JOURNAL OF AGRICULTURAL PRODUCTION

Year: 2024 | Volume: 5 | Issue: 3 | e-ISSN 2757-6620 | agriprojournal.com

PRENSIP

JOURNAL OF AGRICULTURAL PRODUCTION

2024 • VOLUME 5 • ISSUE 3

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JOURNAL OF AGRICULTURAL PRODUCTION

2024 ● VOLUME 5 ● ISSUE 3

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JOURNAL OF AGRICULTURAL PRODUCTION
ISSN: 2757-6620
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RESEARCH ARTICLE

Effects of Rooting Mediums and Growth Regulating Agents on Rooting Parameters of Lavender and Lavandin Cuttings (*Lavandula sp.*)

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ARTICLE INFO

Article History
Received: 17.05.2024
Accepted: 28.06.2024
First Published: 30.09.2024

Keywords
Cutting propagation
IBA
Lavandula × intermedia Emeric ex Loisel
Lavandula angustifolia
NAA
Rooting medium

ABSTRACT

This study was conducted in Çanakkale province (North-West Anatolia) to determine the optimal auxin dosages (IBA at 2000 ppm and NAA at 1000 ppm) and rooting media (soil, peat, and cocopeat) for the cutting propagation of lavender, which holds significant industrial and commercial value. The research utilized three varieties belonging to the *Lavandula angustifolia* Mill species: Hemus, Sevtopolis and Drujba, as well as the Super A variety from the *Lavandula × intermedia* Emeric ex Loisel species. The experiment was organized with three replications, following a split-plot trial design divided into random plots. Results indicated that the Super A variety of *Lavandula × intermedia* Emeric ex Loisel, when cultivated in cocopeat medium with IBA at 2000 ppm, achieved the highest rooting rate (88.33%) and rooting status (17.67 units). The Sevtopolis variety of the *Lavandula angustifolia* species exhibited the highest values for root number (15.33), root length (9.88 cm), shoot number (20.93), and shoot length (15.35 cm) when grown in cocopeat medium with IBA at 2000 ppm. Based on these findings, the use of cocopeat medium and IBA at 2000 ppm auxin application is recommended for the effective propagation of lavender by cuttings.

Please cite this paper as follows:

Karakaş, İ., & İzci, B. (2024). Effects of rooting mediums and growth regulating agents on rooting parameters of lavender and lavandin cuttings (*Lavandula sp.*). *Journal of Agricultural Production*, *5*(3), 138-152. https://doi.org/10.56430/japro.1485102

1. Introduction

It is estimated that there are 422,000 blooming plants globally, with 72 thousand of these species employed for medicinal reasons (Arslan et al., 2015; Schippmann et al., 2006). It has been announced that medicinal and aromatic plants had a market share of 60 billion dollars in world trade in 2000 and 100 billion dollars in 2015. Türkiye's share in this market is 2.5 billion TL (İpek, 2017). The world's import value of essential oil derived from these plants is 4.6 billion dollars. Türkiye's imports total 26.7 million dollars, while its exports total 33.3 million dollars. Plant essential oils such as rose, laurel, and thyme play a vital role in Turkish exports, whereas essential oils such as mint, orange, lavender, and citrus fruits dominate imports (Temel et al., 2018).

The fragrant plant lavender is a semi-shrub, perennial, essential oil plant in the Lamiaceae family (Aslancan & Sarıbaş, 2011; Guenther, 1972). Lavender essential oil, fresh flowers and dried products are grown for food and other purposes. Lavender is a very valuable essential oil plant due to the essential oil obtained from its flowers; it is also widely used in

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perfume, cosmetics, aroma, pharmaceutical and detergent industries (Arabacı & Bayram, 2005). Lavender is utilized as an aesthetic plant in landscaping, beekeeping, and eco tourism, as well as an aromatic and essential oil plant. Lavender essential oil is commonly used in cosmetics, perfumes, pharmaceuticals, and soaps (Ceylan, 1996).

The Lamiaceae (Labiatae) family contains roughly 200 genera and 3000 species worldwide. It comprises 45 genera and 546 species growing natively in Türkiye, has a 42.2% endemism rate, and is one of the Lamiaceae family's significant gene centers. There are three distinct lavender species in the globe, each with a high economic and commercial value (Cesur Turgut et al., 2017). Lavander (*L. angustifolia* Mill. = *L. officinalis* L. = *L. vera* DC), Lavandin (*L. intermedium* Emeric ex Loisel. = *L. hybrida* L.), and Spike lavander (*L. spica* = *L. latifolia* Medik.) are three species with substantial economic benefits (Arabacı et al., 2007). These plants are native to the Mediterranean's hilly areas, but they are now commonly grown throughout Southern Europe, Australia, and the United States. Essential oil quality is better in lavender variations, while essential oil output is higher in lavandin kinds (Kara & Baydar, 2013).

Lavender cultivation, which is particularly concentrated in and around Türkiye's Isparta province, has poor practices in other locations, despite its potential (Baydar, 2010; Kara, 2011). However, *L. angustifolia* cultivation, which has high essential oil quality and economic returns, is not common in our country. Instead of *L. angustifolia*, *L. stoechas*, known as French lavender, whose essential oil is obtained from its leaves and flowers and has a strong smelling, grows naturally in our country (Ayanoğlu et al., 2000; Baytop, 1999).

Lavender essential oil is economically very important and has a huge demand worldwide. Lavender essential oil is used in the production of high-priced perfumes, fragrances and essences, pharmaceutical products, and also in the cosmetics and food industries due to its phenolic metabolites (Prusinowska & Śmigielski, 2014). In our nation, which has diverse vegetation, the popularity of lavender, an essential oil plant that can be farmed, is growing by the day, and so is the need for seedlings. To compete in both domestic and international markets, plants that meet the criteria must be grown. Gaining a foothold in domestic and international markets requires providing seedlings with high yield and quality (Çiçek, 2019).

The most pressing issue in lavender is providing high-yielding, high-quality propagation material. The plant reproduces vegetatively (by cuttings) and generatively (by seeds). *Lavandula* species can be propagated by seeds but this should not be the preferred method of propagation. Asexually propagated lavender plants provide more homogeneous products, and quality clones increase the possibility of obtaining higher quality essential oil (Tyub et al., 2007). Moreover, growth is slow in seed propagation, and it is recommended to propagate by cuttings, as problems such as low germination, weed control and cultural operations are difficult, and the plant's development is slow in the early stages and it exhibits a lot of variation in growth rate and oil composition. Plants grown by seeds lack standardization and homogeneity, complicating cultural methods, and the presence of genotypic variations may not give the superior quality essential oil that the species requires globally. Moreover, poor rooting and limited market availability have been problems for superior clones of *Lavandula* species (Calvo & Segura, 1988).

Propagation by cuttings can be defined as the technique of obtaining a new plant with a piece of stem, root or leaf, called cutting, obtained from the mother plant to be produced. Propagation by cuttings is used in many medicinal and aromatic plants and ornamental plants. It is the simplest, cheapest and fastest production technique performed outdoors or indoors in a greenhouse environment. Since propagation by cuttings is taken from the mother plant, whose yield and quality are known, it guarantees high yield and quality in new plantations (Ürgenç, 1992). High quality is the most important criterion in essential oil plants. For these reasons, propagation by cuttings is one of the common methods applied to perennial, semi-shrub and bushy plants. However, there are some problems in *L. angustifolia* and *Lavandula* × *intermedia* Emeric ex Loisel. The most important of these problems is rooting, and to solve this problem, different studies have been conducted. According to the results, it has been reported that the rooting rate varies depending on the type of cutting taken (Bona et al., 2012a).

The oldest known hormones that are effective for rooting are the auxin group hormones. With its discovery in 1930, auxin began to be used frequently on rooting (Halloran & Kasım, 2002). Auxin increases carbohydrate transport in shoots, leaves and cuttings and ensures the formation of adventitious roots and also functions in the initiation of rooting (Hartmann et al., 2011). Synthetic hormone auxin forms frequently used in rooting are IBA (Indole Butyric Acid) and NAA (Naphthalene Acetic Acid). The most commonly used one is IBA. IBA is not toxic in large concentrations and is effective in promoting rooting in many plant species (Özbek et al., 1961). IBA also has a stimulating effect on rooting by providing low mobility and chemical stability in plant tissues (Çiçek, 2019; Hartmann et al., 2011). Plants produce auxin in their roots and young leaves, but it is more advantageous to use the application of synthetic auxins to prevent the death of cuttings (Çetin, 2002). In addition, in different studies conducted on the rooting of cuttings, it was found that various hormones, holding times in hormones, and different medium used were effective in rooting and the best rooting was obtained from IBA (Ayanoğlu et al., 2000; Bhat et al., 2008; Bona et al., 2012b). Not only IBA but also NAA is used in the rooting of cuttings, and various studies are carried out on the hormones used, the doses and holding times of these hormones.

However, in lavender, there are almost no studies on the hormone doses used, different media, cuttings types, different hormone applications, and the holding time in the hormone with high practical applicability, and different types. Especially those with high essential oil yield and essential oil quality; research is limited in terms of the applicability of rooting studies on Hemus, Sevtopolis and Drujba varieties belonging to the *L. angustifolia* species and Super A variety belonging to the *Lavandula* × *intermedia* Emeric ex Loisel. species. The aim of this research was to determine the rooting performance of Hemus, Sevtopolis and Drujba varieties of *L. angustifolia* and Super A variety cuttings of *Lavandula* × *intermedia* Emeric ex Loisel. by treating them in Soil, Peat and Cocopeat media and with IBA 2000 ppm, NAA 1000 ppm hormones.

2. Materials and Methods

Cuttings of different lavender species used in the research were obtained from an organic farming enterprise and privately owned nurseries in Çanakkale, Ezine, Yaylacık village in late February 2020. Cuttings to be used in the research (1+0) were bare selected from rooted seedlings. Cuttings of Hemus, Sevtopolis and Drujba cultivars belonging to *L. angustifolia* species and Super A cultivar belonging to *Lavandula* × *intermedia* Emeric ex Loisel. species were used in the research. Plants in the 3rd year of plantation were used as rootstock. The greenhouse trial was carried out in a privately owned greenhouse located at the airport in Çanakkale Province, Merkez District, Barbaros Neighborhood, in 2020. The research was carried out in a greenhouse located at the airport location of Çanakkale Province, Merkez District, Barbaros Neighborhood, and 6-liter rooting pots were used in the experiment. The pots were thoroughly washed and disinfected before being filled with soil, peat and coco peat.

Soil, peat and cocopeat were used as rooting media. Based on an examination of the rooting media's basic structure, the high clay soil can be made more pliable and aerated with the use of the peat medium. Peat is inherently acidic, which decreases pH in highly alkaline soils. Peat also lessens the amount of nutrients that leach or run off. It has the advantage of having excellent soil and air holding properties and making water available when needed, allowing peat, fertilizer and water to pass to plants gradually. Peat soil has a fibrous structure (Anonymous, 2020). Cocopeat is an all-purpose growing medium made from coconut shells. Its air-filled porosity and high water holding capacity make it an ideal growing medium for herb plants. It is 100% organic and environmentally friendly. Its pH value is 5.7-6.5, EC level is <1 mS/cm, ideal for plant growth. It does not contain soil-borne pathogens and weeds. It is easy to use in seedling trays because it has a fluffy structure, does not clump and is not compressed. It supports fast, healthy root growth with constant moisture and oxygenation. It has tremendous oxygenation properties that are important for healthy root growth. Its high water holding capacity can retain moisture up to eight times its volume without sacrificing air supply. It is a tremendous soil conditioner and the presence of organic compounds in cocopeat encourages root growth and may offer some natural resistance to plant diseases. Cocopeat's physical and biochemical properties make it resistant to bacterial and fungal growth (Hartmann & Kester, 1983). The soil used as rooting medium was taken from the agricultural land next to the airport in Çanakkale Province, Merkez District, Barbaros Neighborhood, where the cuttings will be rooted and transferred to the field. Characteristics of the trial area and rooting soil, soil depth (cm) 0-30, pH 7.6, lime (%) 5, EC (mmhos) 193, sand (%) 17, clay (%) 43, silt (%) 44, compound silty-clay, organic matter mmount (%) was recorded as 2.88.

Cuttings obtained from an organic farm in Çanakkale Ezine Yaylacık village and from private sector nurseries were taken from three-year-old shoots of Hemus, Sevtopolis and Drujba varieties of *Lavandula angustifolia* species and Super A variety of *Lavandula* × *intermedia* Emeric ex Loisel. species. The rooting phase trial of the research was established using the Random Plots Trial Design with 3 factors and 3 replications. According to the Experimental Design of Divided Plots in Random Plots, cuttings of plant materials were used in the main plots. Three different media on the lower plots and two different auxins (2000 ppm IBA doses) on the bottom plots. The NAA dose was 1000 ppm, and the control group without any treatment was established in 3 replications. The shoots taken from the rootstock plants were cut to a length of about 10-12 cm, and all the leaves of the lower ¾ part of each cutting were stripped and prepared for planting. The prepared cuttings were kept in a beaker for a while, with the bottoms remaining in the water. Afterwards, the rooting pots were thoroughly washed and sterilized.

In terms of the methods used, the hormone dose applied to the cuttings and the holding times varied. However, studies on minute and slow immersion methods rather than fast immersion and second-based studies are limited and almost non-existent. Therefore, in this research, the cuttings were prepared for planting by keeping them in the rooting hormone for five minutes to ensure better penetration of the cuttings with the rooting hormone, better coverage of the roots, and the hormone doses used were not too high. In the study, cuttings were prepared for planting by keeping their bottom parts in concentrations of IBA (control-0 and 2000 ppm) and NAA (control-0 and 1000 ppm) for 5 minutes. Then, the cuttings were planted in 6-liter rooting pots filled with soil, peat and coco peat and were watered regularly twice a week. In the research, the cuttings were checked regularly during the rooting period, and no diseases or pests were observed. Rooting performances and rooting-related parameters of the cuttings, removed from the rooting medium after an average of 60-70 days, were measured and analyzed.

2.1. Features Examined During the Rooting Stage

The following measurements and examinations were made after the roots of the cultures, planted in rooting medium and kept in this medium for an average of 2 months, were washed with sterile pure water.

Rooting rate: The number of rooted and unrooted shoots after each application was determined and expressed as a percentage.

Rooting status: In each application, the number of cuttings showing or not showing rooting was determined.

Number of roots: The number of roots in cuttings showing or not showing rooting in each application was determined.

Stem length: The lengths of the roots in cuttings showing or not showing rooting in each application were measured in cm.

Shoot length: After culture, the shoot lengths of the plants were measured in cm.

Number of shoots: The number of shoots developed in each application has been determined.

2.2. Statistical Analysis and Evaluations

Variance analysis was performed according to the split-plot trial design in which random parcels were divided. According to this analysis, the average values of the application that were statistically significant were grouped according to DUNCAN. Statistical evaluations were made using the TOTEMSTAT package program (Dumanoğlu et al., 2019).

3. Results and Discussion

3.1. Rooting Rate

The effect of different rooting media, cultivars and IBA 2000 ppm and NAA 1000 ppm doses on the rooting rate of lavender cuttings was statistically significant ($p \leq 0.01$) and it was observed that it differed according to the cultivars (Table 2). The highest rooting rate among the varieties was obtained in Super A variety with 88.33%, while the lowest rooting rate was obtained a rate of 48.67% was obtained in Sevtopolis and Drujba cultivars. When the general effects of different rooting media are examined, it is seen that the highest rooting rate is obtained from the cocopeat medium with 88.33%, and the lowest rooting rate is obtained from the soil medium with 48.67%. In addition, when the main effects of hormone applications were examined, it was observed that the highest rooting rate was obtained in the IBA 2000 ppm application with 88.33%, and the lowest rooting rate was obtained in the 0 (control) application with 48.67%. When we look at the rooting rate averages of hormone applications in general, we can say that the IBA 2000 ppm dose is more effective (Table 1).

Table 1. Effect of IBA 2000 ppm and NAA 1000 ppm dose on the rooting rate (%) of lavender in different medium.

		Control	**IBA**	**NAA**	**Average**
Hemus	Soil	50.33	72.33	71.67	64.77
	Peat	53.67	76.00	71.33	67.00
	Cocopeat	57.00	86.00	81.67	74.88
	Average	53.66	78.111	74.889	68.88 BC
Sevtopolis	Soil	48.67	67.67	67.33	61.22
	Peat	49.67	72.00	72.33	64.66
	Cocopeat	53.33	82.67	78.33	71.44
	Average	50.55	74.111	72.667	65.77 C
Drujba	Soil	48.67	73.33	71.67	64.55
	Peat	49.67	77.67	75.00	67.44
	Cocopeat	58.67	86.33	83.33	76.11
	Average	52.33	79.111	76.667	69.37 B
Super A	Soil	52.67	78.00	74.67	68.44
	Peat	53.67	82.00	77.67	71.11
	Cocopeat	62.00	88.33	86.00	78.77
	Average	56.11	82.77	79.44	72.77 A
	Variety Average	53.16 C	78.52 A	75.91 B	69.20
	Rooting Medium Average	64.75 B	67.55 B	75.30 A	

* There is no statistical difference between averages indicated with the same letter.

When the variety×medium interaction was examined in the study, the effect on the rooting rate of lavender cuttings was statistically insignificant (Table 2). In the average of the cultivar×medium interaction, the rooting rate ranged from 50.55 to 56.11% and the highest rooting rate was 62.00% in the Super A variety and cocopeat medium, the lowest rooting rate with 48.67% was obtained in the Sevtopolis and Drujba cultivars and soil medium. According to the general averages, we can say that the Super A variety and cocopeat medium stand out compared to the others (Table 1). When the variety×hormone interaction was examined in the study, the effect on the rooting rate of lavender cuttings was statistically insignificant.

In variety×hormone interaction averages rooting rate varies between 72.66 and 82.77%. The highest rooting rate of 88.33% was observed in the Super A variety with the IBA 2000 ppm dose, the lowest rooting rate of 48.67% was observed in the Sevtopolis and Drujba cultivars with the 0 (control) combination. Generally, we can say that the Super A variety and the IBA 2000 ppm dose stand out. In the study, when the medium×hormone interaction was examined, the effect on the rooting rate of lavender cuttings was statistically insignificant (Table 2). The average rooting rate in the medium×hormone interaction ranged from 50.55 to82.77%. The highest rooting rate of 88.33% was observed in the cocopeat medium with the IBA 2000 ppm interaction, and the lowest rooting rate of 48.67% was observed in the soil medium with the0 (control) combination. Generally, we can say that cocopeat medium and IBA 2000 ppm dose application stand out (Table 1).

When the variety×medium×hormone interaction was examined in the study, the effect on the rooting rate of lavender cuttings was statistically insignificant (Table 2). In the average of the variety×medium×hormone interaction the rooting rate ranged from 61.22 to 78.77%, the highest rooting rate of 88.33% was observed in the Super A variety, cocopeat medium and IBA 2000 ppm combination, while the lowest rooting rate of 48.67% was observed in the Sevtopolis and Drujba cultivar, from the soil medium and 0 (control) combination (Table 1). Generally, it is seen that the Super A variety, cocopeat medium and IBA 2000 ppm application stand out. When IBA, NAA and different medium applications are examined, it is seen that the IBA 2000 ppm dose stands out and the applied hormone and doses increase rooting compared to 0 (control) and cocopeat medium stands out in different medium used. In studies examining the effects of different rooting media on the rooting rate, Türkmen (2019) reported that the highest rooting rate of 57% of the yellow-girl tea plant was obtained from sand medium applied with 1000 ppm IBA. Bayraktar et al. (2018) investigated the effects of different greengouse media and hormones on the rooting yew (*Taxus baccata* L.) and found that the best results were obtained in greenhouse 2 with the IBA 5000 ppm treatment in perlite rooting medium. Kumar and Ahmed (2013) investigated the effects of different rooting media and different IBA doses on the rooting of cuttings in pelargonium (*Pelargonium graveolens* L.) and reported that the highest rooting rate of 94.05% was obtained in cocopeat medium.

Table 2. Mean square values obtained as a result of variance analysis of the examined parameters.

	Source of Variation	Rooting Rate	Rooting Status	Number of Roots	Root Length	Shoot Number	Shoot Length
AVERAGE OF SQUARES	Recurrence	3.398	0.298	1.869	0.573	0.034	0.019
	Variety	221.741**	13.699**	14.154**	7.938**	13.680**	17.511**
	Error 1	13.769	0.969	0.327	0.002	0.185	0.046
	Medium	1076.120**	51.826**	10.145**	4.566**	16.180**	9.485**
	Variety x Medium	1.972	0.892	0.598	0.049**	0.336	0.031*
	Error 2	27.301	1.418	0.795	0.001	0.594	0.010
	Hormone	7005.398**	309.716**	644.500**	303.705**	1309.345**	581.694**
	Variety x Hormone	7.843	0.574	3.940**	0.695**	3.351**	1.089**
	Medium x Hormone	22.037	0.400	1.419	0.433**	1.141*	0.579**
	Variety x Medium x Hormone	7.185	0.646	1.222	0.026**	0.327	0.014
	Error 3	18.412	1.369	0.866	0.001	0.436	0.019
	1. CV(Variety) = 0.74%	CV(Medium) = 1.35%	CV(Hormone) = 0.41% Rooting Rate				
	2. CV(Variety) = 7.15%	CV(Medium) = 8.66%	CV(Hormone) = 8.51% Rooting Status				
	3. CV(Variety) = 5.97%	CV(Medium) = 9.31%	CV(Hormone) = 9.72% Number of Roots				
	4. CV(Variety) = 0.72%	CV(Medium) = 0.43%	CV(Hormone) = 0.47% Root Length				
	5. CV(Variety) = 3.02%	CV(Medium) = 5.41%	CV(Hormone) = 4.64% Shoot Number				
	6. CV(Variety) = 2.46%	CV(Medium) = 1.14%	CV(Hormone) = 1.60% Shoot Length				

*, **: Respectively, significant at p≤0.05 and p≤0.01 levels.

