility of materials belonging to *C. maxima* and *C. moschata* species obtained from Türkiye and abroad were evaluated. Commercial Gordion F1 cultivar was used as the scion.

2.2. Method

To determine Fusarium resistance level, 48 genotypes were tested, including *C. moschata* lines, *C. maxima* × *C. moschata* hybrids, which provide high grafting compatibility with hybrid cucumber scions. In this study, *Fusarium solani* f.sp. *cucurbitae* isolate was grown on liquid synthetic media prepared according to Pitrat et al. (1991) and seedling root dipping (10^6 conidia/ml) (Figure 1) was used as the inoculation method (Gordon et al., 1989; Zink and Gubler, 1985).

The severity of the disease was evaluated according to the scale (0–4) proposed by Erper et al. (2015). 0 = Healthy seedling, white roots, no symptoms, 1 = Slightly infected seedlings, normal seedling development, slight discoloration of stem veins, 2 = 50% infection in roots, disease severity was determined according to the disease scale values (3 = Severe infection of roots, stem deformity, moderate or severe yellowing and wilting of cotyledons, 4 = Dead plant). The % disease severity of the cultivars was determined by using the disease scale values obtained using the Tawsend-Heuberger formula (% Disease severity: $\Sigma(n.v)/V.N\times100$, n: Amount of samples corresponding to a certain disease degree on the scale, v: Scale value, V: Highest scale value, N: Total number of samples observed) (Townsend and Heuberger, 1943).

Then according to Martyn and McLaughlin (1983) disease resistance level was determined [I= ≤ 20%: Highly resistant (HR), II= 21-50%: moderate resistant (MR), III=51-80%: low resistant (LR), IV= > 80% susceptible]. According to this grouping four moderate resistant genotypes (Table 1) were grafted onto cucumber hybrid Gordion F1 and evaluated for graft compatibility, plant growth and cucumber yield during 2021 autumn trials as indicated below. Four moderate resistant genotypes, two of which are interspecies hybrids and two of which belong to the species *C. moschata* were grafted onto Gordion F1 hybrid cucumber cultivar. Ungrafted Gordion F1 was used as control (Figure 2).

2.2.1. Cucumber grafting and transfer to nursery conditions

Rootstock and scion seeds were sown in a private seedling company. Rootstock seeds were planted on 28 August, and scion seeds were planted 2 days later. Grafting was carried out approximately ten days after sowing the rootstock seeds, when both rootstock and scion seedling were at the first true leaf stage (Figure 3). The slant cut grafting technique was used. It is important to remove the first leaf and lateral buds when a cotyledon of rootstock is cut on a slant. After grafting, grafted seedlings are heated at 24-28 °C in the growth unit covered with plastic film. The newly inoculated plants were exposed to 100% relative

Figure 1. Inoculation.

Figure 2. Rootstock genotypes and scion.

Figure 3. Grafted seedlings.

Table 1. Genotypes disease severity (%) and resistance level.

Genotypes	Disease severity index[a] (%)	Disease resistance level[b]
13	38	MR
37	31	MR
13×18	45	MR
15×18	45	MR

[a] Calculated according to Townsend-Heuberger formula [b] I= ≤ 20%: Highly resistant (HR), II= 21-50%: moderate resistant (MR), III= 51-80%: low resistant (LR), IV= > 80%: susceptible (S).

humidity and temperatures ranging from 24 to 28°C. During the graft healing, the relative humidity was gradually reduced within 7-10 days by opening the sides of the plastic tunnel at regular intervals. At the end of this period, the seedlings were transferred to normal nursery conditions and grown nearly 20 days until the planting stage. Survival rate of grafted seedlings were determined as % before the grafted plants were planted in the greenhouse.

2.2.2. Growing conditions and experimental design

The experiment was conducted in autumn 2021 in an unheated greenhouse in the Batı Akdeniz Agricultural Research Institute in Antalya, Türkiye. Control and grafted plants were transferred to the greenhouse on the 30th of September. The experiment was conducted in a completely randomized block design with three replications (ten plants for each treatment). Rows were one meter apart and the distance between plants was 0.50 m. and a drip irrigation system was employed. During the growing season necessary cultural practices were carried out.