Alp et al. (2010) reported the highest rooting rate at 53.3% in green, semi-wood and wood cuttings of garden roses taken at different periods in 0 (control), 1000, 1500 and 2000 ppm IBA applications and a minimum of 2000 ppm IBA in wood cuttings of *R. chinensis* var. Arslanoğlu and Albayrak (2011) documented that the highest rooting rate in rosemary and lavender to stem suttings in IBA applications at 1000, 2000, 4000 and 6000 ppm doses. Our findings also show that the highest rooting rate obtained in lavender at 2000 IBA doses is similar to the studies in the above literature. In another study, lavender (*Lavandula angustifolia* Mill) semi-wood, green bottom and green tip cuttings were treated with control-0, 2000, 4000, 6000, 8000, and 10000 ppm IBA doses, and the highest rooting rate was observed in the green bottom cutting type with 8000 and 10000 ppm IBA (Çiçek, 2019). Kara (2011) stated that the highest rooting rate of 95.13% was obtained in the *L.*

angustifolia var. Silver variety from the application of different IBA doses (0, 1000, 2000, 3000, and 4000 ppm) to different lavender and lavandin cuttings and planted with 4000 ppm IBA in March. This difference may be due to different plants, the use of different rooting media, differences in cutting types or holding times.

Shahhoseini et al. (2015) reported that the highest rooting rate for rosemary (*Rosmarinus officinalis* L.) semi-wood cuttings with IBA and NAA (0, 2000, 3000, 4000, 5000 ppm) doses in 1-minute immersion application was 84% with NAA 1000 ppm. The results showed that the highest rooting rate of NAA 1000 oom 84% does not agree with our findings. When all the findings are examined; it is seen that plant species, rooting products and doses, rooting medium and techniques used differ, affect the results and may change.

3.2. Rooting Status

According to the results of variance analysis performed on the effects of IBA 2000 ppm and NAA 1000 ppm doses on the rooting status of lavender cuttings under in vivo conditions in different media; variety, medium and dose of IBA 2000 ppm and NAA 1000 ppm were found to be statistically significant at the level of $p \leq 0.01$, while the interaction effects of variety × medium, variety × hormone, medium × hormone and variety × medium × hormone dose were found to be statistically insignificant (Table 2). The highest rooting rate was obtained in the Super A variety with 17.67 units, while the lowest rooting rate was obtained in the Sevtopolis and Drujba varieties with 9.73 units. When the general effects of different rooting media are examined, it is seen that the highest rooting status was obtained from cocopeat media with 17.67 units, and the lowest rooting status was obtained from soil media with 9.73 units. In addition, when the main effects of hormone applications were examined, it was that the highest rooting status was obtained from IBA 2000 ppm application with 17.67 units, and the lowest rooting rate was obtained in IBA 0 (Control) application with 9.73 units (Table 3). In general, when the rooting status averages of the varieties are examined, the highest values were obtained in the Super A variety compared to the others. When the averages effects of hormone applications on rooting status are examined in general, it can be said that there is a difference between the effects of IBA 2000 ppm and NAA 1000 ppm doses, and considering that different environments generally affect the rooting status, the effects of soil, peat and cocopeat on rooting status vary and there are differences between them.

The effect of lavender cuttings on rooting status was found to be statistically insignificant in terms of variety×medium interaction (Table 2). Rooting status ranged between 10.11 and 10.73 in the average of variety×medium interaction, and the highest rooting status was obtained from the Super A variety and cocopeat combination with 12.40. The lowest rooting status was obtained from the combination of Sevtopolis variety and Drujba varieties in soil medium with 9.73 (Table 3). The effect of the variety×hormone interaction on the rooting status of lavender cuttings was found to be statistically insignificant (Table 2). In the average of variety×hormone interaction, rooting status varied between 10.63 and15.70, the highest rooting status was obtained from the Super A variety and IBA 2000 ppm combination with 17.67. The combination of Sevtopolis and 0 (Control) gave the lowest rooting status with 9.73, while the combination of Drujba and 0 (Control) gave the same result.

Table 3. Effect of IBA 2000 ppm and NAA 1000 ppm dose on the rooting status (number) of lavender in different medium.

		Control	**IBA**	**NAA**	**Average**
Hemus	Soil	10.07	14.47	14.33	12.95
	Peat	10.73	15.20	14.27	13.40
	Cocopeat	11.40	17.20	16.33	14.97
	Average	10.73	15.62	14.97	13.77 A
Sevtopolis	Soil	9.73	13.53	13.47	12.24
	Peat	9.93	14.40	14.47	12.93
	Cocopeat	10.67	16.53	15.67	14.28
	Average	10.11	14.82	14.53	13.15 B
Drujba	Soil	9.73	14.67	14.33	12.91
	Peat	9.93	15.53	15.00	13.48
	Cocopeat	11.73	17.27	16.67	15.22
	Average	10.46	15.82	15.33	13.87 A
Super A	Soil	10.53	15.60	14.93	13.68
	Peat	10.73	16.40	15.53	14.22
	Cocopeat	12.40	17.67	17.20	15.75
	Average	11.22	16.55	15.88	14.55 A
	Variety Average	10.63 B	15.70 A	15.18 A	13.84
	Rooting Medium Average	10.45 C	13.51 B	15.06 A	

* There is no statistical difference between averages indicated with the same letter.

In terms of medium×hormone interaction, the effect on the rooting status of lavender cuttings was statistically insignificant (Table 2). In the medium×hormone interaction averages, the rooting status varied between 10.11 and 16.55, and the highest rooting status was obtained from the interaction of cocopeat medium and IBA 2000 ppm with 17.67. The lowest rooting status was obtained from soil and 0 (control) interaction with 9.73 units (Table 3). When the variety×medium×hormone interaction was examined, the effect on rooting status of lavender cuttings was statistically insignificant (Table 2). The rooting status ranged from 12.24 to 15.75 when cultivar, medium, and hormone interactions were considered. The Super A variety, cocopeat medium, and IBA 2000 ppm dosage with 17.67 units provided the maximum rooting status. The lowest rooting status was obtained from the combination of Sevtopolis and Drujba varieties with soil medium and 0 (control) with 9.73 units (Table 3). When the general averages are examined, it is seen that cocopeat medium and IBA 2000 ppm applications have positive effects on the results. In general, it was observed that the application of cocopeat and IBA 2000 ppm doses were effective in the Super A variety.

3.3. Number of Roots

According to the results of the variance analysis on the root number effect of lavender cuttings in vivo conditions, in different media and at the dose of IBA 2000 ppm and NAA 1000 ppm; cultivars, medium, IBA 2000 ppm and NAA 1000 ppm dose and variety×hormone interaction was found to be statistically significant at 0.01 level ($p \leq 0.01$). The interaction effects of variety×medium, medium×hormone and variety×medium×hormone dose were insignificant. The effect of different rooting media and IBA 2000 ppm and NAA 1000 ppm dose and the effect of variety×hormone interaction on root number of lavender cuttings was statistically significant ($p \leq 0.01$) and it was observed that it changed according to cultivars (Table 2). While the highest root number was obtained from the Sevtopolis variety with 15.33, the lowest root number was obtained from the Super A variety with 4.47. In terms of the general effects of different rooting media, the highest root number was obtained in the cocopeat medium with 15.33, while the lowest root number was obtained from the soil medium with 4.47. In addition, the highest root number was obtained from IBA 2000 ppm application with 15.33, while the lowest root number was obtained from 0 (control) application with 4.47 in terms of the main effects of hormone applications (Table 4). When the impacts of hormone application on the effect of root number are analyzed, it was observed that IBA 2000 ppm dose and NAA 1000 ppm dose applications have positive impacts compared to 0 (control) group, and the number of roots increased.

Table 4. Effect of IBA 2000 ppm and NAA 1000 ppm dose on the number of roots (number) of lavender cuttings in different medium.

		Control	**IBA**	**NAA**	**Average**
Hemus	Soil	4.60	12.00	9.27	8.62
	Peat	5.00	11.67	10.27	8.97
	Cocopeat	4.87	13.07	10.67	9.53
	Average	4.82	12.24	10.06	9.04 C
Sevtopolis	Soil	4.60	13.73	11.40	9.91
	Peat	4.93	14.47	12.27	10.55
	Cocopeat	5.27	15.33	12.67	11.08
	Average	4.93	14.51	12.11	10.519 A
Drujba	Soil	5.13	12.47	10.07	9.22
	Peat	5.07	13.13	11.20	9.80
	Cocopeat	5.13	14.13	11.53	10.26
	Average	5.11	13.24	10.93	9.76 B
Super A	Soil	4.47	12.20	9.60	8.75
	Peat	5.07	12.87	10.27	9.40
	Cocopeat	5.00	13.40	10.87	9.75
	Average	4.84	12.82	10.24	9.30 C
	Variety Average	4.92 C	13.20 A	10.83 C	9.65
	Rooting Medium Average	9.12 B	9.43 B	10.16 A	

* There is no statistical difference between averages indicated with the same letter.

The effect of lavender cuttings on root number was statistically insignificant in terms of the variety×medium interaction (Table 2). In the average of the cultivar×medium interaction, the number of roots varied between 4.82 and 5.11, and the highest number of roots was 15.33, obtained from the combination of Sevtopolis cultivar and cocopeat medium. The lowest root number was obtained from the combination of the Super A variety and soil medium with 4.47 (Table 4). When the variety×hormone interaction was examined, the effect on the rooting status of lavender cuttings was found to be statistically significant ($p \leq 0.01$) (Table 2). In the average of variety×hormone interaction, the highest number of roots was obtained from the combination of Sevtopolis cultivar and IBA 2000 ppm dose with 15.33 roots, followed by the combination of Drujba cultivar and IBA 2000 ppm dose. On the other hand, the lowest number of roots was obtained from the Super A variety and 0 (control) with 4.47, followed by the interaction of Hemus with the Sevtopolis variety and 0 (control) with 4.60 (Table 4).

When the medium×hormone interaction was examined, the effect of lavender cuttings on the root number was found to be statistically insignificant (Table 2). In the medium×hormone interaction, the average number of roots ranged between 4.82 and 14.51, and the highest root number, 15.33, was obtained from the combination of cocopeat medium and IBA 2000 ppm dose. The interaction between the soil medium and 0 (control) was found to produce the lowest root number, with 4.47 roots (Table 4). When the variety×medium×hormone interaction was examined, the effect of lavender cuttings on root number was found to be statistically insignificant (Table 2). Considering the average values of the effect of variety×medium×hormone interaction on root number, the number of roots varied between 8.62 and 11.08, and the highest root number was obtained as 15.33from the interaction of Sevtopolis variety, cocopeat medium and IBA 2000 ppm dose. The lowest root number was obtained from the combination of Super A variety, soil medium and 0 (control) with 4.47 (Table 4).

The effect of different medium and hormone applications on the number of roots in the cultivars and Hemus cultivar was below average compared to other cultivars, and the highest averages belonged to the Sevtopolis variety. When the effect of different mediums on the number of roots was examined, it was observed that the soil medium remained below the average values, and the highest average values were obtained from the cocopeat medium followed by the peat medium. When the impact of various hormone applications on the root number is examined, it was seen that the 0 (control) application was significantly below the average compared to the others, and the highest average values were obtained from the NAA 1000 ppm application and the IBA 2000 ppm application. However, the preferred application should be the combination of Sevtopolis variety, cocopeat medium and IBA 2000 ppm.

Polat and Yıldırım (2017) reported that the highest root number was obtained in the application of IBA 2000 ppm dose with 1.61. Yusnita et al. (2018) documented that the highest root number was 17.8-25.5 in 2000 and 4000 ppm applications. The findings obtained at the 2000 IBA are consistent with our findings with the highest root number. Ayanoğlu and Özkan (2000) reported the highest root number (22.35 units) was obtained from 100 ppm and was observed in the rapid dipping method. This may be due to plant variety, use of different rooting media, different cutting types, or holding times. When all the findings are examined, it is seen that the plant species, rooting products and doses, rooting media and the techniques used differ, have an effect on the results and can vary.

3.4. Root Length

The effect of different rooting media, IBA 2000 ppm and NAA 1000 ppm doses and the interaction of variety×hormone, medium×hormone and variety×medium×hormone on root length of lavender cuttings were found to be statistically significant ($p \leq 0.01$) and differed according to cultivars (Table 2). While the highest root length was obtained in the Sevtopolis variety with 9.88 cm, the lowest root length was obtained in the Hemus variety with 2.34 cm. When the general effects of different rooting media were examined, the highest root length was obtained in the cocopeat medium with 9.88 cm, while the lowest root length was obtained in the soil medium with 2.34 cm. In addition, when the main effects of hormone applications were examined, the longest root length was obtained from IBA 2000 ppm application with 9.88 cm, while the lowest root length was obtained from 0 (control) application with 2.34 cm. In general, it is seen that the effect of different media and hormone applications on root length in cultivars gives very positive and important results (Table 5).

According to the results of variance analysis regarding the effects of IBA 2000 ppm and NAA 1000 ppm doses on root length of lavender cuttings in different media in vivo conditions; it was observed that interactions of cultivars, medium, IBA 2000 ppm and NAA 1000 ppm doses, variety×medium, medium×hormone and variety×medium×hormone were statistically significant at $p \leq 0.01$ level. When the variety×medium interaction was examined, the effect of lavender cuttings on root length was found to be statistically significant ($p \leq 0.01$) (Table 2). The average root length of the variety×medium interaction ranged between 2.48 and 3.09 cm and the highest root length of 9.88 cm was obtained from the interaction of the Sevtopolis variety and cocopeat medium. The lowest root length of 2.34 cm was obtained from the interaction of the Hemus cultivar with the soil medium. In general, it was observed that the combination of cocopeat and peat medium used compared to the soil, especially in Sevtopolis and Drujba varieties, gave very positive and significant results on root length compared to the soil medium (Table 5).

Table 5. Effect of IBA 2000 ppm and NAA 1000 ppm dose on root length (cm) of lavender cuttings in different medium.

		Control	IBA	NAA	Average
Hemus	Soil	2.34	7.43	5.34	5.04
	Peat	2.46	7.31	5.78	5.18
	Cocopeat	2.66	8.27	6.30	5.74
	Average	2.48	7.66	5.80	5.32 D
Sevtopolis	Soil	2.86	9.30	6.45	6.20
	Peat	3.10	9.61	7.23	6.64
	Cocopeat	3.32	9.88	7.68	6.96
	Average	3.09	9.59	7.12	6.60 A
Drujba	Soil	2.54	8.13	5.73	5.46
	Peat	2.73	8.35	6.29	5.79
	Cocopeat	2.85	8.74	6.74	6.10
	Average	2.70	8.40	6.25	5.78 B
Super A	Soil	2.46	7.86	5.55	5.29
	Peat	2.62	8.22	6.20	5.68
	Cocopeat	2.82	8.64	6.64	6.03 C
	Average	2.63	8.23	6.13	5.66
	Variety Average	2.72 C	8.47 A	6.32 B	5.84
	Rooting Medium Average	5.50 C	5.82 B	6.21 A	

* There is no statistical difference between averages indicated with the same letter.

When the variety×hormone interaction was examined, the effect of lavender cuttings on root length was found to be statistically significant ($p \leq 0.01$) (Table 2). The average root length of the variety×hormone interaction ranged from 2.72 to 8.47 cm and the highest root length was 9.88 cm, obtained from the interaction of the Sevtopolis with IBA 2000 ppm. It was observed that the lowest root length was 2.34 cm and was obtained from the interaction of Hemus cultivar with 0 (control). In general, the highest average values were obtained in the Sevtopolis cultivar in IBA 2000 ppm application (Table 5).

The effect of lavender cuttings on root length was found to be statistically significant in terms of the medium×hormone interaction ($p \leq 0.01$) (Table 2). The medium×hormone interaction averages showed that the root length varied between 2.48 and 9.59 cm and the highest root length of 9.88 cm was observed from the combination of cocopeat medium and IBA 2000 ppm. The lowest root length was obtained in soil medium and 0 (control) interaction with 2.34 cm. Regarding the effect of the medium and hormone applications generally used in the experiment, the interaction of cocopeat medium and IBA 2000 ppm stands out and is seen to have more positive effects (Table 5).

When the variety×medium×hormone interaction was examined, the effect of lavender cuttings on root length was found to be statistically significant ($p \leq 0.01$) (Table 2). Root length ranged from 5.04 to 6.96 cm. The interaction of the Sevtopolis cultivar with the cocopeat medium and IBA 2000 ppm dose resulted the longest root length, measuring 9.88 cm. The lowest root length, 2.34 cm, was obtained from the interaction of Hemus cultivar with soil medium and 0 (control) (Table 5). The IBA 2000 ppm dose produced more effective results in the Sevtopolis cultivar, followed by the Drujba cultivar, while the cocopeat medium was more effective in promoting root length compared to the other media.

When IBA, NAA, and different medium applications are examined, it is observed that the IBA 2000 ppm dose stands out, and the applied hormones and doses significantly increase rooting compared to 0 (control). Additionally, cocopeat medium also stands out among the different media used. Polat and Yıldırım (2017) investigated the effects of 1:1 perlite–cocopeat mixture medium and different IBA doses on the cuttings of several jujube genotypes. they found that the highest root length, 21.91 mm, was obtained in February with the IBA 2000 ppm dose. Similarly, Demirbaş (2019) examined the effects of cuttings of oil rose (*Rosa damascena* Mill.), a plant known for its difficult rooting, across different periods and different hormone doses. The highest root length was achieved with 2000 ppm. Our findings are similar with these studies.

Kara et al. (2011) investigated the effects of different cutting periods and IBA doses on the rooting of cuttings from rosemary (*Rosmarinus officinalis*), hyssop (*Hyssopus officinalis*), and sage (*Salvia officinalis*). The highest root lengths were recorded as 7.1 cm for rosemary, 6.1 cm for hyssop, and 5.1 cm for sage, using a 4000 ppm IBA dose in cuttings taken in March. These differences may be attributed to the plant species, the use of different rooting media, variations in cutting types, different hormones, doses, or holding times.

3.5. Shoot Number

It was observed that the effects of different rooting media, the IBA 2000 ppm and NAA 1000 ppm doses, and the interactions of variety×medium, variety×hormone, medium×hormone, variety×medium×hormone on shoot number of lavender cuttings varied depending on the variety. While the highest number of shoots was observed in the Sevtopolis variety (20.93), the lowest shoot number was recorded for the Super A variety (6.93). When examining the general effects of different rooting media, cocopeat produced the highest shoot number, matching the 20.93 shoots of the Sevtopolis variety, while soil medium produced the lowest number (6.93 shoots). In terms of hormone application, the IBA 2000 ppm dose resulted in the highest shoot count of 20.93, while the control (0 ppm) yielded the lowest (6.93 shoots). These findings suggest that different rooting media and hormone applications significantly influence the shoot number of lavender varieties, generally leading to positive results (Table 6).

Statistically, the effects of different rooting media, the IBA 2000 ppm and NAA 1000 ppm doses, and the interaction between variety and hormone significantly affected the shoot number ($p \leq 0.01$). The medium and hormone interaction was also statistically significant ($p \leq 0.05$), though the interaction between variety, medium, and hormone was insignificant. Analyzing the variety and medium interaction showed an insignificant effect on the shoot number, with averages ranging from 7.40 to 7.68. (Table 2). The highest number of shoots (7.93) was achieved with the Drujba variety in cocopeat medium, while the lowest (6.93) was seen in Super A variety with soil medium (Table 6).

Table 6. Effect of IBA 2000 ppm and NAA 1000 ppm dose on the number of shoots (number) of lavender cuttings in different medium.

		Control	**IBA**	**NAA**	**Average**
Hemus	Soil	7.00	17.67	13.67	12.77
	Peat	7.60	17.80	15.47	13.62
	Cocopeat	7.60	19.20	16.00	14.26
	Average	7.40	18.22	15.04	13.55 C
Sevtopolis	Soil	7.00	20.20	16.93	14.71
	Peat	7.60	20.67	17.60	15.28
	Cocopeat	7.80	20.93	18.00	15.57
	Average	7.46	20.60	17.51	15.19 A
Drujba	Soil	7.40	17.73	14.87	13.33
	Peat	7.73	19.47	16.60	14.60
	Cocopeat	7.93	20.13	16.93	15.00
	Average	7.68	19.11	16.13	14.31 B
Super A	Soil	6.93	18.00	14.60	13.17
	Peat	7.87	18.73	15.40	14.00
	Cocopeat	7.67	19.53	16.07	14.42
	Average	7.48	18.75	15.35	13.86 BC
	Variety Average	7.51 C	19.17 A	16.01 B	14.23
	Rooting Medium Average	13.50 C	14.37 B	14.81 A	

* There is no statistical difference between averages indicated with the same letter.

When the variety×hormone interaction was examined, the effect of lavender cuttings on the number of shoots was statistically significant ($p \leq 0.01$) (Table 2). The number of shoots in the variety × hormone interaction ranged from 7.51 to 19.17, with the highest shoot number of 20.93 obtained from the combination of the Sevtopolis variety and the IBA 2000 ppm dose, while the lowest number of shoots, 6.93, was recorded for the Super A variety with no hormone application (Control). In general, the application of IBA 2000 ppm significantly increased the number of shoots compared to the control (Table 6).

When the medium×hormone interaction was examined, the effect of lavender cuttings on the number of shoots was statistically significant at the $p \leq 0.05$ level (Table 2). In the medium×hormone interaction, the number of shoots varied between 7.40 and 20.60, with the highest number of 20.93 shoots obtained from the combination of cocopeat medium and IBA 2000 ppm dose. The lowest shoot number, 6.93, was recorded in the soil medium with no hormone application (Control). The application of cocopeat medium and IBA 2000 ppm hormone dose stood out, as the soil medium and control combination yielded values well below the average (Table 6).

When the variety×medium×hormone interaction was examined, the effect of lavender cuttings on the number of shoots was statistically insignificant (Table 2). The number of shoots in this interaction ranged between 13.17 and 15.57, with the highest number of 20.93 obtained from the combination of the Sevtopolis cultivar with cocopeat medium and IBA 2000 ppm dose (Table 6). The lowest number of shoots, 6.93, was recorded for the Super A variety in the soil medium without hormone application (control). Overall, the use of the medium and hormones positively influenced the number of shoots, with the highest values observed in the combination of IBA 2000 ppm and cocopeat medium. Based on these results, the combination of the Sevtopolis variety with IBA 2000 ppm and cocopeat medium, as well as the Hemus variety with cocopeat medium and IBA 2000 ppm, can be recommended.

3.6. Shoot Length

The effects of different rooting media, IBA 2000 ppm, and NAA 1000 ppm doses, as well as the interaction of variety×medium, variety×hormone, medium×hormone, variety×medium×hormone on the shoot length of lavender cuttings varied across different varieties. The highest shoot length, 15.35 cm, was obtained from the Sevtopolis variety, while the lowest shoot length, 4.19 cm, was recorded for the Hemus variety. Regarding the general effects of rooting media, the cocopeat medium produced the highest shoot length at 15.35 cm, and the soil medium produced the lowest at 4.19 cm. Additionally, in terms of hormone application, the IBA 2000 ppm dose resulted in the highest shoot length (15.35 cm), whereas the control treatment (0 ppm) resulted in the lowest shoot length (4.19 cm) (Table 7). Overall, the use of different rooting media and hormone applications generally increased shoot length and had positive effects on the cultivars.

Table 7. Effect of IBA 2000 ppm and NAA 1000 ppm dose on shoot length (cm) of lavender cuttings in different medium.

		Control	IBA	NAA	Average
Hemus	Soil	4.19	11.31	7.05	7.51
	Peat	4.42	11.77	7.67	7.95
	Cocopeat	4.75	12.38	8.25	8.45
	Average	4.45	11.82	7.65	7.97 D
Sevtopolis	Soil	5.09	13.94	8.59	9.20
	Peat	5.60	14.37	9.43	9.80
	Cocopeat	5.90	15.35	10.13	10.46
	Average	5.52	14.55	9.38	9.82 A
Drujba	Soil	4.57	12.25	7.57	8.13
	Peat	4.78	12.70	8.33	8.60
	Cocopeat	5.07	13.37	8.90	9.11
	Average	4.80	12.77	8.26	8.61 B
Super A	Soil	4.47	11.93	7.31	7.90
	Peat	4.66	12.37	8.01	8.34
	Cocopeat	4.98	12.95	8.71	8.88
	Average	4.70	12.41	8.01	8.37 C
	Variety Average	4.87 C	12.89 A	8.33 B	8.69
	Rooting Medium Average	8.20 C	8.66 B	9.22 A	

* There is no statistical difference between averages indicated with the same letter.

The effect of different rooting media, varieties, and IBA 2000 ppm and NAA 1000 ppm doses on shoot length of lavender cuttings were statistically significant for both cultivar×hormone and medium×hormone interactions ($p \leq 0.01$), while the variety×medium interaction was significant at the $p \leq 0.05$ level. The variety×medium×hormone interaction, however, was statistically insignificant. The variety×hormone interaction significantly affected shoot length ($p \leq 0.01$) (Table 2). The average shoot length in this interaction ranged from 4.87 to 12.89 cm, with the highest value, 15.35 cm, obtained from the interaction between the Sevtopolis cultivar and IBA 2000 ppm. The lowest shoot length, 4.19 cm, was recorded for the Hemus cultivar with the control treatment (Table 7). In general, all varieties showed increased shoot length when treated with IBA 2000 ppm or NAA 1000 ppm, compared to the control.

The medium×hormone interaction significantly affected the shoot length of lavender cuttings ($p \leq 0.01$) (Table 2). The average shoot length for this interaction ranged from 4.45 to 14.55 cm. The highest shoot length of 15.35 cm was obtained from the combination of cocopeat medium and IBA 2000 ppm, while no hormone application. The use of cocopeat medium, combined with the IBA 2000 ppm dose, proved particularly effective in increasing shoot length (Table 7).

The variety×medium×hormone interaction did not have a statistically significant effect on the length of lavender cuttings (Table 2). The average shoot length for this interaction ranged from 7.51 to 10.46 cm. The highest shoot length, 15.35 cm, was recorded for the interaction between the Sevtopolis variety, cocopeat medium, and IBA 2000 ppm (Table 7). The lowest shoot length, 4.19 cm, occurred with the Hemus variety, soil medium and, no hormone application. When considering the results and averages overall, the variety×hormone and medium×hormone interactions had statistically significant effects on the shoot length of lavender cuttings ($p \leq 0.01$). The IBA 2000 ppm dose showed a pronounced effect on increasing shoot length across varieties (Table 2). Furthermore, the use of cocopeat medium in combination with IBA 2000 ppm promoted rooting. Based on their effects on shoot length, the recommended applications are cocopeat medium and IBA 2000 ppm, with the most effective combination being Sevtopolis variety with cocopeat medium and IBA 2000 ppm.

Upon examining the effects of IBA, NAA, and different medium applications, the IBA 2000 ppm dose emerged as particularly effective, significantly increasing shoot length compared to the control (0 ppm). The cocopeat medium also stood out among the various media tested. In line with these findings, Uzunoğlu and Mavi (2016) observed that in ornamental pomegranate (*Punica granatum* L.) cuttings treated with IBA (0, 500, 1000, 2000, 3000 ppm) the best results for shoot length were obtained with 3000 ppm IBA. However, Çiçek (2019) reported contrasting findings in lavender (*Lavandula angustifolia* Mill) cuttings, where 4000 ppm IBA applied to bottom cuttings produced the longest shoots. These variations indicate that plant species, rooting products and doses, rooting medium, and techniques can all influence the results, and differences in these factors may lead to changes in outcomes.

Various studies have shown differences in the rooting status, rooting rates, number of roots, root lengths, number of shoots, and shoot lengths of the cuttings. These variations may be attributed to physiological and chemical factors, as well as the differing regenerative abilities and genetic structures of the species and their environmental conditions. Factors such as the age of the mother plant, the timing and type of cutting, and the plant's nutrient status, hormonal levels, and anatomical structure can also influence these outcomes (Ahmed et al., 2002; Gil-Albert & Boix, 1978; Hartmann et al., 1997; Kara, 2011; Schaberg et al., 2000).

4. Conclusion and Recommendations

In recent years, the cultivation of medicinal and aromatic plants has been steadily increasing. Alongside yield, the quality of these plants is of great importance, as the primary value of medicinal and aromatic plants lies in the active substances they contain. As a result, scientific research on advanced breeding techniques aimed at improving quality has accelerated, leading to significant advancements. In Türkiye, lavender cultivation has expanded to various regions, particularly Isparta. This study, briefly summarizes the findings on the effects of different rooting media and doses of IBA 2000 ppm and NAA 1000 ppm on the rooting of cuttings from various lavender varieties. The study found that the highest rooting rate of lavender cuttings, using different rooting media and doses of IBA 2000 ppm and NAA 1000 ppm, was observed in the Super A variety at 88.33%, while the lowest rooting rate was seen in the Sevtopolis and Drujba varieties at 48.67%. When examining the overall effects of different rooting media, the highest rooting rate was achieved with cocopeat medium at 88.33%, while the lowest rooting rate was recorded in soil medium at 48.67%. When analyzing the effects of IBA and NAA, and different media applications, the IBA 2000 ppm dose emerged as particularly effective, significantly increasing rooting compared to control (0 ppm). The cocopeat medium also stood out among the tested media.

Among the tested varieties, the highest rooting status was recorded in the Super A variety with 17.67 units, while the lowest rooting status was noted in the Sevtopolis and Drujba varieties, both at 9.73 units. When examining the overall effects of different rooting media, the highest rooting status was achieved with the cocopeat medium at17.67 units, while the lowest rooting status was observed in the soil medium at 9.73 units. The IBA 2000 ppm dose was particularly effective, significantly enhancing rooting compared to control (0 ppm), Additionally, the cocopeat medium proved to be the most effective among the various media tested. Among the tested varieties, the highest number of roots was recorded in the Sevtopolis variety at 15.33, while the lowest number of roots was noted in the Super A variety at 4.47. When analyzing the overall effects of different rooting media, the highest number of roots was also observed in cocopeat medium at 15.33, while the lowest number of roots was found in the soil medium at 4.47. The IBA 2000 ppm dose proved to be particularly effective, significantly enhancing the number of roots compared to control (0 ppm), with the cocopeat medium demonstrating superior results among the various media used.

Among the varieties, the highest root length was recorded in the Sevtopolis variety at 9.88 cm, while the lowest root length was noted in the Hemus variety at 2.34 cm. When examining the overall effects of different rooting media, the highest root length was achieved in the cocopeat medium, also measuring 9.88 cm, whereas the lowest root length was observed in the soil medium at 2.34 cm. Furthermore, when evaluating the effects of different hormone applications, the longest root length was obtained from the IBA 2000 ppm application, measuring 9.88 cm, while the lowest root length was noted in the control group (0 ppm) at 2.34 cm. Overall, the impact of various media and hormone applications on root length among the different varieties yielded very positive and significant results. Similarly, among the varieties, the highest number of shoots was observed in the Sevtopolis variety at 20.93, while the lowest number of shoots was recorded in the Super A variety at 6.93. When analyzing the general effects of different rooting media, the highest number of shoots was obtained from the cocopeat medium, also at 20.93, while the lowest number of shoots was found in the soil medium at 6.93. Furthermore, when assessing the effects of different hormone applications, the highest number of shoots, 20.93, was achieved with the IBA 2000 ppm dose, while the lowest number of shoots was noted in the control group (0 ppm).

Among the varieties, the highest shoot length was obtained in the Sevtopolis variety with 15.35 cm, while the lowest shoot length was obtained in the Hemus variety with 4.19 cm. When the general effects of different rooting medium were examined, the highest shoot length was obtained from the cocopeat medium with 15.35 cm, while the lowest shoot length was obtained from the soil medium with 4.19 cm. When the general effects of different hormone applications are examined, the highest shoot length of 15.35 cm was obtained from the IBA 2000 ppm dose application, while the lowest shoot length of 4.19 cm was obtained from the 0 (Control) application. When all the results are examined, in the propagation of lavender by cuttings, cocopeat medium and IBA 2000 ppm dose application, where the highest rooting parameters are obtained, can be recommended for cuttings taken from different varieties, especially Sevtopolis and Super A varieties. the cuttings of the plantations to be created with these varieties, using cocopeat medium, and 2000 ppm IBA applied to the cuttings will increase rooting and make a big difference.

Acknowledgment

This paper is a part of the MSc thesis of the first author.

Conflict of Interest

The authors declare that they have no conflict of interest.

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http://agriprojournal.com
JOURNAL OF AGRICULTURAL PRODUCTION
ISSN: 2757-6620
PRENSIP
https://prensip.gen.tr

RESEARCH ARTICLE

The Effect of Plant Growth Promoting Bacteria Strains on Yield and Some Quality Parameters of Eggplant (*Solanum melongena* L.)

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ARTICLE INFO

Article History
Received: 02.05.2024
Accepted: 28.06.2024
First Published: 30.09.2024

Keywords
Fertilizer
PGPB
Solanum melongena L.
Yield

ABSTRACT

This research was carried out under field conditions to determine the effect of PGPB on the yield and quality of Pulsar F1 eggplant variety under Igdir ecological conditions in 2021. The experiment was conducted with 8 treatments (*Brevibacillus reuszeri* strain IT 119, *Kluyvera cyrocrescens* strain IT 160, IT 119 + IT 160, IT 119 + Fertilizer, IT 160 + Fertilizer, IT 119 + IT 160 + Fertilizer, Fertilizer and Negative Control) with 3 replications according to the randomized block design. Bacterial strains were applied by inoculating 100 ml (10^6 cfu/ml) to the root zone of the plants during seedling stage. PGPB treatments increased total yield, discard yield, marketable yield, plant height, plant stem thickness, number of leaves, plant root wet weight, plant root dry weight, stem wet weight, stem dry weight, number of fruits per plant, fruit wet weight, fruit dry weight and root length compared to negative and positive control groups. The treatments had no effect on fruit diameter. Although both strains were found to be successful, especially *Kluyvera cyrocrescens* strain IT 160 alone and in combination showed positive effects in terms of the parameters examined in eggplant plants and has the potential to be used as biofertilizer.

Please cite this paper as follows:
Aydın, M., & Figen Dönmez, M. (2024). The effect of plant growth promoting bacteria strains on yield and some quality parameters of eggplant (*Solanum melongena* L.). *Journal of Agricultural Production*, *5*(3), 153-162. https://doi.org/10.56430/japro.1477206

1. Introduction

Eggplant is an economically important vegetable species in the Solanaceae family, grown as perennial in tropical regions and as annual in temperate regions. It is one of the most widely produced vegetables in the world and has a wide variation both genotypically and phenotypically. Today, the most widely known and cultivated eggplant species all over the world is *Solanum melongena* L. (Kassi et al., 2019).

With the emergence of many diseases in today's living conditions, great attention has started to be paid to healthy nutrition, and the consumption of products in this regard has increased rapidly. From this point of view, contrary to popular belief, eggplant is as valuable as other vegetables and it is an important part of healthy nutrition due to its vitamins, minerals, low protein, carbohydrate and fat content, fibrous structure and antioxidant source (Kocayiğit, 2010). In addition to being utilised as a vegetable in different ways, it has been used as a medicinal plant since ancient times and today it is used as an ornamental plant in the pharmaceutical sector and in landscaping (Demir, 2020).

Due to the phenolic and antioxidant compounds it contains, its importance in terms of human health has been better understood in recent years. It protects our body against chronic diseases and cleans it from harmful substances; it has painkillers, anti-inflammatory, and calming properties; and it is one of the most important vegetables used in diets in the fight against obesity with its approximately 90% water content.

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Thanks to the antioxidants it contains, it has been reported that it blocks the formation of free radicals that damage the cell membrane and cause oxidation of malignant LDL cholesterol, leading to an increased risk of heart disease and stroke. It is also used in the treatment of diabetes, arthritis, asthma, bronchitis, stomach, and liver diseases.

In fact, it has become a vegetable which is accepted as the "King of Vegetables" in India due to its economic and nutritious nature and its use in the treatment of diseases (Fategbe et al., 2013; Külahlıoğlu, 2016).

Globally, since the beginning of modern agriculture, farmers have endeavoured to improve the quality and yield of eggplant by selecting varieties and using techniques such as irrigation, fertilisation and control of pathogens/pests. However, many agricultural soils do not contain sufficient amounts of one or more of the nutrients such as nitrogen, phosphorus, potassium and iron, and therefore plant growth is insufficient. Unfortunately, farmers have become more dependent on chemical sources of nitrogen, phosphorus, etc. to eliminate this problem and to obtain higher plant yields. As a result of this, chemical fertilizers pose a great risk to human and environmental health due to both the cost of production and the decrease in non-renewable resources such as oil and natural gas used to produce them.

In order to greatly increase agricultural productivity and to do so in a sustainable and environmentally friendly manner, many of the current approaches to agriculture, including the use of chemical fertilizers, herbicides, fungicides, and insecticides, need to be reconsidered (Lucy et al., 2004). At this point, the use of plant growth-promoting bacterial (PGPB) strains as an integral component of agricultural practice is a technology whose time has come. In both managed systems and natural ecosystems, the beneficial bacteria-plant relationship plays a key role in promoting plant health and growth and increasing yields (Compant et al., 2010). Such plant growth-promoting bacteria are capable of enhancing plant growth and protecting plants from diseases and abiotic stresses through a wide variety of mechanisms.

Some important properties such as biological nitrogen fixation, phosphate solubilisation, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, production of siderophores and phytohormones are considered as characteristics of plant growth promoting bacteria (Souza et al., 2015). In addition, they indirectly contribute to plant growth by showing biocontrol activity against pathogens causing diseases in plants with their enzymes, antimicrobial substances, and superiority in competition (Innerebner et al., 2011; Whipps, 2001). These two complementary properties make PGPBs valuable for agriculture. The combination of different methodologies such as identification of PGPB strains, determination of plant growth promoting properties of bacteria, seed inoculation tests under laboratory conditions and field cultivation trials are important for the development of new and effective inoculants for agriculture. Effective bacterial inoculants to be used in vegetable cultivation will contribute to reducing or eliminating the use of fertilizers, improving the physical, chemical, and biological properties of soil, promoting plant growth, overcoming food safety challenges, reducing environmental pollution, and increasing productivity.

In this study, it was aimed to determine the effects of 2 bacterial strains and chemical fertiliser on yield and quality parameters of Pulsar F1 eggplant cultivar, which is the most preferred eggplant variety by the producers in Iğdır province, under field conditions, which ranks first in eggplant production in Eastern Anatolia Region in addition to TÜİK (2019).

2. Materials and Methods

2.1. Bacteria and Plant Material Used in the Study

In the study, *Brevibacillus reuszeri* strain IT 119 isolated from the rhizosphere of *Atriplex nitens* and *Kluyvera cryocrescens* strain IT 160 isolated from the stem of tomato plant were used as plant growth promoting bacteria. The catalase test, nitrogen fixation, phosphate solubilisation and ACC-deaminase activity of both bacterial strains were found positive (Temel, 2023). Additionally, hypersensitivity tests were performed on these strains in tobacco and it was determined that they did not cause necrosis. Pulsar F1 eggplant variety was grown to determine the effect of bacterial strains on plant yield and quality parameters.

2.2. Field Trial

The experiment was established in 2021 in the Iğdır Merkez Özgür neighborhood according to the coincidence blocks experimental design with 3 replications. A total of 288 eggplant seedlings were planted with 12 plants in each replicate. Plotting was done as 60 cm above the row, 100 cm between the rows and 120 cm between the plots, and 140 cm between the treatments in the block. All cultural treatments were carried out regularly during the experiment. The water requirement of the plants was met by the drip water method.

Before the first planting, 1 ton of burnt barnyard manure was applied to a 500 m^2 area as base fertilizer. Before the first seedling planting, 5 kg of compound fertilizer 18-18-18 (N-P-K) from the Gübretaş company was applied by drip irrigation. White Lily Exelans NK liquid organomineral fertilizer was applied 3 weeks after the seedling planting during the development period of the plants. Only water was given to the plants in the negative control group.

Bacterial strains maintained at -80 °C were inoculated in Nutrient Agar medium and left to grow in an incubator set at 27 °C. After incubation, a loop of 48 hours fresh culture of the bacterial strains was taken and transferred to Nutrient Broth medium. Bacterial inoculum was prepared by incubating the liquid media containing bacteria overnight at 150 rpm/min at 27 °C on a shaker. After incubation, the bacterial solutions were diluted with water and the concentration of the mixture was adjusted to 10^6 cells/ml using a turbidimeter. The prepared bacterial solutions were inoculated 100 ml into the root zone of the seedling plants.

There were 8 treatments in the experiment: IT 119, IT 119 + Fertiliser, IT 160, IT 160 + Fertiliser, IT 119 + IT 160, IT 119 + IT 160 + Fertiliser, Negative Control (Water only) and Positive Control (Fertiliser only).

In the study, total yield (kg/parcel), discard yield (kg/parcel) and marketable yield (kg/parcel) were determined according to Başer (2015), plant height and stem thickness were determined according to Kanber (1997), number of leaves (number/plant), plant root dry weight, plant stem wet weight and plant stem dry weight were determined according to Yücel (2020) and root length was determined according to Özgen (2019). For fruit quality parameters, fruit length, fruit diameter and fruit weight were measured after each harvest during the growing season. The measurements were made on 6 randomly selected fruits from each replicate of each plot after harvest. Then, average fruit length, average fruit diameter and average fruit wet weight values were calculated by averaging the measured values. Fruit dry weight (%) was obtained by calculating the ratio of dry weight to wet weight as % after weighing the fresh weight of two fruits taken after harvesting during the vegetation period and then dried in an oven at 70 °C until the weight was constant.

2.3. Statistical Evaluation

The data obtained in the experiment were subjected to analysis of variance using SPSS (version 26.0) statistical package programme and the differences between treatments were determined by Duncan Multiple Comparison Test with 1% error probability.

3. Results and Discussion

3.1. Effect of Treatments on Yield Parameters of Pulsar F1 Variety

The effects of treatments on total yield, discard yield and marketable yield are presented in Table 1.

Table 1. Effect of treatments on yield parameters.

Treatments	TY (kg)	DY (kg)	MY (kg)
IT 119	59.66±0.12[a*]	3.13±0.06[d]	56.53±0.02[a]
IT 119 + G	55.83±0.07[b]	4.04±0.02[c]	51.79±0.17[b]
IT 160	59.77±0.07[a]	2.97±0.01[d]	56.80±0.23[a]
IT 160 + G	43.95±0.12[d]	4.09±0.05[c]	39.86±0.02[d]
IT 119 + IT160	39.94±0.29[f]	3.00±0.12[d]	36.94±0.06[e]
IT 119 + IT160 + G	44.74[c]±0.01[c]	3.03±0.01[d]	41.71±0.29[c]
Negative Control	31.09±0.58[g]	9.24±0.58[b]	21.84±0.12[g]
Positive Control	42.37±0.09[e]	12.54±0.06[a]	29.83±,0.58[f]
F	1890.616[**]	293.238[**]	2527.054[**]

*Values are the average of three replicates. There is no statistical difference between the values shown with the same letter in the same column. ** $p \leq 0{,}01$. **TY:** Total yield, **DY:** Discard yield, **MY:** Marketable yield, **G:** Fertiliser.