2.2.3. Vegetative growth, fruit and yield traits

The vegetative growth parameter including plant height (cm) was measured at 60 days after transplanting (DAT) on five random plants per experimental plot. Rootstock and scion hypocotyls diameter were measured at 30 days after transplanting by using a caliper above the grafting zone. Earliness of flowering was counted as the number of days from transplanting to first flowering. All plots were harvested, starting from the end of October, to measure the early and total yields. The yield obtained from the first four harvests was evaluated as early yield whereas the total yield included the entire harvest period. Five fruits from each plot were chosen to determine quality measurements. Average fruit length (cm), average fruit diameter (mm), fruit-shape index, flesh hardness (kg cm^2) were recorded. Data for all measurements were subjected to analyses of variance in MSTAT-C package program and the differences between the means were compared by using LSD test.

3. Results and Discussion

The effects of grafting on different rootstocks on survival rate, vegetative growth, fruit yield and quality were examined.

3.1. Survival rate

The survival rate of the plants grafted on different rootstocks is presented in Figure 4. Survival rate was calculated by counting the survived plants. The highest percentage of successful grafting was recorded Gordion/Strongtosa (%98) and Gordion/37. Survival rate changed depending on rootstock and scion compatibility (Lim et al.,1994; Oda et al., 1993; Davis et al., 2008). The suitable rootstock requires a strong affinity with the scion guarantee the survival rate of graft (Traka-Mavrona et al., 2000). Tamilselvi and Pugalendhi (2017) attributed the compatibility to vascular regeneration across the graft interface between the scion and the rootstock. Different researchers have reported that the grafted combinations have a special character other than rootstock compatibility (Salehi-Mohammadi et al., 2009).

3.2. Vegetative growth

The results of the vegetative growth of cucumber plants were observed 60 days after transplanting (Figure 5). No symptoms of Fusarium wilt were observed during the trial. The longest plant height was obtained from the commercial RS841 rootstock, followed by the 15×18 rootstock. Hybrid rootstocks had higher plant height than other

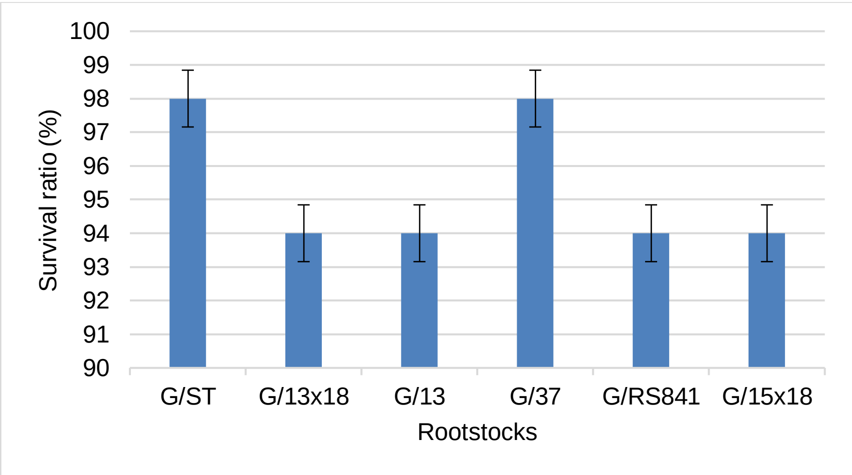

Figure 4. Survival ratio of cucumber plant cultivars grafted onto different rootstocks.

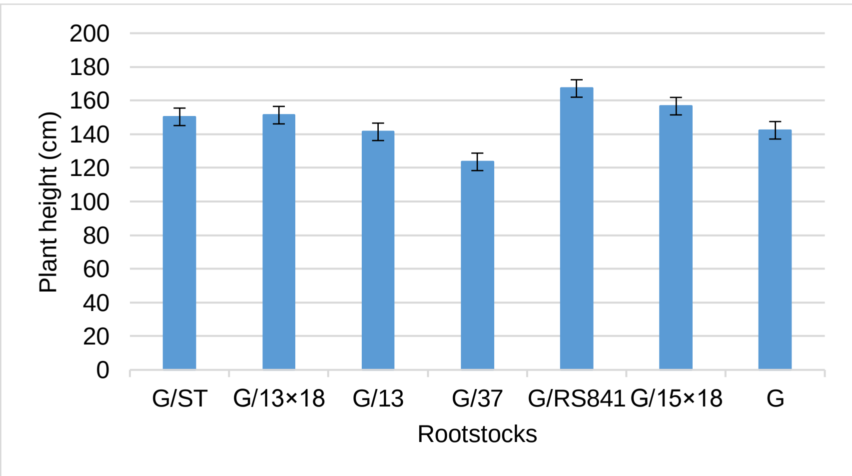

Figure 5. Plant height 60 days of transplanting in grafted and ungrafted cucumbers.

rootstocks used. The other studies using Cucurbita rootstocks have also reported positive results in cucumber grafting onto Cucurbita rootstocks, supporting our study (Colla et al., 2012; Colla et al., 2013; Savvas et al., 2013; El-Sayed et al., 2014).