When Table 1 was examined, it was observed that the total yield of eggplant s harvested during the vegetation period varied between 31.09 and 59.77 kg/parcel. The highest total yield was obtained in IT 160 and IT 119 treatments, while the lowest yield was determined in the plants in the negative control group. It was observed that single application of bacterial strains gave better results than the application of fertiliser and mixture of each other. During the vegetation period, it was found that the total discard yield of the eggplant s harvested from the experiment varied between 2.97 and 12.54 kg/parcel. The highest discard yield was found in the positive control group with 12.54 kg/parcel. The lowest discard yield was 2.97, 3.00, 3.03 and 3.13 kg/parcel in IT 160, IT 119 + IT 160, IT 119 + IT 160 + Fertiliser and IT 119 treatments, respectively. When the effect of treatments on marketable yield was evaluated, it was determined that marketable yield varied between 21.84and 56.8 kg/parcel. The highest yield was determined as 56.80 and 56.53 kg/parcel in plants treated with IT 119 and IT 160 strains, respectively.

The effects of treatments on plant height, plant stem thickness and number of leaves are given in Table 2.

Table 2. Effect of treatments on plant height, plant stem thickness and number of leaves.

Treatments	PH (cm)	PST (mm)	NL
IT 119	98±0.58[c*]	15.70±0.06[d]	250±0.58[a]
IT 119 + G	104±2.31[ab]	22.00±0.58[a]	172±0.58[f]
IT 160	100±0.58b[c]	22.08±0.01[a]	238±0.58[c]
IT 160 + G	102±1.15[abc]	21.43±0.01[ab]	235±0.58[d]
IT 119 + IT160	105±2.31[a]	20.40±0.58[c]	245±0.58[b]
IT 119 + IT160 + G	104±1.15[ab]	21.20±0.58[b]	229±0.58[e]
Negative Control	55±0.58[d]	12.04±0.01[f]	45±0.58[h]
Positive Control	99±0.58[c]	15.05±0.01[e]	169±0.58[g]
F	152.328[**]	351.187[**]	14472.375[**]

*Values are the average of three replicates. There is no statistical difference between the values shown with the same letter in the same column. ** p≤0,01. **PH:** Plant height, **PST:** Plant stem thickness, **NL:** Number of leaves, **G:** Fertiliser.

The effects of treatments on plant height, stem thickness and number of leaves were found statistically significant (p≤0.01). As a result of the treatments, the longest plant height (105 cm) was obtained in IT 119 + IT 160 treatment, while the shortest plant height (55 cm) was measured in the negative control group. Table 2 shows that the stem thickness of eggplant plants varied between 12.04and 22.08 mm. The highest plant stem thickness was obtained in plants inoculated with IT 160 bacteria alone and IT 119 strains together with fertiliser. When the values of the number of leaves were analysed, the highest number of leaves (250) was determined in the IT 119 application and the lowest number of leaves (45) was determined in the plants in the negative control group.

The effects of the treatments on plant root wet weight, root dry wet weight, plant stem wet and stem dry weights were found statistically significant (p≤0.01) and the results are presented in Table 3.

Table 3. Effect of treatments on plant height, diameter and number of leaves.

Treatments	PRWW (gr)	PRDW (gr)	PSWW (gr)	PSWW (gr)
IT 119	97±0.58[e*]	32.8±0.12[e]	901±0.12[f]	313.3±0.58[c]
IT 119+G	233±0.58[b]	90.3±0.17[a]	1597±0.17[a]	448.1±1.15[a]
IT 160	153±0.58[c]	28.8±0.12[e]	1002±0.12[e]	346.3±0.58[bc]
IT 160+G	236±0.58[a]	88.2±0.58[b]	1103±0.58[d]	362.5±1.15[b]
IT 119 + IT160	128±0.58[d]	46.2±0.12[d]	1197±0.12[b]	362.3±1.15[b]
IT 119+IT160+G	78±0.58[f]	65.0±0.46[c]	1159±0.46[c]	359.8±1.15[b]
Negative Control	46±0.58[h]	15.3±0.12[g]	195±0.12[h]	147.4±0.58[e]
Positive Control	71±0.58[g]	24.6±0.35[f]	679±0.35[g]	250.8±1.15[d]
F	15731.786[**]	8946.604[**]	177166.640[**]	58.766[**]

*Values are the average of three replicates. There is no statistical difference between the values shown with the same letter in the same column. ** p≤0,01. **PRWW:** Plant root wet weight, **PRDW:** Plant root dry wet weight, **PSWW:** Plant stem wet weight, **PSDW:** Plant stem dry weight, **G:** Fertiliser.

When Table 3 is examined, the highest root wet weight (236 g) was determined in the plants where IT 160 strainin was applied together with fertiliser. The lowest root wet weight (46 g) was measured in the plants in the negative control group. Similarly, the highest root wet weight (233 g) was determined in the plants in which IT 119 strain was applied together with fertiliser. The highest root dry weight (90.3 g) was measured in IT 119 + fertiliser application. Again, the second highest value (88.2) was obtained in the plants where IT 160 strainin was applied together with fertiliser. Compared to the negative control, all of the treatments had a positive effect on root dry weight. It was observed that the results of plant stem wet weight varied between 195 and1597 g. It was determined that the best treatment result belonged to IT 119 + Fertiliser treatment. The highest plant stem dry weight (448.1 g) was obtained from the plants in which IT 119 strain was applied together with fertiliser. IT 160 + Fertiliser, IT 119 + IT 160 and IT 119 + IT 160 + Fertiliser treatments were in the same group and gave good results.

The effect of the treatments on the number of fruits per plant is presented in Table 4.

Table 4. Effect of treatments on number of fruits per plant and root length.

Treatments	FNP (Number)	RL (cm)
IT 119	18.0±0.57[a*]	36±0.58[b]
IT 119 + G	17.8±0.11[a]	28±0.58[d]
IT 160	18.0±0.57[a]	43±0.58[a]
IT 160 + G	18.5±0.29[a]	31±0.58[c]
IT 119 + IT160	19.3±0.11[a]	36±0.58[b]
IT 119 + IT160 + G	19.5±0.29[a]	32±0.58[c]
Negative Control	13.0±0.57[b]	20±0.33[f]
Positive Control	14.0±0.57[b]	25±0.58[e]
F	30.602**	164.753**

*Values are the average of three replicates. There is no statistical difference between the values shown with the same letter in the same column. ** p≤0,01. **FNP:** Fruit number per plant, **RL:** Root length, **G:** Fertiliser.

It was observed that the application of the strains both with each other and as a mixture with fertiliser gave the best results. The highest number of fruits per plant was obtained as 19.5 in IT 119 + IT 160 + Fertiliser treatment. It was determined that root length varied between 20 and 43 cm in the experiment. The longest root length (43 cm) was measured in IT 160 bacteria treatment. The shortest root length (20 cm) was recorded in the plants in the negative control group.

3.2. The Effect of Applications on Fruit Quality Parameters

The effects of treatments on fruit length, fruit diameter, fruit wet weight and fruit dry weight are given in Table 5.

Table 5. Effect of treatments on fruit length, fruit diameter, fruit wet weight and fruit dry weight.

Treatments	FL (cm)	FD (cm)	FWW (gr)	FDW (%)
IT 119	17.0±0.58[ab*]	2.2±0.58[n.s.]	135±0.58[c*]	19±0.58[c]
IT 119+G	15.5±1.15[abc]	2.1±0.58	141±0.58[b]	18±0.58[cd]
IT 160	16.0±0.58[ab]	2.1±0.58	143±0.58[a]	26±0.58[a]
IT 160+G	14.8±1.15[bc]	2.2±0.58	111±0.58[e]	21±0.58[b]
IT 119+IT160	18.5±0.58[a]	2.3±0.58	103±0.58[g]	21±0.58[b]
IT 119+IT160+G	18.0±1.15[a]	2.1±0.58	113±0.58[d]	17±0.58[de]
Negative Control	13.0±0.58[c]	2.2±0.58	70±0.58[h]	16±0.58[e]
Positive Control	17.0±1.15[ab]	2.2±0.58	107±0.58[f]	19±0.58[c]
F	3.863**	1.500	1762.232**	29.089**

*Values are the average of three replicates. There is no statistical difference between the values shown with the same letter in the same column. **p≤0,01. **FL:** Fruit length, **FD:** Fruit diameter, **FWW:** Fruit wet weight, **FDW:** Fruit dry weight, **G:** Fertiliser, **n.s.:** Insignificant value.

The effect of bacterial treatments on fruit length was found to be statistically significant at p≤0.01 level (Table 5). It was determined that fruit length varied between 13-18.5 cm. As a result of IT 119 + IT 160 and IT 119 + IT 160 + Fertiliser treatments, the longest length measurement was recorded as 18.5 and 18.0 cm, respectively. The shortest fruit length was determined in the plants in the negative control group. The effect of bacteria treatments on fruit diameter was found statistically insignificant. The highest fruit wet weight was 143 g in IT 160 treated plants, while the lowest fruit wet weight was 70 g in the negative control group. Fruit dry weight percentage varied between 16 and 26 in the experiment.

In this study, the effect of single and multiple inoculation of *B. reuszeri* strain IT 119 and *K. cryocrescens* strain IT 160 on the growth of Pulsar F1 eggplant variety was evaluated. Single inoculation of bacterial strains resulted in the highest values for total yield, marketable yield, number of leaves, root length, fruit wet weight and fruit dry weight, while the highest values were obtained for root wet weight, root dry weight, stem wet and dry weight when bacteria were applied together with fertilizer. The maximum value for plant height was measured in plants where two bacterial strains were applied together. It was found that single bacterial inoculation and bacteria + fertilizer application increased plant stem thickness. The longest fruit length was obtained as a result of the combination of both bacterial strains with each other and with fertilizer. When the results were evaluated in general, it was seen that bacteria treatments gave better results than fertilizer applications and bacteria increased the effectiveness of fertilizer. Similar data to the findings of this study were obtained as a result of different PGPR applications to eggplant plants. In a study conducted by Consentino et al. (2022), it was concluded that in eggplant inoculated with *Azospirillum brasilense*, total yield increased by 5% and marketable yield increased by 9% compared to the control, and the bacteria treatment significantly affected the average mass of marketable fruit. In the study conducted by Guedes et al. (2018), longer plant height was obtained in eggplant inoculated with *Pseudomonas fluorescens* compared to plots with barnyard manure. Castro et al. (2005) observed a positive correlation between bacterial application and eggplant growth and determined that bacterial applications contributed to plant growth rate and increased stem diameter. *Pseudomonas putida* SAB10 and *P. palleroniana* SAW21 inoculated eggplant plants increased plant height, stem wet weight and stem dry weight compared to the control group (Fathalla & Sbary, 2020). Saputri et al. (2023) reported that PGPR applied together with barnyard manure caused faster decomposition of organic matter in barnyard manure, which increased the growth and yield of eggplant plants. It was found that the biopreparation formulated with a cellular concentration of 10^7 CFU mL-1 of *Brevibacillus borstelensis* B65 had properties that stimulated the growth of eggplant grown in a mixture of soil and humus, and the physiological activity of bacterial strains stimulated the growth of the root system of plants. It was also found that *B. borstelensis* B65 treatment increased the mass of eggplant roots, stems and leaves, causing a highly significant increase in root and leaf weight (Vinent et al., 2014). Fu et al. (2010) revealed that high salinity in the root medium was detrimental to the growth and development of eggplant, but eggplant inoculated with *Pseudomonas* showed a positive effect on plant growth under salt stress.

The effect of the bacteria in the study on the growth of different plant groups was tested and similar successful results were obtained. *K. cryocresscens* strain RCK-113C treatment was found to maximally increase the chlorophyll content (50.02), leaf length (26.03 cm), leaf area (268.38 cm^2), flower wet and dry weight (15.54 g and 0.88 g) of hyacinth. In addition, maximum onion diameter (42.57 mm), onion length (40.01 mm) and onion weight (12.01 g) were determined. The reasons for the increases in plant growth were attributed to increased nutrient uptake, provision of plant growth hormones, improvement of chlorophyll content and organic acids by bacterial treatments (Parlakova Karagöz et al., 2019). It was reported that *K. cryocresscens* treatment was more effective on leaf width and plant height of hyacinth plant compared to control and fertilizer treatments, the harvest period was prolonged and maximum leaf length was obtained as a result of the application (Bintaş et al., 2021), while the same bacterial strain increased magnesium and potassium content in leaves and onions (Bintaş et al., 2020). Parlakova Karagöz (2020) concluded that the bacterial formulation consisting of a combination of *B. megaterium* TV-91C, *P. agglomerans* RK-92 and *K. cryocresscens* TV-113C strains can be successfully used to improve plant growth and plant aesthetics in poinsettia cultivation. It was observed that the application of nitrogen-fixing and phosphorus-solubilizing *K. cryocresscens* strains to Ceyhan 99 bread wheat variety at different salt concentrations increased both phosphorus and nitrogen amounts in the soil (Söğüt & Çığ, 2019). *K. cryocresscens* M7 strain isolated from industrial wastes was found to be resistant to the antibiotics vancomycin, ampicillin, carbenicillin and streptomycin and was also found to be a beneficial bacterium capable of removing nickel from soil (Bisht & Kumar, 2023). *Enterobacter cloacae*, *Klebsiella pneumoniae* and *K. cryocrescens* strains were found to have promising potential as seed inoculants for African Cabbage (*Cleome gynandra* L.) (Shipoh, 2021). It has been stated that *K. cryocrescens* strain may have potential for biofertilizer production required in organic agriculture due to its ability to make water-insoluble phosphates soluble and directly promote plant growth through biological N2 fixation (Parlakova Karagöz et al., 2019). *K. cryocrescens* has been found to be able to dissolve tricalcium phosphate $Ca_3(PO_4)_2$ and hydroxyapatite $[Ca_{10}(PO_4)_6(OH_2)]$ (Sharma et al., 2013; Vazquez et al., 2000) and has the capacity to produce siderophores. Therefore, it was emphasized that *K. cryocrescens* not only promotes growth, but also antagonistic to phytopathogenic microorganisms, so it can be used as a potential candidate for developing bacterial inoculants (López et al., 2019).

Brevibacillus reuszeri is a spore-forming bacterium (Sano & Anraku, 2018) with high chitinase activity (Gürkök & Görmez, 2016). Bacteria in the genus *Brevibacillus* are one of the most common gram-positive bacterial species recorded from various environmental habitats (Allan et al., 2005) and have been found to produce many bioactive metabolites (Bartel et al., 2019). Strains belonging to the genus *Brevibacillus*, which attract attention with their high growth rate, better transformation efficiency through electroporation, ability to synthesize indole-3-acetic acid and extracellular protease production, act as biocontrol agents with their ability to suppress different phytopathogenic species and are used as a source of various enzymes in biocontrol studies (Edwards & Seddon, 2001; Gupta et al., 2000). *Brevibacillus* species also play an important role in bioremediation to combat pollution caused by toxic metals and to reduce environmental pollution in agricultural soils, water and atmosphere. In different studies, *Brevibacillus* applications as PGPR were found to have a remarkable effect on root biomass (Burd et al., 2000; Grichko et al., 2000). Karlidag et al. (2009) found that bacterial applications together with fertilizer were the most effective application in increasing the growth and yield of strawberry plants. The yield and plant growth enhancing effects of bacteria on strawberry were explained by the nitrogen fixing and phosphorus solubilizing capacity of bacteria. Nehra et al. (2016) found that another *Brevibacillus* species, *B. brevis*

SVC(II)14, accelerated the growth and development of cotton plants and increased root development. The mobility of Strainin and its ability to survive at high temperatures (52 °C) showed that it is a suitable inoculant to improve cotton growth. Shi et al. (2022) found that *B. velezensis* YH20 significantly increased plant height and dry weight of *Carya illinoinensis* (Pecan) compared to the control group and had the most significant effect on growth promotion. *B. reuszeri* MPT17 strains were found to have the most significant effect in promoting plant root growth. Furthermore, compared to the control, the levels of available phosphorus and potassium in rhizosphere soils and the total potassium content in plant roots were improved as a result of *B. reuszeri* MPT17 treatment. *B. reuszeri* strain MPT17 was also found to promote the growth of *Pinus massoniana* in another study (Li et al., 2015).

It has been stated that nitrogen, phosphorus and potassium are the main essential nutrients of plants and their amounts in soil directly affect plant growth and development (Emmanuel & Babalola, 2020; Saxena et al., 2020). A significant positive correlation was found between soil nutrient content and biomass with PGPR applications (Song et al., 2021). Shi et al. (2022) found that there is a correlation between soil nutrient content and seedling biomass, phosphorus content in soil affects plant biomass accumulation, and the significant increase in biomass after inoculation with PGPR may be related to changes in soil nutrient levels. Many studies have shown that PGPRs, especially *Bacillus* species, become the dominant member of the microbial community in the inoculated medium, secrete bacteriostatic substances, aid plant nutrient uptake and promote plant growth by regulating endogenous hormones (Poveda & Gonzalez-Andres, 2021). The effects of PGPR applications on plants are the result of synergistic/antagonistic interactions between inoculated strains, i.e., induction or suppression of native microbiota and indigenous microbial populations (Trabelsi & Mhamdi, 2013).

4. Conclusion

As a result of both agricultural practices involving the intensive use of chemical fertilisers and the growing population and industrialisation, the world's atmospheric, terrestrial and aquatic systems are no longer sufficient to absorb and break down the increasing amount of waste produced. As a result, the environment is becoming increasingly contaminated with various toxic metals and compounds. Remediation of contaminated soils and waters is therefore essential. One way to address this problem is the use of plant growth-promoting bacteria as part of agricultural practices in protocols to improve plant and soil health. In both managed and natural ecosystems, the beneficial bacteria-plant relationship plays a key role in supporting plant health and growth and increasing yields. Plant growth-promoting bacteria are bacteria that can enhance plant growth and protect plants from diseases and abiotic stresses through a wide variety of mechanisms. Some important properties such as biological nitrogen fixation, phosphate and potassium solubilisation, ACC deaminase activity can be considered as the characteristics of plant growth promoting bacteria. The bacterial strains in this study were previously determined to fix nitrogen, solubilise phosphorus and possess ACC deaminase enzyme. The use of these bacterial strains in maintaining soil fertility and promoting plant growth is promising for the conservation of agricultural resources.

Bacterial strains isolated from plants resistant to extreme conditions in Iğdır province were used for the first time in this study in eggplant cultivation, which is important for the province. It was determined that single or mixed bacterial applications increased the yield and quality parameters of eggplant compared to fertiliser application. However, it was observed that *K. cyrocrescens* strain IT 160 was the most effective stimulant bacterium in increasing eggplant yield and increased the yield by 41%. The application of *K. cyrocrescens* strain IT 160 as a bioinoculant in eggplant cultivation will reduce the use of chemical fertilisers, reduce production costs and environmental pollution and contribute to the increase of agronomic efficiency.

Acknowledgment

We would like to thank Iğdır University Scientific Research Projects for their contribution to the support of the project numbered ZİF0322Y03. This study is derived from the Master's thesis titled "The Effect of Bacterial Strains (PGPB) Stimulating Plant Growth on Yield and Some Quality Parameters of Eggplant Plant (*Solanum melongena* L.)".

Conflict of Interest

The authors declare that they have no conflict of interest.

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http://agriprojournal.com
JOURNAL OF AGRICULTURAL PRODUCTION
ISSN: 2757-6620
PRENSIP
https://prensip.gen.tr

RESEARCH ARTICLE

Effects of Various Fat Sources Added into the Diets of Laying Hens on Calcium and Phosphorus Metabolism

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ARTICLE INFO

Article History
Received: 17.04.2024
Accepted: 30.06.2024
First Published: 30.09.2024

Keywords
Animal fat
Laying hen
Mineral bioavailability
Serum calcium-phosphorus level
Vegetable oils

ABSTRACT

This research was conducted to determine the effects of different vegetable oils and animal fat added into laying hens' rations at different levels during the late laying period on serum calcium and phosphorus levels. A total of 54 sixty-seven weeks old Isa Brown hens were weighed and randomly distributed into nine dietary treatment groups as C (control fed with only basal diet), T2 (basal diet + 2% tallow), M2 [basal diet + 2% tallow and linseed oil mixture (50/50)], S2 (basal diet + 2% sunflower oil), L2 (basal diet + 2% linseed oil), T4 (basal diet + 4% tallow), M4 [basal diet + 4% tallow and linseed oil mixture (50/50)], S4 (basal diet + 4% sunflower oil) and L4 (basal diet + 4% linseed oil), respectively. Each treatment group consisted of 6 subgroups, comprising of 1 bird each. At the end of the study, serum calcium and phosphorus levels (mg/dl) of C T2, M2, S2, L2, T4, M4, S4, L4 groups were 18.63 and 5.85, 18.03 and 4.52, 18.60 and 5.00, 16.62 and 4.42, 17.55 and 4.33, 16.25 and 5.63, 17.48 and 3.95, 17.47 and 3.28, and 21.43 and 5.12, respectively. The highest calcium level was observed in L4 group (21.43 mg/dl), while the highest phosphorus level (5.85 mg/dl) was detected in the control (C) group. As a result of the research, no significant effect ($p>0.05$) was found in terms of different fat sources and levels among the groups during the late laying period on the serum calcium and phosphorus levels of laying hens.

Please cite this paper as follows:

Çelebi, Ş., & Karaoğlu, M. (2024). Effects of various fat sources added into the diets of laying hens on calcium and phosphorus metabolism. *Journal of Agricultural Production*, *5*(3), 163-169. https://doi.org/10.56430/japro.1469620

1. Introduction

The poultry sector is an important branch of animal husbandry in Türkiye. According to the latest data of TÜİK (2021), 35% of poultry poulation consists of laying hens and approximately 20 billion (1.2 million tons) eggs are produced annually. However, the inadequacy of animal feed resources in terms of both quantity and quality is often the biggest obstacle to the development of this sector (Ergün & Bayram, 2021; Kaya et al., 2019). To maintain their productivity healthly and to produce quality products without loss, the nutrients needed by laying hens must be provided in sufficient quantities and in usable form with diets.

Ca and P are the main structural elements of the skeletal system in animals and are indispensable for the normal course of vital functions. Due to the relationship between them, Ca and P are handled together in animal nutrition and the ratio is desired to be 2:1 or 3:1 for optimum utilization. In addition to being structural elements, Ca and P, which exist as ions and other compounds, play a role in many physiological processes (Matuszewski et al., 2020). Ca, the main component of bone tissue and egg shell production, is essential for poultry due to

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its role in acid-base balance and enzymatic system. It affects eggshell quality, bone mineralization and growth (Li et al., 2020). Many researches have shown that the shell of each egg contains 2.2 g of calcium. Deficiency in Ca causes deformation in the skeletal system, tibial dyschondriplasia, rickets, nerve damage and a decrease in egg production and its shell quality (Xing et al., 2020). Also, P is an important element in living organisms beceouse of metabolic functions. It plays a big role in phosphorylation reactions in biological systems such as synthesis of some energetic compounds like ATP, ADP, phosphocreatine and similar metabolites in addition to formation of bone and egg shell, acid-base balance, carbohydrate, lipid and protein metabolism (Çelebi et al., 2005).

Ca and P concentrations in the body and their status in stores have a dynamic structure and are subject to regulation. Due to this regulation through hormonal control, bone mineral content constantly changes. This Ca-P exchange between tissues and stores is particularly important in high-producing animals such as dairy cows and laying hens, where the need for these minerals is several times higher than the other animals (Matuszewski et al., 2020).

Egg shell quality problems in laying hens are important and increase with age. In many feeding studies conducted to improve shell quality, it has been reported that the factors affecting bioavailability are as important as the amount and sources of calcium and phosphorus (Bintaş & Özdoğan, 2017).

Oils are routinely added to mixed feeds in today's feed industry because they are a cheap energy source, prevent dust formation, reduce heat stress and have many other important benefits (Çelebi & Macit, 2008; Kurt & Küçük, 2010).

Fats used to increase efficiency in poultry rations provide a significant portion of dietary energy, as well as being a source of other nutrients such as vitamins. However, there are many research evidences that fats or oils have positive or negative effects on the digestion and absorption of inorganic substances in animal nutrition (Kurt & Küçük, 2010). Studies have shown that oils rich in unsaturated fatty acids are better digested and absorbed by poultry than saturated fats (Atteh & Leeson, 1984; Leeson & Atteh, 1995). Since oils are water-insoluble compounds, an emulsion step is necessary for their absorption. Because of phospholipid group of oils has surface-active properties, they are important in the emulsification of lipids and can affect the absorption of nutrients in the small intestine (Huang et al., 2007). Indeed, previous studies on poultry (Atteh & Leeson, 1984) have shown that soap formation occurs from dietary fatty acids with minerals during the digestion process, and that the level of digestion and absorption of soap significantly affects the utilization of the relevant fatty acids. Atteh and Leeson (1985) also observed that increasing the calcium content of the diet aggravated the soap formation problem.

In the presence of fats, the absorption of calcium from the digestive system is partially inhibited. Because Ca^{++} ions and fatty acids form insoluble soaps, which negatively affects the utilization of both calcium and fats. If fat is added to poultry rations, it is necessary to increase dietary calcium to improve feed conversion rate (Tabeidian et al., 2010; Utlu & Çelebi, 2004).

Another important factor affecting digestion is whether fats are saturated or unsaturated. There are studies showing that unsaturated fats are better used than saturated fats in animal metabolism and have higher ME (Çelebi & Macit, 2008).

The main factors affecting the digestion and absorption of fats in chickens are the fatty acid composition of the dietary fat, the mineral content of the diet, especially calcium, and the type, breed and age of the poultry (Atteh & Leeson, 1985; Baucells et al., 2000). Examining the effects of age on fat utilization in poultry, Duckworth et al. (1950) found that the ability of chicks to digest tallow increased with age up to 4 weeks. In another study, Renner and Hill (1960) observed that tallow could be used in 8-week-old chickens as much as in adult chickens. Similarly, Fedde et al. (1960) determined that 1-week-old chicks could digest beef tallow at a rate of 53%, and this rate increased to 80% when they were 12 weeks old. On the other hand, Whitehead and Fisher (1975), who investigated the effect of fat type and animal age on the evaluation of fats, observed that the digestibility of tallow was 57% in 2-weeks-old chicks and 74% in 8-weeks-old chickens, while they reported that there was no difference in the use of corn oil and lard in old hens.

Additionally, apart from the studies conducted with vegetable oils and animal fats, many recent studies showed that the addition of essential oils to poultry diets in even very small amounts increases serum Ca and P levels by reducing the pH of the digestive system increasing the intestinal surface area and the amount of endogenous enzymes (Sevim et al., 2020).

In present study, it was aimed to determine whether the inclusion of various vegetable and animal fat sources with different degrees of saturation in the diet at different levels affects serum calcium and phosphorus levels, in order to provide a solution to the egg shell problems that increase with age in laying hens.

2. Materials and Methods

The data of this study was obtained from the laying hens reared at the Research and Application Farm of the Agricultural Faculty, Atatürk University. Isa Brown layers (n=54, 67 weeks of age) were weighed and randomly assigned to nine dietary treatment groups and reared in individually cages (50 cm × 46 cm × 46 cm; length × width × height). Each treatment group was replicated six times as subgroups, comprising of 1 bird each. The layers at the late laying period were fed once daily with an isonitrogenous but not isocaloric commercial layer feed for 8 weeks. As shown in Table 1, control group was fed with basal diet (C) containing about 16 % CP, 2650 kcal ME/kg and 3.77% Ca and 0.29% available P. Feeds and water were offered *ad-libitum* and all hens were exposed to 16 h of light day^{-1}.

The experimental diets were made by adding the sunflower, linseed and tallow fats and their mixture (w/w) to the basal diet at 2% and 4% levels. Control group was fed with only basal diet, the experimental groups were fed with basal diet + 2% tallow (T2), basal diet + 2% mixture of tallow and linseed oil (w/w) (M2); basal diet + 2% sunflower oil (S2), basal diet + 2% linseed oil (L2), basal diet + 4% tallow (T4), basal diet + 4% mixture of tallow and linseed oil (w/w) (M4), basal diet + 4% sunflower oil (S4), and, basal diet + 4% linseed oil (L4). Table 1 shows the ingredients and chemical composition of experimental diets. The basal diet was formulated according to the recommendations of the National Research Council (NRC, 1994; Table 1). At the end of the eight weeks feeding period, blood samples for determination of plasma Ca and P values were obtained from the wing veins of six hens per treatment, the plasma was separated by centrifugation blood for 10 min. at 2000 x g and stored at -20 °C for further measurements. The Ca and P were analyzed on auto analyzer by using commercial kits.

Table 1. Ingredients and chemical composition of basal and experimental diets fed to laying hens.

	Experimental groups[1]								
Ingredients (%)	**C**	**T2**	**M2**	**S2**	**L2**	**T4**	**M4**	**S4**	**L4**
Corn	38.30	37.30	37.30	37.30	37.30	36.30	36.30	36.30	36.30
Wheat	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Barley	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Soybean meal	12.00	13.00	13.00	13.00	13.00	14.00	14.00	14.00	14.00
Full Fat soybean	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Sunflower meal	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Meat-bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Tallow	-	2.0	-	-	-	4.0	-	-	-
Tallow and linseed oil (w/w)	-	-	2.0	-	-	-	4.0	-	-
Sunflower oil	-	-	-	2.0	-	-	-	4.0	-
Linseed oil	-	-	-	-	2.0	-	-	-	4.0
Marble meal	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lysine-HCl	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Chemical Analysis									
ME (kcal kg^{-1})[3]	2650	2800	2800	2800	2800	2950	2950	2950	2950
Crude protein (%)	16.0	16.07	16.07	16.07	16.07	16.10	16.10	16.10	16.10
Crude fiber (%)	4.63	4.68	4.68	4.68	4.68	4.70	4.70	4.70	4.70
Crude ash (%)	12.40	12.40	12.40	12.40	12.40	12.40	12.40	12.40	12.40
Ether extract (%)	2.70	4.65	4.65	4.65	4.65	6.60	6.60	6.60	6.60
Ca (%)	3.77	3.77	3.77	3.77	3.77	3.77	3.77	3.77	3.77
P (%)	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29

[1]C: Control, basal diet; T2: basal diet + 2% tallow; M2: basal diet + 2% mixture of tallow and linseed oil (w/w); S2: basal diet + 2% sunflower oil; L2, basal diet + 2% linseed oil, T4: basal diet + 4% tallow; M4: basal diet + 4% mixture of tallow and linseed oil (w/w); S4: basal diet + 4% sunflower oil; L4, basal diet + 4% linseed oil.
[2]Each 2.5 kg vitamin premix contains: Vitamin A, 8.000.000 IU; vitamin D3, 2.000.000 IU; vitamin E, 20 mg; K3, 3000 mg; B1, 1500 mg; B2, 4000 mg; B12, 500 mg; nicotinamide, 6000 mg; Ca D-pantothenate, 6000 mg; B6, 2500 mg; choline-Cl, 200 000 mg; biotin, 1000 mg.
[3]Calculated values.

2.1. Statistical Analysis

All data were analyzed with SPSS (1999) statistical software package for Windows 10.0 version by using one-way analysis of variance (ANOVA).

3. Results and Discussion

The results related to the effects of the inclusion of different fat sources at different levels to diets of laying hens on serum Ca and P levels are presented in Table 2. All literature reports regarding serum Ca and P levels presented here are expressed as mg/dl.

Table 2. The effect of different fat sources in diets on serum parameters of laying hens.

Treatment Groups	Parameters	n	Serum (mg/dl) Ca $\bar{X} \pm S_{\bar{x}}$	P $\bar{X} \pm S_{\bar{x}}$
C	Control (only basal diet)	6	18.63 ± 025	5.85 ± 0.54
T2	BD + 2% tallow	6	18.03 ± 0.46	4.52 ± 0.54
M2	BD + 2% mixture (1% T + 1% L)	6	18.60 ± 0.42	5.00 ± 0.77
S2	BD + 2% sunflower	6	16.62 ± 0.89	4.42 ± 0.57
L2	BD + 2% linseed	6	17.55 ± 0.33	4.33 ± 0.38
T4	BD + 4% tallow	6	16.25 ± 1.11	5.63 ± 1.10
M4	BD + 4% mixture (2% T + 2% L)	6	17.48 ± 0.91	3.95 ± 0.79
S4	BD + 4% sunflower	6	17.47 ± 1.50	3.28 ± 0.62
L4	BD + 4% linseed	6	21.43 ± 2.35	5.12 ± 0.73
Significance			*ns*	*ns*

C: Control, fed with only basal diet, T2: Basal diet + 2% tallow, M2: Basal diet + 2% tallow and linseed oil mixture (50/50), S2: Basal diet + 2% sunflower oil, L2: Basal diet + 2% linseed oil, T4: Basal diet + 4% tallow, M4: Basal diet + 4% tallow and linseed oil mixture (50/50), S4: Basal diet + 4% sunflower oil, and L4: Basal diet + 4% linseed oil, BD: Basal diet, ns: non significant, ($p>0.05$).

The inclusion of various vegetable oil and animal fat sources, having different degrees of saturation or unsaturation, to the diets of aged hens during the late laying period did not affect investigated serum parameters. There were no significant ($p>0.05$) differences among the groups in terms of serum Ca and P levels.

In contrast to the current study, Bölükbaşı et al. (2005) investigated the effects of dietary Ca and vitamin D_3 supplementation on plasma Ca and P levels of late-laying hens and determined that plasma Ca (9.7-17.07) and P values (3.35-4.95) were affected ($p<0.01$) by dietary treatments.

Smilarly, Utlu and Çelebi (2004) stated that adding sunflower oil at different levels (0, 2, 4 and 6%) to the young goose (goslings) diets did not have significant effect on serum Ca (10.5-11.7) and P (8.9-10.5) levels. The findings of the research were compatible with the results of present study.

As shown in Table 2, the group fed with diet including 4% linseed oil showed the highest serum Ca level (21.43). On the other hand, the highest serum P level (5.85) was found in the control group. It was observed that by increasing the amount of saturated fat in the diet, serum Ca levels decreased (T2: 18.02 vs. T4: 16.25), and on the contrary, with the addition of highly unsaturated vegetable oils to diet serum Ca levels (S4: 17.47 vs S2: 16.62 and L4: 21.43 vs L2: 17.55) increased. Although not in all groups, serum phosphorus levels increased with increased intake of saturated tallow and unsaturated vegetable oils compared to some groups fed with diets including their lower levels.

In studies conducted out on essential oils, it has been reported that the biological use of calcium increases (Mountzouris et al., 2011) and its fecal excretion decreases (Olgun & Yıldız, 2014) with the addition of essential oil to the diet. In addition, there are also studies reporting that essential oils do not affect plasma minerals (Ali et al., 2007) or reduce their levels (Capkovicova et al., 2014).

Sevim et al. (2020) reported that the highest serum calcium concentration was obtained in the groups fed diets supplemented with orange peel oil (OPO) at 100, 200, 300 and 400 mg/kg, in Japanese quail. The difference between these groups and the groups fed with diet supplemented with OPO at 0 (control) and 50 mg/kg levels was found to be significant ($p<0.01$). Serum phosphorus concentration increased with the addition of OPO to the diet.

Although Bintaş and Özdoğan (2017) found that the addition of boron and zeolite to the diet did not affect serum calcium and phosphorus in old laying hens, Kaya et al. (2019) found that rosa canina seed, which they added to the rations of old laying hens at different levels, significantly increased the blood Ca level (19.08-19.83) compared to the control (12.11) group. Serum Ca and P levels of this study were similar to findings (Ca: 17-25, P: 2.79-5.27) of Kaya et al. (2019) and Nie et al. (2018).

Eren et al. (2003) researched the effect of adding different levels of boron to laying hen diets on serum Ca and P levels at different weeks and reported that increases in serum Ca levels (17.98-25.36) (except for P: 6.75-12.61) were not affected by the treatment, even if it caused changes.

Usayran et al. (2001) investigated the effects of inclusion 4% fat and 4 different levels of non-phytate phosphorus (NPP) to the diet on egg quality in laying hens exposed to high environmental temperatures (35±1 °C) and found that continuous exposure to high environmental temperature reduced serum Ca levels (15.66 vs 17.0) and P (6.91 vs 7.01) along with performance characteristics, but by adding fat improved serum P level (7.01 vs 5.66). Oil supplementation significantly improved P levels, but had no effect on Ca in serum. The trial results showed that there was no significant interaction amongst temperature, NPP supplementation and dietary fat addition for any of the measured criteria.

Abdel-Wareth and Lohakare (2014) examined the addition of the peppermint leaves into the diet at different levels of laying hens during the late laying period and did not observe any significant difference in serum calcium (13.14) and phosphorus (7.16-7.35) amongst treatments and the values remained within the normal range. Also, Atteh and Leeson (1985) investigated the response of laying hens to dietary saturated and unsaturated fatty acids in the presence of varying dietary calcium levels and found that there were no effects on bone Ca and P retentions.

In another study, serum Ca and P levels of hens during the late laying period by supplementing the diet with vitamin D (3000 IU/kg feed) and different levels of P was examined by Çelebi et al. (2005), the highest serum Ca (10.05) level was found in the vitamin D supplemented group; the lowest Ca level (7.90) was observed in the vitamin D-free, P-supplemented (0.45% of feed dry matter) group, which had the highest serum P content (8.42). Likewise, the lowest serum P concentration (4.60) was found in the control and only vitamin D supplemented group (Çelebi et al., 2005). These means related to Ca and P were lower the current research results supplemented with different oils and ratios.

The incompatibility of the results of current research with the findings of other studies may be due to the negative effect of saponification, which occurs as a result of the interaction of oils with different properties and minerals added to rations of animal of different species, breeds and ages on the digestion and absorption of Ca and P. Also, the addition of saturated or unsaturated fats affects the absorption of any of these minerals positively or negatively, it may cause an imbalance by disrupting the ideal ratio between Ca and P resulting in a decrease in bioavailability.

4. Conclusion

Serum Ca and P levels were not affected by the addition of different vegetable oil and animal fat sources at different levels to laying hen diets during the late laying period. On the other hand, the use of high-saturation fats such as tallow in increasing amounts in the diet resulted in a decrease in serum Ca and P levels. However, as the amount of highly unsaturated vegetable oils in the diet increased serum Ca levels also increased, as observed in the group fed with diet containing linseed oil.

Compliance with Ethical Standards

The study was carried out at the Research and Application Farm of the Agricultural Faculty, Atatürk University, before 2006.

Conflict of Interest

The authors declare that they have no conflict of interest.

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http://agriprojournal.com
JOURNAL OF AGRICULTURAL PRODUCTION
ISSN: 2757-6620
PRENSIP
https://prensip.gen.tr

RESEARCH ARTICLE

Analysis of Microbiological and Certain Qualitative Properties of Pastırma Marketed in Kastamonu

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ARTICLE INFO

Article History
Received: 04.07.2024
Accepted: 05.08.2024
First Published: 30.09.2024

Keywords
Kastamonu
Microbiology
Pastırma
Residual nitrite
Salt

ABSTRACT

This research implicates the physical, chemical and microbiological analysis of pastırma samples taken from eight different companies at three separate times of the year marketed in Kastamonu. Microbiological properties, the amount of free fatty acids, salt and residual nitrite were analyzed on the samples; pH and moisture properties tests were carried out. It is stated that the number of *Micrococcus/Staphylococcus* and lactic acid bacteria formed the dominant flora and the number of lactic acid bacteria and *Micrococcus/Staphylococcus* were 6.07-7.93 and 6.52-7.22 log cfu/g respectively. The number of yeast-mold and total aerobic mesophilic bacteria were detected as 4.09-6.51 and 6.81-7.76 log cfu/g respectively. Although it has been observed that the number of Enterobacteriaceae, which is an important criterion for quality in pastırma, is below 10^2 cfu/g in general, some of the samples obtained from companies C, D, F, G, K showed values above <2 log cfu/g. In the samples, pH values were determined as 5.67-6.13 on average, moisture values were determined as 47.04-52.12% on average and salt was determined as 2.98 to 8.78% by mass. The average free fatty acid and residual nitrite values were found to be 0.031-0.118% and 0.159-10.241 ppm respectively.

Please cite this paper as follows:

Hazar Suncak, F. Y., & Gider, T. (2024). Analysis of microbiological and certain qualitative properties of pastırma marketed in Kastamonu. *Journal of Agricultural Production*, *5*(3), 170-175. https://doi.org/10.56430/japro.1509921

1. Introduction

Pastırma is a traditional meat product which the meat is obtained from certain parts of beef and then cured, dried, and covered with pasta called "çemen" (Hazar et al., 2017; Kaban, 2013; Öz & Kaya, 2019). The components used in the curing process are among the important factors that affect the quality of pastırma. Salt, nitrate and/or nitrite are used in the curing process. In addition to these ingredients, spices can also be used to enhance the flavor, smell, taste, appearance, and texture of the product during the curing process (Aksu et al., 2016; Kaban, 2013). The use of salt alone in pastırma production results in a firm texture and dark color (Hazar et al., 2017; Tekinşen & Doğruer, 2000).

Nitrite is used in meat products to prevent the formation of harmful bacteria that can cause food poisoning, inhibit the development of bacteria and their toxin production, as well as to promote color formation and prevent oxidation due to its antioxidant properties (Akköse et al., 2017; Honikel, 2008). However, nitrite also plays an important role in the formation of carcinogenic nitrosamines, which are formed as a result of the reaction between nitrogen oxide generated by the reduction of nitrite and amino groups (Sallan et al., 2019). The formation of these compounds is a complex process and depends on the

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level of nitrite used during the curing stage, production steps, microorganisms with decarboxylase activity, residual nitrite content, water activity and pH value (Sallan et al., 2020).

Salt ratio used in the curing stage of pastırma varies between 8-10% (Kaban, 2013). It plays a crucial role in reducing water activity, ensuring microbiological stability, dissolving muscle proteins that are important for texture development, and enhancing the salty taste in the final product (Hastaoğlu & Vural, 2018). On the other hand, salt has numerous negative effects on human health (Hastaoğlu & Vural, 2018). The World Health Organization (WHO) emphasizes that adults should consume less than 2 g of sodium per day (5 g of salt) (WHO, 2023).

Another important step in the production of pastırma is the covering process of meat with paste, known as "çemen". The main purpose of covering with pasta is to provide flavor, taste and texture to the product, as well as to enhance its color (Nizamlıoğlu et al., 1998). In the production of çemen, fenugreek seed flour (*Trigonella foenum graecum*), fresh garlic, red pepper and water are used (Kaban, 2013). Garlic present in çemen helps prevent the growth of various microorganisms and molds (Gökalp et al., 2012; Tekinşen & Doğruer, 2000). Kastamonu Pastırma is produced under natural conditions using traditional methods based on knowledge and skills of the craftsmen. What sets it apart from other pastırma varieties is the use of Taşköprü (Kastamonu) garlic in çemen production (Türker et al., 2019). Taşköprü garlic from Kastamonu is known for being rich in mineral substances (especially selenium) and vitamins. It is also characterized by its large size, resistance to climatic conditions and long shelf life (Doğantürk, 2016).

Within the scope of this study, samples of pastırma were collected from 8 different companies involved in the production and sale of pastırma in Kastamonu at 3 different time points. These samples were analyzed for moisture, pH, free fatty acids, residual nitrite, salt, and microbiological parameters. It is stated that the pH value of pastırma should be between 5.5 and 6 (Kaban, 2013). Additionally, according to the Turkish Food Codex Meat and Meat Products Regulation, the moisture content of pastırma should be below 50% (Tarım ve Orman Bakanlığı, 2019). Whether Kastamonu pastırma meets these criteria or not has been determined through scientific data. Furthermore, determining the microbiological characteristics of a food product is crucial for ensuring its quality and safety. This study aimed to reveal the physical, chemical and microbiological properties of pastırma in the Kastamonu market.

2. Materials and Methods

In the study, samples of pastırma (sırt type) were collected from eight different companies involved in the production and sale of pastırma in the Kastamonu market at three different time points. The collected pastırma samples were tested for pH, moisture content, salt, free fatty acid and residual nitrite levels, as well as microbiological parameters including *Micrococcus/Staphylococcus*, lactic acid bacteria, Enterobacteriaceae, total aerobic and mesophilic bacteria and yeast-mold.