Flower formation can be affected by rootstock in cucurbits (Traka-Mavrona et al., 2000). Hybrid rootstocks have lower abortion rates (Bayoumi et al., 2021). Earliness of flowering is presented in Table 2 which has significant differences between the grafted rootstock-scion genotypes. The rootstock 15×18 was the earliest flowering in the rootstocks. It was followed by rootstock 37, RS841, 13×18 and Strongtosa. Rootstock 13 delays the grafted cucumber flowering. Plant height and rootstock/stem diameter of cucumber (*Cucumis sativus*) plants grafted onto different cucurbitaceous rootstocks were presented in Table 3. Data of Table 3 indicated that the hybrid rootstocks showed significant increment in plant lenght. The highest plant length was obtained from RS 841 rootstock. Data presented in Table 3 shows that grafting cucumber scions onto RS 841 rootstock significantly increased in plant length (167.18 cm) compared to ungrafted plants. In stem diameter there was no statistically significant difference between the combinations. The largest stem diameter was obtained from Strongtosa rootstock (9.63 mm).

3.3. Physical characteristics of cucumber fruits

The effect of six rootstocks on cucumber fruit characteristics (fruit lenght, fruit diameter, fruit-shape index, fruit firmness, panel test) was presented in Table 4. Among the rootstocks, the highest fruit length (14.32 cm) was obtained from Strongtosa rootstock. In the contrary, the smallest

Table 2. Effect of rootstocks on the earliness of flowering.

Rootstocks	Earliness of flowering (days)
Strongtosa	11.0 b
13×18	11.0 b
13	14.0 a
37	9.0 c
RS 841	11.0 b
15×18	8.0 d
Gordion (Control)	14.0 a
LSD	2.92
CV (%)	14.0
Standard deviation	2.26

*Means followed by different letter within same column (factors) are significantly different at $P < 0.05$

Table 3. Effect of rootstocks on vegetative growth of grafted cucumber plants.

Rootstocks	Plant length (cm)	Stem diameter (mm)
Strongtosa	150.33 ±7.6 bc	9.63±1.1
13×18	151.33±6.8 bc	8.93±1.3
13	141.41±7.7. c	9±0.1
37	123.58 ±9.8 d	8.23±0.4
RS 841	167.18±9.9 a	8.93±0.5
15×18	156.66±12.1 ab	9.53±0.8
Gordion (Control)	142.33 ±0.6 bc	
LSD	15.03	n.s.
CV (%)	5	9
Standard deviation	13.72	0.50

*Means followed by different letter within same column (factors) are significantly different at $P < 0.05$

Table 4. Effect of rootstocks on fruit length, fruit diameter, fruit shape index, fruit firmness and panel test

Rootstocks	Fruit lenght (cm)	Fruit diameter (mm)	Fruit-shape index	Fruit Firmness (kg cm^{-2})	Panel test
Strongtosa	14.32±14.3	26.25±1.1	5.45±0.2	2.44±0.16	4.33±0.99 ab
13×18	13.70±13.7	23.58±3.5	5.86± 0.7	2.37±0.15	3 ±1.06b c
13	14.03±14	24.65±3.7	5.75± 0.7	2.39±0.11	2.33±0.71 c
37	13.53±13.5	24.51±0.5	5.52± 0.1	2.45±0.11	2.66±0.74 c
RS 841	13.60±13.6	25.05±0.8	5.42± 0.1	2.41±0.06	3.33±0.83 abc
15×18	13.83±13.8	22.41±2.5	6.20± 0.4	2.52±0.06	4.66±1.20 a
Gordion (Control)	14.08±14.1 ns	26.43±1.2 ns	5.33± 0.2 ns	2.54 ±0.08 ns	3.33±1.25 abc
LSD					1.41
CV (%)	4	9	7	4	23
Standard deviation	0.29	1.42	0.31	0.06	0.85