2.1. Analysis of pH

In order to determine the pH value, 10 g of pastırma sample was weighed and 100 ml of distilled water was added on each sample. The samples were homogenized for 1 minute using an Ultra-Turrax (ISOLAB, I.622.01.001, Germany). Before the pH value of the homogenized samples was measured using a pH meter (WTW, Germany), it was calibrated using suitable buffer solutions (pH 7.00 and pH 4.00).

2.2. Moisture Analysis

For the purpose of determining the moisture content, 10 grams of pastırma sample was weighed into nickel containers. The samples were then dried in a drying cabinet (Megaterm, E420P) at 100-102 ºC until a constant weight was reached (18-24 hours). The amount of moisture of pastırma was determined based on the initial and final weights of the samples.

2.3. Residual Nitrite and Salt Analysis

In order to specify the residual nitrite and salt content, 10 grams of samples were mixed with 10 ml of saturated borax solution. The mixture was homogenized using an Ultra-Turrax device (ISOLAB, I.622.01.001, Germany). The homogenate was then incubated in a boiling water bath (Nüve, ST30) at 100 °C for 15 minutes. After incubation, Carrez I and Carrez II solutions were put in the mixture and mixed together. The sample solution was transferred to a 200 ml measuring flask in order to get the appropriate volume. The measuring flask was stirred and kept waiting for 30 minutes at room temperature. The sample solution was then filtered twice using a filter paper (Whatman 595, diameter 150 mm, nitrate/nitrite-free). In order to determine the residual nitrite content, 10 ml of the filtrate was mixed with 10 ml of Griess reagent and incubated at room temperature in a lightless conditions for 30 minutes. After the 30-minute incubation period, the absorbance was measured at 540 nm with use of a spectrophotometer (PhotoLab7600 WTW, Germany) that was calibrated to the appropriate wavelength. The residual nitrite content was determined by taking dilution factor, sample volume and standard curve into account (Tauchmann, 1987). For salt analysis, 20 ml of the filtrate obtained from the residual nitrite analysis was taken and 10% potassium chromate solution was added on. Then, it was titrated using 0.1 N $AgNO_3$ solution in order to calculate the salt content.

2.4. Free Fatty Acid

The sample of 17.5 g pastırma was taken for determining the amount of free fatty acids and 0.875 g of Na_2SO_4 and 35 ml of chloroform were added on it. The mixture was then stirred for 5 minutes and filtered through Whatman No. 4 filter paper. After filtration, 25 ml of the filtrate was taken and titrated with 0.01 N potassium hydroxide-ethanol solution in the presence of phenolphthalein indicator. The amount of potassium hydroxide-ethanol solution used in the titration was benefited for result calculation and the content of free fatty acids was given as grams of oleic acid per 100 g of fat (Wang, 2001).

2.5. Microbiological Analysis

MRS (De Man, Rogosa Sharpe, Merck) Agar medium was used for lactic acid bacteria enumeration in pastırma samples. Prepared from appropriate dilutions, spread method was applied on MRS Agar plates and after 2 days of anaerobic incubation at 30°C, the level of lactic acid bacteria was specified by considering catalase (-) colonies.

In order to determine the total number of aerobic mesophilic bacteria, PCA (plate count agar) plates, which were prepared previously from appropriate dilutions, were inoculated using spread method. After 2 days of incubation at 30 °C, the total number of aerobic mesophilic bacteria was determined.

RBC (Rose Bengal Chloramphenicol) Agar medium was used for yeast-mold enumeration. Petri plates were waited for 5 days at 25 °C after inoculation from appropriate dilutions using spreading method. Yeast and mold colonies developed as a result of incubation were counted and the level of yeast-molds in the samples was determined.

The number of *Micrococcus/Staphylococcus* was determined using previously prepared and sterilized MSA (Mannitol Salt Agar Oxoid) medium. The inoculated plates were incubated aerobically for 2 days at 30 °C and the level of *Micrococcus/Staphylococcus* was calculated regarding the catalase (+) cocci.

Enterobacteriaceae number in the samples was detected by spreading 0.1 mL of proper dilutions on VRBD (Violet Red Bile Dextrose) Agar plates. These plates were incubated for 2 days at 30 °C anaerobically. As a result of incubation, the level of Enterobacteriaceae was determined by counting red colonies larger than 1 mm.

2.6. Statistical Analysis

In the study, pastırma from different companies were purchased at different times and analyzed. The trial was set up and carried out with 3 replications according to the randomized complete blocks trial plan. The results were applied to analysis of variance. The average of main sources of variation found to be statistically important were compared with the Duncan multiple comparison test (SPSS 20.0).

3. Results and Discussion

3.1. pH

The pH values of pastırma samples taken from different companies at three different times are given in Table 1. It was determined that the highest pH value belonged to company E and the lowest to company C. It is thought that the reason why the pH values of the companies differ from each other is due to the differences in the production process of each company (drying time, the amount of salt used in curing, etc.) and the difference in raw materials. According to the Turkish Food Codex Communiqué on Meat, Prepared Meat Mixtures and Meat Products, it is stated that the pH value in pastırma can be at most 6.0 (Tarım ve Orman Bakanlığı, 2019). Among the pH values determined in the study, the value belonging to 4 companies was found above this upper limit.

3.2. Moisture

When the average moisture values of different companies are examined, it is observed that the moisture value is highest in companies A, C and D; however, the lowest in companies B and G. According to the Turkish Food Codex Communiqué on Meat, Prepared Meat Mixtures and Meat Products, the amount of moisture in pastırma excluding fenugreek, can be up to 50% by mass (Tarım ve Orman Bakanlığı, 2019). It is thought that the moisture value varies depending on the fact that some companies do not sufficiently dry pastırma. What is more, the thickness of pastırma and their drying times may differ. Stability of meat products categorized in medium moisture food class such as pastırma can be achieved by reducing the amount of usable free water in its structures during the drying and curing stages. Otherwise, both the microbiological quality decreases and an undesirable product is obtained in terms of legislation (Işıksal et al., 2009). Aksu and Kaya (2001) determined the moisture content to be in the range of 38.92-51.81% in their study on pastırma samples obtained from Erzurum market.

3.3. Residual Nitrite and Salt

Nitrite/nitrate prevents both the growth of microorganisms and pathogens that cause spoilage and delays the bitterness caused by the deterioration of fats, furthermore it forms the meat color and flavor of cured meat (Hui, 2012). However, in order for these effects to occur, nitrate must be reduced to nitrite in processes while using nitrate. Microorganisms with nitrate reductase activity have positive effect in reducing nitrate to nitrite (Akköse et al., 2017). The nitrite concentration in raw nitrate-cured meat products is quite low. Therefore, NO+ formation is very unlikely. However, nitrosamines may form in the products heated above 130 °C (Honikel, 2008). Due to the carcinogenic properties of nitrosamines, which are formed as a result of the reaction of nitrite with secondary amines in meat products, their use was restricted in the United States in the

1960s (Toldrá et al., 2009). Therefore, the level of use in food is limited by some countries (Aksu et al., 2005). According to the Turkish Food Codex Additives Regulation, the highest nitrite usage amount in non-heat-treated meat products is given as 150 ppm (Tarım ve Orman Bakanlığı, 2019). The mean residual nitrite amount was detected between 0.159-10.241 mg/kg (Table 1).

The use of salt in the production of cured meat products affects the chemical and microbiological properties of the product. It limits the growth of some pathogenic and spoilage microorganisms (Benetini et al., 2012). Salt values of pastırma samples are given in Table 1. It has been observed that the highest salt value belongs to companies B and F; however, the lowest average value belongs to company C. According to the Turkish Food Codex Communiqué on Meat, Prepared Meat Mixtures and Meat Products, the highest salt value in pastırma can be 10% (in dry matter) at most (Tarım ve Orman Bakanlığı, 2019). Among the salt values determined in the study, the values belonging to some companies were found above this upper limit. In another study on pastırma, the amount of salt was found to be in the range of 2.74-9.36% (Aksu & Kaya, 2001; Doğruer et al., 1995). In a study conducted by İhtiyar (2019) on Kastamonu pastırma, the lowest amount of salt was found to be 3.31% in Sırt Pastırma and 7.74% in average Kuşgömü Pastırma.

3.4. Free Fatty Acid Value

It is stated that free fatty acids are important factors in flavor formation, physicochemical properties of fatty tissue and nutritional value of dry-cured ham, depending on their composition (Liu et al., 2019). Free fatty acid values in pastırma were found to be between 0.021 g oleic acid/100g and 0.242 g oleic acid/100g, depicted in Table 1. Similar to this study, the amount of free fatty acid in pastırma treated with different salt formulations was found to be 0.095-0.122 g oleic acid/100 g (Yalınkılıç et al., 2023).

Table 1. Moisture, pH, salt, free fatty acid and residual nitrite results of pastırma purchased from different companies at three different times (mean value ± standard deviation).

Companies	pH	Moisture (%)	Salt (%)	Free Fatty Acid (g oleic acid/100 g)	Residual Nitrite (ppm)
A	5.78±0.05d	51.96±3.02a	5.92±0.39b	0.049±0.019d	0.610±0.374d
B	5.74±0.1e	47.04±2.39c	6.66±1.14a	0.049±0.010d	0.159±0.183d
C	5.67±0.57f	51.68±0.74a	3.86±1.28f	0.082±0.007b	3.027±3.321c
D	6.01±0.12b	52.12±2.73a	4.71±0.55e	0.118±0.095a	10.241±4.280a
E	6.13±0.24a	49.85±3.08b	5.00±0.68de	0.066±0.045c	0.467±0.343d
F	6.03±0.27b	49.74±2.58b	6.72±1.50a	0.067±0.062c	5.787±8.038b
G	5.74±0.02de	47.61±1.68c	5.25±0.83cd	0.031±0.010e	0.175±0.074d
K	5.89±0.03c	49.96±2.71b	5.54±1.09c	0.096±0.061b	0.291±0.190d

Averages marked with different letters in the same column are different from each other.

3.5. Microbiological Properties

The average values of the number of total aerobic mesophilic bacteria (TAMB) of pastırma obtained from the market are presented in Table 2. The highest number of TAMB was found in company C with an average of 7.76 log cfu/g. It was specified that the number of yeast-mold in pastırma was in the range of 3.46-7.21 log cfu/g. Kaban (2013) stated that the number of yeast and mold in pastırma increased during the drying stages and decreased in the fenugreek and final drying stages. Additionally, it has been emphasized that garlic, which constitutes 35% of the çemen mixture, protects the pastırma against mold.

Lactic acid bacteria and catalase-positive cocci are significant groups of microorganisms found in pastırma. Lactic acid bacteria contribute to the development of sensory and textural properties of the product with their low proteolytic activities and acid production. While lactic acid bacteria and *Micrococcus/Staphylococcus* bacteria are generally present in the raw material at the level of 2-3 log cfu/g, they show a significant increase during the production process (Kaban, 2013). Lactic acid bacteria, an important group in pastırma, also play a role in the development of the sensory properties of the product thanks to their low lipolytic activity (Öz et al., 2017). The highest lactic acid bacteria count in pastırma belonged to company C with the average value of 7.93 log cfu/g. Furthermore, the value of other companies did not fall below the level of 10^6 cfu/g. Catalase positive cocci, which form the dominant flora in pastırma, have nitrate reductase activity as well as catalase activity. In addition, these microorganisms are crucial in forming color and flavor, color stability and oxidation delaying thanks to their lipolytic and proteolytic activities (Kaya & Kaban, 2010). These acid-sensitive bacterias can form the dominant flora in pastırma due to the appropriate pH (Kaban, 2013). The average number of *Micrococcus/Staphylococcus* in the samples varied between 6.50-7.52 cfu/g (Table 2).

The Enterobacteriaceae family, which includes many foodborne pathogens and unwanted microorganisms, mostly cannot survive in pastırma (Fettahoğlu et al., 2019; Hazar et al., 2017; Kaban, 2009, 2013; Öz et al., 2017). In the study, microorganisms belonging to the Enterobacteriaceace family were detected at different levels in the samples of companies C, D, F and K. In the samples of companies A, B and E, the number of Enterobacteriacea was found below the detectable limit (<2).

Table 2. Microbiological analysis results of pastırma purchased from different companies at three different times (cfu/g) (mean value ± standard deviation).

Companies	TAMB	Yeast-Mold	Lactic Acid Bacteria	*Micrococcus/Staphylococcus*
A	7.22±0.20bc	6.12±0.49b	6.78±0.34b	7.22±0.14a
B	6.87±0.62d	6.10±0.95b	6.07±0.99d	6.74±0.70b
C	7.76±0.45a	4.09±0.40d	7.93±0.60a	6.50±0.40c
D	7.27±0.35bc	4.14±0.59d	6.90±1.05b	7.05±0.59a
E	7.18±0.64bc	6.51±0.50a	6.30±0.55cd	7.10±0.67a
F	7.09±0.55c	5.51±1.50c	6.22±0.60d	6.62±0.77bc
G	6.81±0.52d	6.20±0.53b	6.50±0.53c	6.52±0.61bc
K	7.41±0.48b	6.41±0.77a	6.20±0.90d	7.07±0.30a

Averages marked with different letters in the same column are different from each other.

4. Conclusion

This study was focused on the analysis of characteristics of Kastamonu pastırma, taken from eight different companies at three different times in Kastamonu region. In the analysis, pastırma samples are examined in terms of moisture, pH, free fatty acids, residual nitrite, salt, and microbiological parameters. As a result, it was seen that some pH and moisture values exceeded the limits specified in the Turkish Food Codex Communiqué on Meat, Prepared Meat Mixtures and Meat Products. Manufacturers should pay more attention to pastırma drying process and take legal limits into consideration. On the other hand, it was concluded that Kastamonu pastırma is safe to consume in terms of salt and residual nitrite. Microbiologically, it has been determined that characteristics of pastırma varies according to companies and time. In addition, it has been observed that microorganisms belonging to Enterobacteriaceae family showed development in pastırma from some companies. This situation also shows that more attention should be paid to hygienic conditions.

Acknowledgment

This study was supported by Kastamonu University Scientific Research Projects Coordination Office (KÜ-BAP01/2020-27).

Conflict of Interest

The authors declare that they have no conflict of interest.

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http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

PRENSİP

https://prensip.gen.tr

RESEARCH ARTICLE

Soil Conservation Strategies for the Reduction of Biodiversity in Mountain Soils: Example of Uludağ National Park/Türkiye

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ARTICLE INFO

Article History
Received: 03.08.2024
Accepted: 09.08.2024
First Published: 30.09.2024

Keywords
Alpine
Erosion
Soil
Soil Conservation
Uludağ

ABSTRACT

Uludağ National Park in Türkiye is famous for its rich biodiversity. The park serves as an essential habitat for a wide variety of flora and fauna and contributes significantly to the conservation of various species. This study examines biodiversity conservation strategies in Uludağ National Park. Soil samples were taken from 17 locations near the summit where endangered endemic plants grow. Analyses of the soil samples reveal the relationships between elevation and soil texture components. According to the soil analysis results, there was a weak positive correlation between elevation and silt content (r = 0.414) and a weak negative correlation between elevation and sand content (r = -0.375). These findings indicate that silt content tends to increase and sand content tends to decrease with increasing elevation. The geomorphological features and soil structure of Uludağ National Park were also an essential part of the research. The region's metamorphic mica schists, granites and marbles affect the soil's physical properties. For example, soils are generally sandy and permeable in areas where granite parent material is present. In contrast, areas where mica-schist parent material is distributed are less resistant and more susceptible to erosion. As a result, effective soil conservation strategies must be implemented to protect biodiversity in Uludağ National Park. These strategies are essential to reduce soil erosion, increase organic matter accumulation and minimise the impacts of climate change. Furthermore, community participation and adaptive management strategies must be adopted for sustainable development and resource management. This study provides essential information for biodiversity conservation in the Uludağ region and contributes to developing conservation strategies.

Please cite this paper as follows:

Sarı, H., & Atmaca, B. (2024). Soil conservation strategies for the reduction of biodiversity in mountain soils: Example of Uludağ National Park/Türkiye. *Journal of Agricultural Production*, *5*(3), 176-184. https://doi.org/10.56430/japro.1527517

1. Introduction

Mountains are vital ecosystems that support a significant part of the world's biodiversity, especially in the tropics, where they act as hotspots of exceptional richness (Rahbek et al., 2019). The complex topography of the mountains creates diverse habitats that harbour a wide variety of species, facilitating isolation and speciation (Lu, 2021). These ecosystems are crucial for biodiversity conservation against species losses caused by climate change and provide opportunities for species to move over short distances through various micro-environmental conditions (Körner, 2014).

Soil conservation in alpine regions is crucial for ecosystem health and sustaining biodiversity. Several studies have emphasised the importance of implementing effective soil conservation measures to reduce soil erosion, increase soil fertility and maintain ecological sustainability in mountainous areas.

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Conservation efforts in mountainous regions are essential for biodiversity conservation. Studies show that protected areas are fundamental for the long-term conservation of mountain biodiversity, especially in tropical regions where human activities affect diversity patterns (Gebert et al., 2019). Furthermore, the spatial relationship between different types of mountainous areas and land use is also essential for sustainable development and resource management (Zhao & Li, 2016). Effective conservation policies, such as those that optimise the conservation of landscape resources in national parks, are vital to maintaining ecological integrity and promoting positive social interactions (Liu & Sun, 2023).

In mountain conservation, it is crucial to assess the impact of human activities on these sensitive ecosystems. Integrating topography and human pressure assessments is vital to accurately assess species vulnerability to climate change and guide conservation strategies (Elsen et al., 2020). Furthermore, understanding the interconnected mechanisms of human-land systems in mountain watersheds can inform land use policies for sustainable development (Wu et al., 2022).

National parks play a crucial role in biodiversity conservation and ecosystem preservation, providing refuge for various species and sustaining essential ecological processes (Siraj et al., 2017). The success of conservation efforts in national parks is influenced by several factors, including monitoring programs tailored to the specific needs of protected areas (Sathyakumar et al., 2014). For example, a case study conducted in the Khangchendzonga National Park and Biosphere Reserve in Sikkim, India, highlights the importance of developing effective monitoring protocols to ensure the long-term conservation of mammals in Himalayan regions (Sathyakumar et al., 2014).

Studies conducted in Türkiye have provided valuable information on the country's biodiversity conservation and ecosystem management. For example, research focusing on mapping ecoregions under climate change in Türkiye highlights the need for large-scale studies to understand and address the impacts of climate change on the country's ecosystems and biodiversity (Ergüner et al., 2018). This study highlights the importance of understanding how climate change affects Türkiye's diverse ecoregions and biodiversity.

Research on forest communities and ecological differentiation in Elmacık Mountain in Düzce highlights the region's rich floristic and vegetation diversity (Aksoy & Çoban, 2017). By analysing the vegetation-environment relationship using multivariate statistical techniques, this study provides valuable information on the ecological dynamics of mountain ecosystems in Türkiye.

In conclusion, biodiversity conservation in national parks requires a multifaceted approach that integrates effective monitoring programs, adaptive management strategies, community engagement and sustainable economic practices. By addressing these various aspects, national parks can continue serving as vital refuges for wildlife, protecting critical ecosystems and promoting the well-being of nature and people.

This study will examine where endemic plants occur at high altitudes and grow only in certain regions. The aim is to comprehensively analyze the soil structure, landforms and ecological characteristics of these areas. The main objective of the research is to identify the conservation measures necessary for the protection and sustainability of these particular areas. The study also aims to develop strategies for creating similar ecosystems or improving existing ones. Considering factors such as soil chemistry, biodiversity, water management and land use, recommendations will be made to protect the ecological balance of these regions.

2. Materials and Methods

2.1. Study Area

While Uludağ was known as "Bithynian Olympos" and "Monk Mountain" in mythological and Ottoman times, it was officially renamed Uludağ in 1925. This important mountain is located in Bursa province, between 28° 58' - 29° 38' east longitude and 39° 45' - 40° 10' north latitude. Uludağ rises with steep slopes and reaches a height of 2,543 meters. The Nilüfer Stream forms the natural boundaries of the mountain to the west and south and the city of Bursa and the district of İnegöl to the north and east.

Study ares is not a single elevation but a mountain range extending northwest-southeast. It covers an area approximately 40 kilometres long and 20 kilometres wide. Uludağ, the highest mountainous mass in the Marmara Region and Western Anatolia, attracts attention with its height and width. It is also an essential region for natural beauty and nature tourism. (Rehder et al., 1994).

2.2. Method

While selecting sample points for the study area, regions near the summit and where endangered endemic species grow were preferred. The location map of the study area is shown in Figure 1.

In the research area, the soils where endemic plants, especially endangered endemic plants, grow were examined, and textural and organic matter analyses were carried out. Soil samples were taken from the area where the plant was grown. Laboratory analyses: Soil reaction (pH) was determined using a pH meter with glass electrode in soil suspensions diluted 1:2.5 with water and N/50 $CaCl_2$ solution (Jackson, 1958). Grain size distribution (texture) was determined using the hydrometer method (Anonymous, 1993). The texture triangle was used for texture class nomenclature (Anonymous, 1996). Lime and salt were determined by the volumetric calcimeter method (Sağlam, 2008). Salt was determined in soil suspensions using the Wheatstone Bridge concentrator (Richards, 1954). Organic matter content (%) was determined by the modified Walkley Black Wet Burning method (Walkley, 1947). Cu, Mn, Zn (ppm) were determined by (DTPA, ICP), Fe, Mg, Ca, K (ppm) were determined by (A. Acetate, ICP) methods.

Figure 1. Location map of the study area.

2.3. Geomorphological Features

When the geomorphological features of Uludağ are examined, a cross-section from the Bursa Plain to the summit is examined, and it is stated that metamorphic and fragmented mica schists are found first. Since mica schists are weaker than marbles regarding abrasion resistance, they formed low areas. The mica schists have a leaf-like structure in thin layers and continue up to about 1100 meters. Gneisses begin to be seen around 900 meters. Towards 1150 meters, granite-like blocks stand out. These blocks rise to 1250 meters and give way to granite blocks in higher regions. After crossing the ridges separated by widening valleys in Sarıalan, the Kırkpınarlar Region is reached. This is an almost flat area where the surrounding hills to the east and west are composed entirely of granite. In the summit region, the granite is overlain by amphibole schist and a thick layer of marble. It is also stated that the first traces of the glacial period in Türkiye were identified in Uludağ by Philipson. This information emphasises the geological diversity and different rock types of Uludağ. It is seen that mica schists are weak against erosion, and different rocks such as granite, gneiss, amphibolous schist and marble are located in different parts of the mountain. It is also stated that Uludağ was affected during glacial periods, and traces of this period are available (Anonymous, 1994).

It is stated that the distribution area of the granite ends near the summit at an altitude of 2300 meters and transitions to the contact metamorphic zone formed as a result of internal eruptions of the granite. The main components of this zone include gneiss, mica schist, granodiorite and marble. It is stated that this zone is 2200-2543 meters high and is one of the characteristic features of karst formation. Karst is a process by which water passes through calcareous rocks to form topographic features such as dissolution, cavities, caves and dolines. It is stated that karst formation is present in this contact metamorphologic zone and that rain and snow water pass through the cracks and dolines in this region and exit the foothills of Uludağ and the Bursa Plain. This information explains the components and characteristics of the contact metamorphic zone through which the granite transgressed due to geological processes in Uludağ. It is also noted that karst formation is essential in the water cycle and hydrological processes (Çepel, 1978).

2.4. Soil Properties

The geological structure, climate and landforms in Uludağ greatly influence soil formation. For this reason, terra rosa, a reddish soil, is generally found at low altitudes, while podsolic brown forest soils are found at higher elevations. The main components of the soil are mineral-rich colluvial soils, usually composed of gravel and blocks on deep slopes. This is especially true in the erosion zone. In areas with hard limestones, typically reddish-coloured terra rosa soils are common (Zech & Cepel, 1977).

The fundamental bedrock of the soils commonly found in Uludağ is granite. Soils developed on granite bedrock are generally found at altitudes of 1300-2200 meters, have a coarse structure, and sand content is generally above 60%. In these soils, the thickness of the dissolved granite zone is the most critical factor affecting the depth of the soil. A thick zone of loose granite provides a favourable environment for root propagation and water accumulation. The soils have good permeability but low base saturation rate and cation exchange capacity. Since the soil pH value is between 3.7 and 4.7, it can be called "Brown Forest Soils" (Çepel, 1978). The soils and landforms of the sample locations are given in Table 1.

Table 1. Topography characteristics of soil samples.

Sample No	Soil type	Landforms	Altitude (meters)
Example 1	Colluvial Soils	Slope	2354
Example 2	Colluvial Soils	Slope	2020
Example 3	Colluvial Soils	Slope	2228
Example 4	Reddish Brown Soils	Ridge	2251
Example 5	Red Brown Mediterranean Soils	Ridge	2272
Example 6	Reddish Chestnut Soils	Slope	2265
Example 7	Reddish Chestnut Soils	Slope	2134
Example 8	Reddish Chestnut Soils	Mountain	2114
Example 9	Saline-Alkali and Saline-Alkali Mixed Soils	Ridge	2090
Example 10	Saline-Alkali and Saline-Alkali Mixed Soils	Ridge	2000
Example 11	Saline-Alkali and Saline-Alkali Mixed Soils	Ridge	2233
Example 12	Saline-Alkali and Saline-Alkali Mixed Soils	Ridge	2242
Example 13	Saline-Alkali and Saline-Alkali Mixed Soils	Ridge	2282
Example 14	Saline-Alkali and Saline-Alkali Mixed Soils	Ridge	2278
Example 15	Chestnut Soils	Mountain	1912
Example 16	Chestnut Soils	Mountain	2354
Example 17	Chestnut Soils	Mountain	2217

Due to the steep, steep and undulating topography, the soil cover is usually very shallow or shallow. Therefore, a complete soil profile does not develop in brown forest soils without limestone. Generally, AC layer is under the well-developed and acid-characterized A1 layer. The main components of these soils are sandy claystone, clayey, sandy, clayey or gravelly deposits. On bare rock and rubble lands, soil development is impossible as no soil layer exists.

2.5. Climate Characteristics

The annual average temperature drops to 3.8 ˚C at the points where the sample points were taken. While the average temperature in Uludağ falls below zero degrees Celsius in December, January, February and March, there is no month in Bursa where the temperature falls below zero degrees Celsius in monthly average values. The coldest month in Bursa is January with an average temperature of 5.4 ˚C, while in Uludağ, it is February with -4.1 ˚C. In July, the hottest month, the average temperature rises to 24.5 ˚C in Bursa and 13.9 ˚C in Uludağ. In terms of monthly average values, there is a difference of 19.1 ˚C between the hottest and the coldest month in Bursa, while this difference is 18 ˚C in Uludağ. When the average high-temperature values are analysed, it is seen that the effective temperature range in Bursa and Uludağ is widening. The month with the lowest average low temperature in Bursa is February, at 1.7 ˚C, while the month with the highest average high temperature is July and August, at 30.6 ˚C. The month with the lowest average values in Uludağ is February, at -7 ˚C. The month with the highest average values is August, with 18.3 ˚C. As can be seen from the experienced values, according to the average low and average high values, a temperature range of 28.9 ˚C is effective in Bursa, and a temperature range of 25.3 ˚C is effective in Uludağ. Looking at the monthly average temperature differences between Bursa and Uludağ, the highest temperature difference between the two stations is observed in June, while the lowest is in October and November. The difference between the two stations increases in the summer period, mainly due to the overheating of Bursa in the summer months.

3. Results and Discussion

Various geological formations belong to the Paleozoic and Paleocene periods in the higher parts of Uludağ. Rocks such as metagranodiorite, marble, and gneiss microsite from the

Paleozoic period cover most of the region. Granite and aplite pegmatite formations represent the Paleocene period. Looking at the sampling points shown on the map, points 1, 4, 5, 7, 8, 9, 12, 13, 14 and 15 are from meta granodiorite areas; points 2, 3 and 6 are from marble areas; points 10, 11 and 17 are from gneiss marcasite areas; points 15 and 17 are from granite areas and points 16 and 17 are from aplite pegmatite areas (Figure 2). These points were selected to examine the effects of different geological structures on soil properties and, thus, on the distribution of endemic plant species.

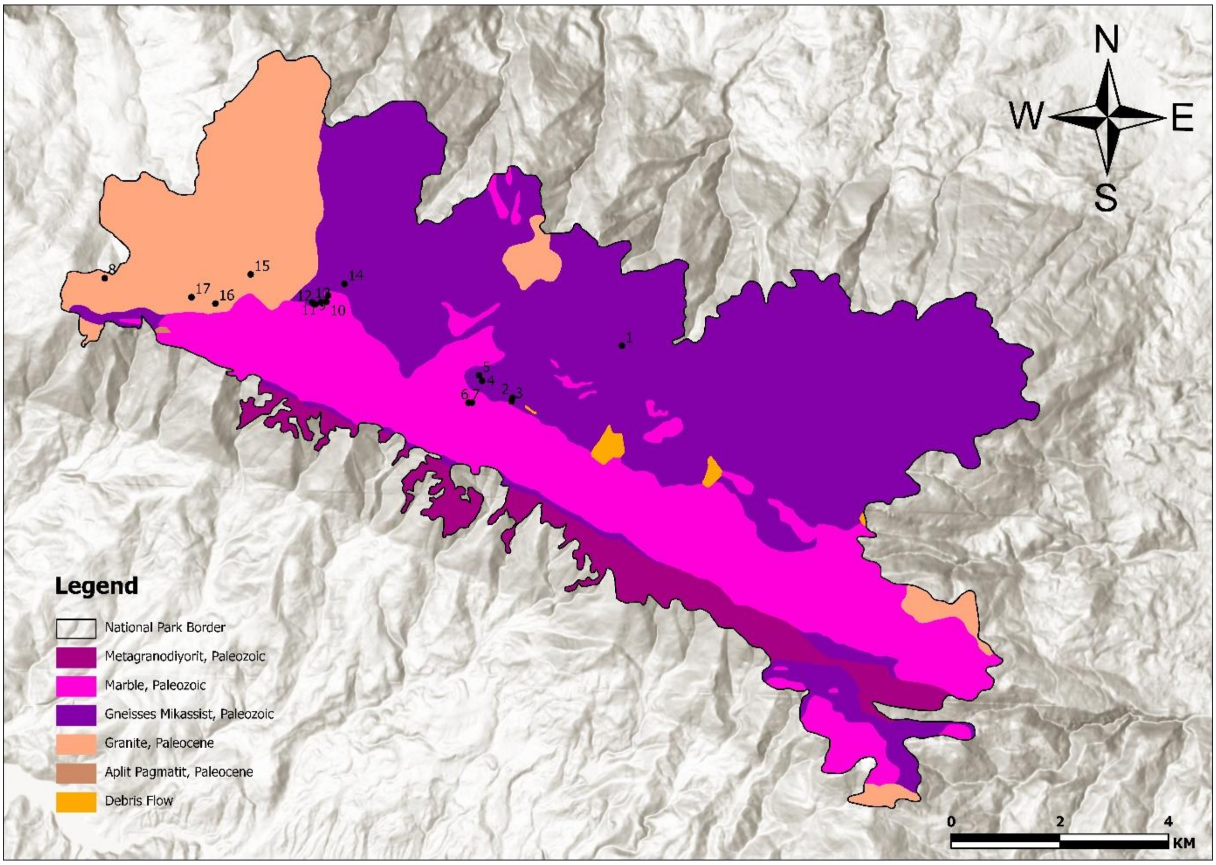

Figure 2. Geological map of study area.

Looking at the landform map of study area (Figure 3), when elevation and slope are considered, the higher regions of study area (high ridges and upper slopes) play an essential role in soil formation. The soils in these regions are generally thinner, stonier, and closer to the bedrock. Therefore, soil formation occurs more slowly.

The mid-slope and upland drainages shown on the map indicate water's flow paths. In these areas, the soil contains less organic matter and is subject to more erosion due to the constant movement of water.

Valleys (valleys) and plains (plains) have deeper soils rich in organic matter. In these areas, the density of vegetation and organic matter accumulation is higher.

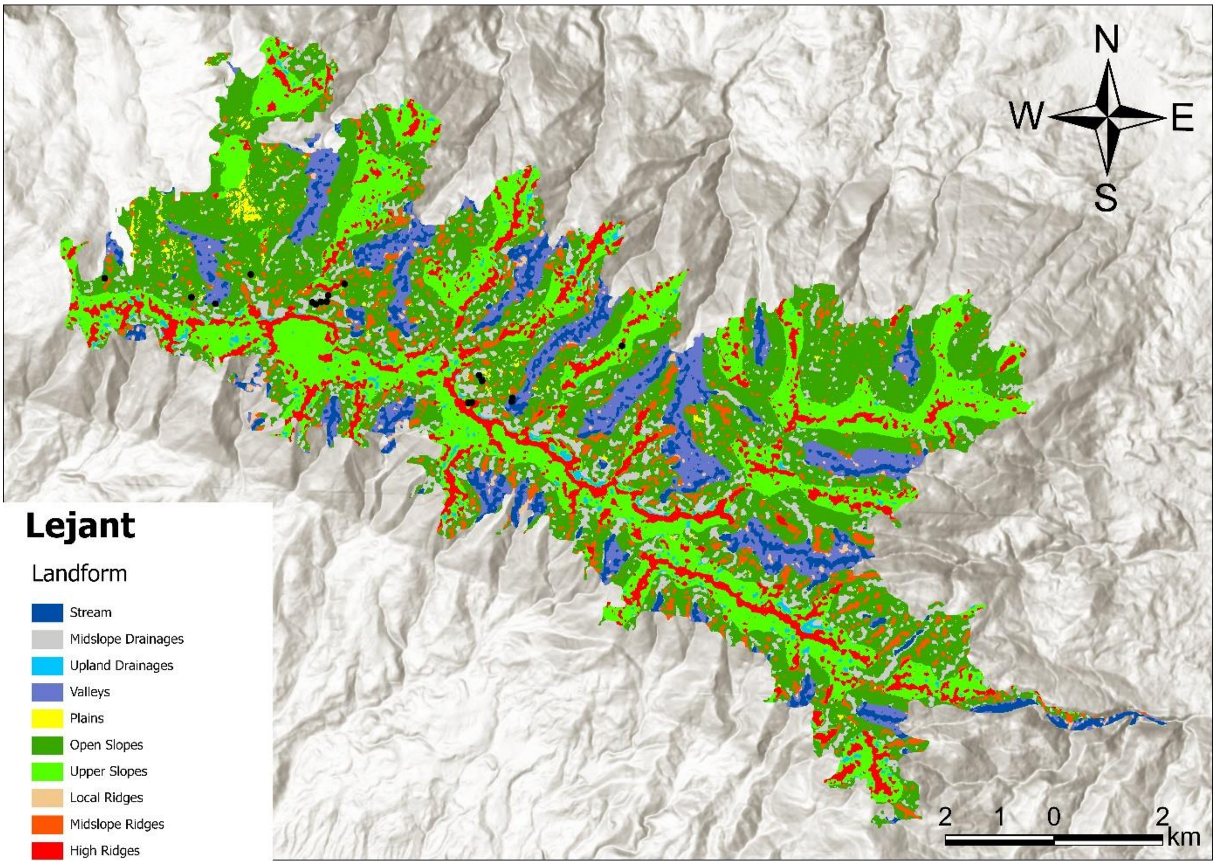

Figure 3. Landform map of study area.

As a result of the analysis of soil samples taken from 17 points in study area, the correlations between elevation and soil texture components were revealed. According to the data, a weak positive correlation (r = 0.414) was found between elevation and silt content and a weak negative correlation (r = -0.375) was found between elevation and sand content. These findings indicate that silt content tends to increase and sand content tends to decrease with increasing elevation. This may reflect environmental conditions favouring the formation of finer-grained soils at higher altitudes.

The literature frequently emphasises that soils' physical and chemical properties change with increasing altitude (Brady & Weil, 2008). Lower temperatures and increased humidity at higher altitudes may affect physical weathering processes and organic matter accumulation, resulting in a change of soil texture in favour of fine-grained fractions (Jenny, 1980). In addition, vegetation is denser in these areas, positively affecting organic matter production and, thus, silt content (Schimel et al., 1994).

The proportions of silt and sand in the soil samples confirm the influence of elevation-related environmental factors. For example, samples at higher altitudes (e.g. Sample 1, Sample 5, Sample 6) have higher silt contents, while samples at lower altitudes (e.g. Sample 8, Sample 9, Sample 10) exhibit higher sand contents. This demonstrates the role of climate and vegetation factors that change with altitude in soil formation and development (Birkeland, 1999).

The high-altitude areas of Uludağ exhibit unique soil properties influenced by various factors such as geology, vegetation and human activities. Studies have shown that soils in the alpine and subalpine belts of Mount Uludağ are affected by disturbances, leading to differences in mineralisation rates and elemental composition (Güleryüz et al., 2006, 2011). The presence of ruderal species such as Verbascum olympicum in degraded areas indicates high rates of nitrification in the soil and adequate nitrate assimilation capacities (Arslan et al., 2013; Güleryüz et al., 2016). Furthermore, the elemental composition of plants growing in contaminated areas in Uludağ Mountain shows the impact of tungsten mining on soil quality (Erdemir, 2017, Kahraman et al., 2017). Soil properties and soil health are essential in biodiversity conservation (Yanardag, 2022; Yetgin, 2023).

Soil properties in the higher elevations of Uludağ are influenced by factors such as organic carbon content, moisture levels and mineral composition, which play an essential role in determining overall soil quality and health (Stanchi et al., 2012; Unsever & Diallo, 2019). The presence of various elements such as copper, iron, zinc and lead in soil can be attributed to both natural geological processes and anthropogenic activities (Güleryüz et al., 2006). Furthermore, using fly ash and lime for soil stabilisation emphasises the importance of soil amendment in improving soil properties.

Soil structure in the highlands of Uludağ is a complex interaction of geological, biological and anthropogenic factors. Understanding the mineralisation rates, elemental composition and pollution levels in these soils is essential for effective land management and conservation efforts in the region. In addition, it has been determined that water resources have decreased in area, and bare rocky lands have increased in area over time (Özsoy, 2021).

These results are essential for understanding the potential impacts of soils on biodiversity in Uludağ National Park. Higher clay content supports plant root growth by increasing water-holding capacity, while lower sand content may help maintain moist conditions by reducing soil water permeability (Hillel, 2003). This could play a decisive role in endemic plant species' settlement and survival strategies.

The distribution patterns of endemic plants in various habitats have been a subject of interest in ecological studies. Observations show that endemic plant species tend to concentrate on specific microenvironments, especially those inhabiting hillsides. These include hollow areas with colluvial material deposited between stones and soils adjacent to lakes. It was also noted that endemic plants thrive in flat areas created by mining activities, where puddles form and where organic matter content is high. This indicates that the presence and distribution of endemic plants are closely linked to their habitats' topographic and edaphic characteristics. In particular, the preference of endemic plants for flat or nearly flat lands with high organic matter content underlines the importance of soil properties in shaping plant distribution patterns. These findings align with the broader literature on ecological niche specialisation of endemic species and highlight the importance of habitat heterogeneity in supporting endemic plant diversity, emphasising the complex relationships between plant communities and their environment.

These correlations between elevation and soil texture components provide critical information for understanding the dynamics of ecosystems in Uludağ National Park and for developing conservation strategies. These findings support the relationships between elevation and soil properties reported in the literature and provide the basis for sustainable biodiversity management in mountain ecosystems (Walker & Del Moral, 2003). Figure 4 gives field views of some of the places where endemic plants are found in Uludağ.

Figure 4. Field views of some of the sites with endemic plants in study area.

4. Conclusion

Given the biodiversity in Uludağ and the role of soil characteristics in plant diversity, it is essential to address soil erosion and encourage soil conservation practices. In particular, creating flat areas such as terracing near lake formations and other places will contribute to accumulating colluvial material in these areas and forming organic matter over time. Vegetation is critical in protecting soils against erosion and contributes to the accumulation of organic matter, vital for plant growth and ecosystem health in alpine soils. Another issue is grazing activities in the area. Managing grazing activities in these areas is another critical aspect of plant conservation, as seasonal grazing can help maintain species composition and soil texture in these sensitive ecosystems. Consequently, a holistic approach integrating soil conservation, biodiversity conservation and sustainable land management practices is essential for effectively conserving plants in alpine mountains. By considering the complex relationships between soil properties, plant communities and ecosystem functions, conservation efforts can be tailored to maintain alpine environments' unique biodiversity and ecological integrity.

Acknowledgment

This study was supported by Namik Kemal University Scientific Research Projects Coordination Unit Project Number: NKUBAP.03.YLGA.23.454.

Conflict of Interest

The authors declare that they have no conflict of interest.

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http://agriprojournal.com
JOURNAL OF AGRICULTURAL PRODUCTION
ISSN: 2757-6620
PRENSIP
https://prensip.gen.tr

RESEARCH ARTICLE

Investigation of the Effects of Erzurum Province Wastewater Treatment Plant on the Karasu River Water Quality

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ARTICLE INFO

Article History
Received: 21.08.2024
Accepted: 03.09.2024
First Published: 30.09.2024

Keywords
Karasu River
Sustainability
Wastewater treatment
Water quality

ABSTRACT

This study was carried out to investigate the effects of the wastewater treatment plant located in the Aziziye District of Erzurum Province on the Karasu River. For this purpose, water samples were taken from 3 stations for a year between 2022-2023. The mean water temperature was measured *in situ* during sampling as 13.8 ˚C, the mean pH value was 7.7, the mean electrical conductivity was 403 µs/cm, and the mean dissolved oxygen value was 6 mg/l. The mean total suspended solid (TSS) amount was determined as 46.7 mg/l, the mean chemical oxygen demand (COD) concentration was 28.9 mg/l, the mean biological oxygen demand (BOD) concentration was 18.5 mg/l, the mean total nitrogen (TN) concentration was 4.47 mg/l, the mean nitrate (NO_3) concentration was 5.35 mg/l, the mean nitrate nitrogen (NO_3-N) concentration was 1.21 mg/l, the mean total hardness (TH) was 155 mg/l, the mean ammonium (NH_4) concentration was 2.75 mg/l, and the mean ammonium nitrogen (NH_4-N) concentration was 2.16 mg/L. As a result, it was determined that the Wastewater Treatment Plant positively affected some water quality parameter values of the Karasu River.

Please cite this paper as follows:

Nuroğlu, Ş., & Kocaman, E. M. (2024). Investigation of the effects of Erzurum Province wastewater treatment plant on the Karasu River water quality. *Journal of Agricultural Production*, *5*(3), 185-191. https://doi.org/10.56430/japro.1536617

1. Introduction

Freshwater resources are one of the most essential natural resources on earth and are vital to humanity. Freshwater resources are utilized in various areas, such as drinking water supply, agricultural irrigation, industrial production and energy production. However, factors such as global climate change, population growth, urbanization and environmental pollution threaten the sustainability of water resources. Therefore, effective and fair management of water resources is of great importance to provide clean and sufficient water for future generations. Protecting water resources plays a critical role in the ecological balance of water, human health and economic development (Tomanbay, 1998). Streams are systems formed by the remaining water after evaporation of the precipitation falling on the earth's surface. Approximately 20% of rainfall evaporates. Most of the remaining part forms streams and flows towards the lakes or seas, while a small part seeps into the soil and constitutes groundwaters (Tanyolaç, 2009).