*Means followed by different letter within same column (factors) are significantly different at $P < 0.05$

fruits (13.53 cm) were obtained from 37 rootstock. Non-grafted Gordion and Strongtosa had the highest fruit diameters (26.43 and 26.25 cm) respectively. Regarding to the effect of different rootstocks on fruit firmness in grafted cucumber data exhibited the highest firmness was obtained from Gordion (2.54 kg cm^2) followed by 15×18 rootstock. Fruit shape index values varied between 5.33 and 6.20. Many studies have reported that grafting has no effect on fruit shape index (Karaağaç and Balkaya, 2013, Rouphael et al. 2008). The effect of rootstock on the taste of cucumber fruits was found to be statistically significant and among the rootstocks, 15×18 rootstock significantly improved the taste (4.66, 4.33) after Strongtosa rootstock. There are many studies on the effect of rootstocks on the physical characteristics of cucumber fruit. Németh et al. (2020) and Zaki et al. (2018) reported that grafting doesn't significantly affect physical parameters such as fruit length, fruit diameter, fruit firmness. In accordance with this study the rootstocks weren't significantly affected fruit length, fruit diameter, fruit firmness. In agreement with this researchers Khapte et al. (2021) reported that grafting did not affect fruit firmness. The taste and aroma are due to the effect of different pumpkin rootstocks (Huang et al., 2016).

3.4. Fruit yield characteristics

The effects of rootstocks on early yield, total yield and number of fruits per plant are shown in Table 5. Early yield, total yield and number of fruits per plant were significantly affected by the rootstocks. In grafted plants yield is a feature that varies depending on the rootstock used, the variety and the environmental conditions (King et al., 2010). Many studies have been carried out on yield in grafted cucumber (Noor et al., 2019; Papadaki et al., 2017; Guan et al., 2020; Aslam et al., 2020). When interspecific winter squash rootstocks were used as rootstocks, significant increases in growth characteristics and yield parameters were obtained. Depending on different rootstock/scion combinations it was determined that early yield was 2450 g to 4146.6 g. All the grafted plants produced significantly higher yield than ungrafted plants. The highest early yield and total yield was obtained from Strongtosa rootstock (4146.6 g, 11281.6 g). Thirteen rootstock exhibit significant decreased with lowest value in early yield (1143.3 g) and total yield (3548.3 g).

There are significant differences in the number of fruits per plant between different rootstock/scion combinations. The number of fruits per plant in cucumbers grafted onto different rootstocks varied between 31.3-118.3. Strongtosa had the highest fruit per plant (118.3), followed by RS 841 (113.6) and 13×18 (100.6) hybrid rootstock. Although higher yields were generally obtained in grafted plants compared to the control group, it is thought that the difference in combinations that did not yield high yields was due to rootstock/scion compatibility. At this point, there are many studies showing whether grafting increases productivity (Velkov and Pevicharova, 2016) or not (Üre and Aktaş, 2019).

4. Conclusion

Grafted seedlings are widely used by watermelon growers in Türkiye. However, its use is not common in cucumber due to rootstock-scion compatibility problems. The selection of rootstock-scion combination is one of the criteria to be considered to increase the yield of grafted cucumber fruits. Moreover, grafting onto Fusarium wilt-tolerant pumpkin rootstocks provides control for this soil-borne pathogen in continuous cultivation in greenhouses.

The use of local landraces as rootstocks will significantly reduce the cost of grafted seedlings. In the present study, the effects of Fusarium tolerant local interspecific cucurbita hybrids on rootstock potential and yield and fruit quality of cucumber were investigated. The present study demonstrate that the fruit yield of cucumber Gordion F1 was increased when grafted onto two *C. maxima* × *C. moschata* hybrid and two *C. moschata* genotypes compared to non-grafted plants. Further studies should look into the effects of grafting on Cucurbita species for cucumber crop improvement in the future.

Table 5. Effect of grafting on early yield, total yield and number of fruits per plant.

Rootstocks	Early yield (g)	Total yield (g)	Number of fruits per plant
Strongtosa	4146.6±1053 a	11281.6 ±819.5 a	118.3± 41a
13×18	3163.3±1565.58 c	10083.3 ±2016.4 b	100.6± 7.5 c
13	1143.3±249.65 e	3548.3± 695 f	31.3±18.90 g
37	3040± 433.27c	5680 ±315.1 e	54± 13.11f
RS 841	3843.3±865.88 b	11186.6± 1940.7 a	113.6 ±37.89 b
15×18	3083.3± 695.27c	6383.3±6392.1 d	60.00 ±1 e
Gordion (Control)	2450±691.77 d	8088.3±3632.3 c	76±7.93 d
LSD	2.92	3.26	0.05
Standard deviation	417.19	2974.22	32.86

*Means followed by different letter within same column (factors) are significantly different at $P<$ 0.05

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