One of the main difficulties in maintaining water quality in rivers is the presence of pollutants. Heavy metals, industrial and man-made pollutants such as chemicals and sewage can enter rivers from a wide variety of sources such as factories, wastewater treatment plants, agricultural activities and urban runoff. Many criteria have been developed to determine the water quality of rivers. The Water Framework Directive,

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prepared by European countries and entered into force in our country in 2005, is among these criteria. This criterion classifies freshwater resources based on water temperature, pH, dissolved oxygen, ammonium-nitrogen, nitrite-nitrogen, nitrate-nitrogen, total phosphorus and chemical oxygen demand parameters. In our country, inland waters are classified into four main classes according to the Water Pollution Control Regulation, prepared in accordance with the mentioned directive, and the Intra-Continental Surface Water Standards included within the same law (Orman ve Su İşleri Bakanlığı, 2015; Varol et al., 2022).

Despite Türkiye being rich in terms of water resources, it is a country that may experience water problems in the near future if the necessary precautions are not taken. The country's average river potential is 186 km^3, and this amount reaches 196 km^3 by adding 10 km^3 of groundwater. Since the annual usable water amount per capita is 1,430 m^3, Türkiye is among the countries experiencing water shortage (Tomanbay, 1998). The Karasu River is of great importance for the province of Erzurum and other regions it passes through, as it hosts agricultural irrigation, energy production and biodiversity. However, problems such as pollution and overuse of rivers can have adverse effects on ecosystems and increase sustainability concerns. Therefore, protecting the natural and economic value of the Karasu River and managing it sustainably is of great importance (Eren & Kaya, 2020).

For this reason, our research was carried out to investigate the effects of the water coming out of the Wastewater Treatment Plant, which was established near the Karasu River and located in the exit section of Erzurum Province, on the water quality of the Karasu River.

2. Materials and Methods

2.1. Study Site

The Karasu River, main branch of the Euphrates River, passes through the Aşkale District of Erzurum and heads towards Erzincan, also receiving the Tuzla Stream coming from the Palandöken Mountain, and after passing the Sansa Strait, it crosses the Erzincan Plain and from there enters the Kemah Strait, after deep and narrow straits, it joins the Murat River at the Keban Dam Lake. Our study covers the region within the borders of Erzurum Province. The map and coordinate data of the study area are presented in Figure 1 and Table 1, respectively.

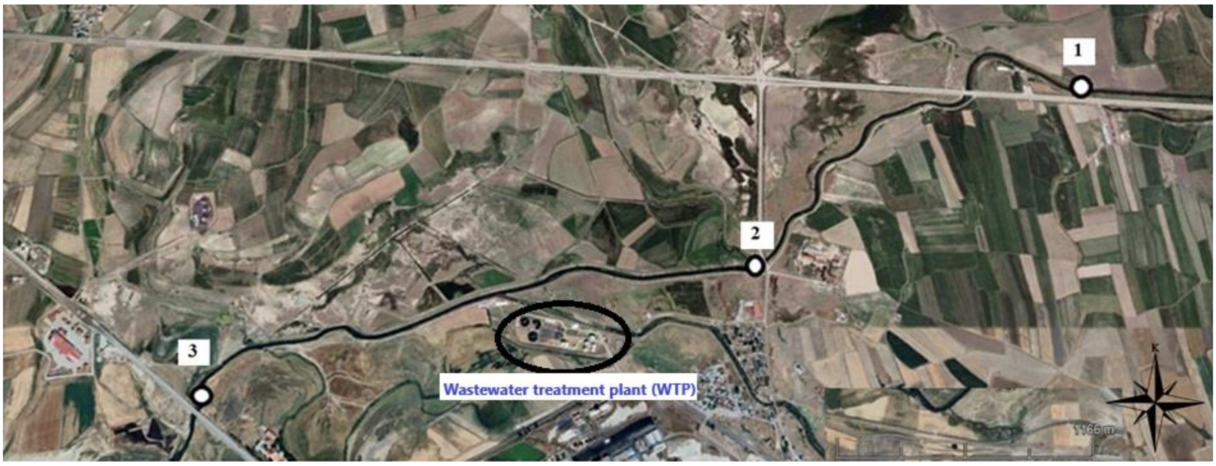

Figure 1. Map showing the Karasu River and stations (Water flow direction from 1 to 3).

Table 1. Coordinates of the stations.

Station	Latitude and Longitude
1	39°58'02"N 41°07'29"E
2	39°57'32"N 41°06'10"E
3	39°57'11"N 41°04'17"E

2.2. Field and Laboratory Studies

In this study, water samples were taken from 3 stations in the Karasu River within the borders of Erzurum Province between October 2022 and September 2023 with polyethylene sampling containers. Water temperature, DO, pH and electrical conductivity were recorded *in situ* using a multi-parameter measuring device (YSI Model).

The total suspended solid amount was determined by the Gravimetric method. Biochemical Oxygen Demand (BOD) was assayed by Manometric/SM 5210 B and Manometry method. BOD analysis was determined time period as 5 days. Chemical oxygen demand (COD) was determined by the Titrimetric/SM 5220 B Ready Kit Method. Total nitrogen (TN) was quantified using the Spectrometric/ISO 29441 Ready Kit Method. Ammonium (NH_4), Ammonium Nitrogen, Nitrate and Nitrate Nitrogen determinations were performed with the Spectrometric/SM 4500 Ready Kit Method. Total hardness was analysed by SM 4500 ready-made kits.

2.3. Data Evaluation

The changes in the data obtained throughout the study based on the season and stations were determined by One-Way (ANOVA) test using IBM SPSS 20. The significance levels of the differences were evaluated according to the DUNCAN test.

3. Results

Throughout the study, the changes in dissolved oxygen, electrical conductivity and water temperature values depending on the season and stations were found to be statistically significant ($p<0.05$). However, while the difference in pH value between seasons was found to be statistically significant ($p<0.05$), the difference between stations was statistically insignificant ($p>0.05$). The lowest pH value was measured as 7.54, and the highest value was 7.95. During the study period, the lowest electrical conductivity value measured during fieldwork was 214, and the highest was 578, whereas the lowest dissolved oxygen value was 3.02 mg/L and the highest was 8.74 mg/L. The lowest water temperature in the Karasu River was determined to be 3.4 °C, and the highest was 22.2 °C (Table 2).

Table 2. Changes in pH, dissolved oxygen, electrical conductivity and water temperature values of Karasu River depending on months and stations (Mean±Standard deviation).

Month	Station	pH	Dissolved oxygen (mg/l)	Electrical conductivity (µS/cm)	Water temperature (ºC)
October	1	7.60±0.33^{Cb}	5.62±0.43^{Ia}	346±4.68^{Kb}	16.5±1.66^{Kc}
	2	7.62 ± 0^{Ca}	5.27±0$^{\text{İc}}$	346±0^{Kb}	16 ± 0^{Aa}
	3	7.63±0.01^{Ca}	5.77±0.54^{Ia}	367.50±23.60^{Ka}	15±1.10^{Ab}
November	1	7.65±0.03^{Ba}	6.26±0.86^{Ia}	424.33±87.26^{Aa}	14.67±1^{Aa}
	2	7.64±0.02^{Ca}	6.17±0.75^{Hb}	402.50±84.25^{Gb}	13.25±2.70^{Bb}
	3	7.66±0.04^{Ba}	6.27±0.69^{Fa}	395.40±76.11^{Hc}	12.32±3.07^{Cc}
December	1	7.65±0.03^{Ba}	6.36±0.66^{Ga}	412.83±79.87^{Ea}	11.83±3.00^{Ca}
	2	7.64±0.05^{Ca}	6.32±0.62^{Ga}	403.14±77.55^{Fb}	10.86±3.70^{Eb}
	3	7.63±0.05^{Ca}	6.32±0.58^{Ea}	397.75±73.77^{Gc}	10.10±4.01^{Fc}
January	1	7.63±0.05^{Ca}	6.33±0.54^{Hb}	409.11±76.72^{Ga}	9.64±3.99^{Fa}
	2	7.62±0.05^{Ca}	6.39±0.55^{Fa}	403.20±74.85^{Eb}	9.08±4.16^{Hb}
	3	7.61±0.05^{Ca}	6.42±0.53^{Da}	400.09±71.95^{Dc}	8.56±4.29$^{\text{İc}}$
February	1	7.61±0.05^{Ca}	6.48±0.54^{Fc}	411.33±78.51^{Fa}	8.33±4.18$^{\text{İa}}$
	2	7.61±0.05^{Ca}	6.65±0.80^{Cb}	405.15±78.40^{Db}	8.22±4.03^{Jb}
	3	7.62±0.05^{Ca}	6.79±0.93^{Aa}	402.29±76.20^{Cc}	8.14±3.89^{Kc}
March	1	7.62±0.05^{Ca}	6.92±1.02^{Ba}	414±85.88^{Da}	8.06±3.77^{Jc}
	2	7.64±0.09^{Ca}	6.86±1.02^{Ab}	413.94±83.10^{Bc}	8.18±3.67^{Kb}
	3	7.65±0.11^{Ba}	6.81±1.00^{Ac}	414.41±80.59^{Bb}	8.27±3.58^{Ja}
April	1	7.66±0.10^{Aa}	6.81±0.98^{Ca}	417.89±79.60^{Ba}	8.38±3.51^{Ic}
	2	7.67±0.12^{Aa}	6.76±0.98^{Bb}	417.63±77.45^{Ab}	8.53±3.47$^{\text{İb}}$
	3	7.69±0.13^{Aa}	6.75±0.95^{Bb}	417.25±75.47^{Ac}	8.67±3.43^{Ia}
May	1	7.69±0.13^{Aa}	6.76±0.93^{Da}	416.33±73.74^{Ca}	8.80±3.40^{Hc}
	2	7.70±0.13^{Aa}	6.74±0.91^{Ba}	407.14±83.61^{Cb}	9.04±3.51^{Ib}
	3	7.70±0.13^{Aa}	6.73±0.89^{Ba}	398.83±90.68^{Fc}	9.27±3.60^{Ha}
June	1	7.70±0.13^{Aa}	6.73±0.87^{Da}	393.63±92.23$^{\text{İa}}$	9.48±3.67^{Gc}
	2	7.70±0.12^{Aa}	6.67±0.90^{Cb}	391.48±90.96$^{\text{İb}}$	9.76±3.84^{Gb}
	3	7.70±0.12^{Aa}	6.63±0.91^{Cb}	389.96±89.50^{Jc}	10.00±3.96^{Ga}
July	1	7.70±0.12^{Aa}	6.62±0.89^{Ea}	391.19±88.03^{Ja}	10.24±4.08^{Ec}
	2	7.70±0.12^{Aa}	6.52±1.00^{Eb}	390.50±86.50^{Jb}	10.64±4.50^{Fb}
	3	7.70±0.12^{Aa}	6.46±1.03^{Dc}	390.31±84.98$^{\text{İc}}$	11.01±4.85^{Ea}
August	1	7.70±0.11^{Aa}	6.39±1.08^{Ga}	394.93±87.21^{Ia}	11.39±5.18^{Dc}
	2	7.70±0.11^{Aa}	6.30±1.18^{Gb}	394.90±85.77^{Ia}	11.68±5.34^{Db}
	3	7.70±0.11^{Aa}	6.22±1.22^{Gc}	394.66±84.42^{Ib}	11.95±5.48^{Da}
September	1	7.70±0.11^{Aa}	6.19±1.23$^{\text{İa}}$	399.55±87.64^{Ha}	12.22±5.61^{Bc}
	2	7.70±0.11^{Aa}	6.09±1.33^{Ib}	399.26±86.35^{Hb}	12.36±5.58^{Cb}
	3	7.70±0.11^{Aa}	6.02±1.38^{Hc}	399.29±85.09^{Eb}	12.48±5.55^{Ba}

A, B, C, D, E, F, G, H, I, J, K, L Capital letters indicate the difference between months, and the difference between means having different letters in the same column is statistically significant ($p<0.05$).
a, b, c Lowercase letters indicate within the same month the difference between stations, and the difference between means having different letters the same column is statistically significant ($p<0.05$).

In this study, the changes in total hardness, COD, BOD, and TSS values depending on the season and stations were found to be statistically significant ($p<0.05$). The lowest total hardness change was measured as 127 mg/l $CaCO_3$, the highest was 195 mg/l $CaCO_3$; the lowest COD value was measured as 14.4 mg/l, the highest was 130 mg/l, the lowest BOD value was measured as 9 mg/l, the highest was 90 mg/l, the lowest TSS was measured as 3 mg/l and the highest was 455 mg/l (Table 3).

Table 3. Changes in total hardness, COD, BOD and TSS values of Karasu River depending on months and stations (Mean±Standard deviation).

Month	Station	Total hardness (mg/l $CaCO_3$)	COD (mg/l)	BOD (mg/l)	TSS (mg/l)
October	1	139.87±107.64^{Jc}	19.18±106.66^{Aa}	16.51±102.11^{Aa}	14.70±98.29^{Ea}
	2	142.00±0^{Kb}	17.40±0^{Ib}	10.00±0Jb	8.00±0^{Kb}
	3	150.00±8.76^{Ka}	17.20±0.22^{İc}	9.50±0.55^{Kc}	6.50±1.64^{Kc}
November	1	165.00±23.54^{Ab}	18.00±1.21^{Ha}	10.00±0.87^{Ja}	11.00±6.87^{İa}
	2	165.25±20.08^{Aa}	17.58±1.29^{Hb}	10.00±0.74^{Ja}	10.25±6.02^{Jb}
	3	160.40±20.44^{Ac}	16.94±1.74^{Kc}	9.80±0.77^{Jb}	10.20±5.33^{İb}
December	1	161.17±18.63^{Ba}	17.22±1.70^{Ja}	10.17±1.10^{İb}	13.50±9.00^{Ga}
	2	158.86±18.13^{Bb}	17.04±1.63^{Jb}	10.14±1.01^{İc}	12.43±8.73^{Fb}
	3	156.50±18.07^{Bc}	17.04±1.52^{Jb}	10.25±0.99^{İa}	11.75±8.34^{Gc}
January	1	155.78±17.12^{Ca}	17.26±1.56^{Ja}	10.44±1.09^{Ib}	12.67±8.28^{Ha}
	2	153.70±17.40^{Gb}	17.23±1.48^{İb}	10.50±1.04^{Ia}	11.90±8.18^{Hb}
	3	153.55±16.58^{Gc}	17.30±1.43^{Ia}	10.55±1.00^{Ia}	11.27±8.04^{Hc}
February	1	154.00±15.92^{Ea}	17.69±1.90^{Ia}	10.83±1.36^{Ha}	10.92±7.78^{Ja}
	2	152.23±16.49^{İb}	17.56±1.88^{Hb}	10.77±1.33^{Hb}	10.31±7.77^{İb}
	3	151.43±16.15^{İc}	17.41±1.89^{Hc}	10.75±1.28^{Hb}	9.93±7.61^{Jc}
March	1	152.87±16.51^{Hc}	17.56±1.90^{İc}	10.87±1.31^{Hc}	9.97±7.34^{Kc}
	2	153.94±16.51^{Fa}	18.71±4.87^{Gb}	11.63±3.23^{Gb}	10.47±7.37^{Ib}
	3	153.82±16.02^{Fb}	19.61±5.96^{Ga}	12.24±3.98^{Ga}	10.85±7.31a^{I}
April	1	153.89±15.56^{Fb}	20.41±6.68^{Gc}	12.72±4.37^{Gc}	11.14±7.20^{Ic}
	2	154.47±15.34^{Ca}	20.76±6.66^{Fb}	12.95±4.36^{Fb}	12.03±7.97^{Gb}
	3	154.45±14.95^{Ca}	21.17±6.74^{Fa}	13.20±4.39^{Fa}	13.33±9.64^{Fa}
May	1	154.62±14.60^{Da}	21.64±6.90^{Fc}	13.52±4.52^{Fc}	15.45±13.43^{Fc}
	2	154.32±14.33^{Db}	26.56±23.72^{Eb}	17.00±16.65^{Db}	35.43±93.19^{Eb}
	3	153.91±14.14^{Ec}	30.63±30.11^{Ca}	19.65±20.54^{Ca}	51.89±119.78^{Ca}
June	1	153.75±13.86^{Ga}	33.10±31.79^{Ba}	21.33±21.68^{Ba}	63.90±130.78^{Aa}
	2	153.12±13.93^{Hb}	32.80±31.18^{Ab}	21.14±21.26^{Ab}	62.14±128.39^{Ab}
	3	152.27±14.31^{Ic}	32.44±30.62^{Ac}	20.90±20.87^{Ac}	60.56±126.12^{Ac}
July	1	152.15±14.05^{İa}	32.23±30.06^{Ca}	20.76±20.49^{Ca}	59.65±123.82^{Ba}
	2	151.25±14.57^{Jb}	31.68±29.65^{Bb}	20.41±20.20^{Bb}	57.77±121.96^{Bb}
	3	151.24±14.32^{Jb}	31.12±29.28^{Bc}	20.05±19.94^{Bc}	55.98±120.19^{Bc}
August	1	152.37±15.34^{Ib}	30.89±28.81^{Da}	19.90±19.62^{Da}	54.45±118.44^{Ca}
	2	152.35±15.08^{Ib}	30.47±28.43^{Cb}	16.91±19.36^{Ec}	52.85±116.82^{Cb}
	3	152.72±14.98^{Ha}	30.05±28.07^{Dc}	19.31±19.12^{Db}	51.45±115.23^{Dc}
September	1	154.00±16.45^{Ec}	29.81±27.67^{Ea}	19.15±18.85^{Ea}	50.32±113.63^{Da}
	2	154.24±16.26^{Eb}	29.55±27.30^{Db}	18.97±18.60^{Cb}	49.01±112.19^{Db}
	3	154.31±16.03^{Da}	29.22±26.97^{Ec}	18.74±18.38^{Ec}	47.76±110.80^{Ec}

A, B, C, D, E, F, G, H, I, J, K, L Capital letters indicate the difference between months, and the difference between means having different letters in the same column is statistically significant ($p<0.05$).
a, b, c Lowercase letters indicate within the same month the difference between stations, and the difference between means having different letters the same column is statistically significant ($p<0.05$).

In this study we conducted in the Karasu River, the changes in total nitrogen, nitrate, nitrate nitrogen, ammonium, and ammonium nitrogen depending on the season and stations were found to be statistically significant ($p<0.05$). The mean total nitrogen concentration was determined as 4.75 mg/L. It was determined that total nitrogen concentration increased in February and March during the research period. In our work, the lowest nitrate concentration was measured as 2.15 mg/L and the highest as 7.06 mg/L. On the other hand, the lowest nitrate nitrogen concentration was determined as 0.48 mg/L and the highest as 2.83 mg/L. Nitrate concentration, similar to the total nitrogen concentration, was found to be higher in February and March than in other months. The mean values of ammonium and ammonium nitrogen concentrations were measured as 3 mg/L and 2.24 mg/L, respectively (Table 4).

Table 4. Changes in TN, NO_3, NO_3-N, NH_4, NH_4-N concentrations of Karasu River depending on months and stations (Mean±Standard Deviation, mg/L).

Month	Station	TN	NO_3	NO_3-N	NH_4	NH_4-N
October	1	4.82±0.40^{Aa}	5.47±0.19^{Aa}	1.96±0.02^{Aa}	3.58±0.67^{Aa}	2.32±0.06^{Aa}
	2	4.36±0^{Fb}	4.94±0^{Kb}	1.12±0^{Eb}	3.05±5.44^{Ac}	2.37±0^{Bc}
	3	4.41±0.05^{Eb}	4.78±0.18^{Jc}	1.08±0.05^{Gc}	3.58±0.58^{Ab}	2.78±0.45^{Ab}
November	1	4.45±0.08^{Ha}	5.54±1.15^{İb}	1.25±0.27^{Fa}	3.19±0.74^{Ba}	2.48±0.57^{Ba}
	2	4.37±0.16^{Eb}	5.48±0.99^{İc}	1.24±0.23^{Da}	3.04±0.69^{Ab}	2.36±0.54^{Bb}
	3	4.39±0.15^{Eb}	5.64±0.94^{Ha}	1.27±0.22^{Ea}	2.95±0.64^{Cc}	2.29±0.49^{Cc}
December	1	4.51±0.31^{Gc}	6.52±2.19^{Eb}	1.47±0.50^{Ca}	2.77±0.71^{Fb}	2.15±0.55^{Ec}
	2	4.58±0.34^{Db}	6.36±2.06^{Ea}	1.43±0.47^{Ba}	2.81±0.66^{Da}	2.23±0.55^{Cb}
	3	4.65±0.37^{Ca}	6.24±1.94^{Ec}	1.40±0.45^{Cb}	2.83±0.62^{Ea}	2.30±0.54^{Ca}
January	1	4.77±0.48^{Eb}	6.83±2.49^{Ca}	1.54±0.58^{Ba}	2.85±0.59^{Ec}	2.31±0.51^{Db}
	2	4.81±0.47^{Bb}	6.70±2.38^{Cb}	1.51±0.55^{Aa}	2.93±0.60^{Cb}	2.36±0.50^{Ba}
	3	4.87±0.49^{Ba}	6.57±2.31^{Cc}	1.48±0.54^{Bb}	2.99±0.61^{Ca}	2.40±0.50^{Ba}
February	1	5.01±0.66^{Ca}	7.06±2.76^{Ba}	1.59±0.64^{Ba}	3.01±0.58^{Db}	2.41±0.48^{Ca}
	2	5.00±0.64^{Aa}	6.93±2.69^{Ab}	1.56±0.62^{Aa}	3.05±0.58^{Aa}	2.43±0.46^{Aa}
	3	5.01±0.61^{Aa}	6.84±2.62^{Ac}	1.54±0.60^{Aa}	3.08±0.57^{Ba}	2.45±0.45^{Ba}
March	1	5.07±0.64^{Ba}	7.03±2.63^{Ba}	1.59±0.60^{Ba}	3.08±0.55^{Ca}	2.44±0.44^{Ba}
	2	4.97±0.72^{Ab}	6.89±2.60^{Bb}	1.56±0.60^{Aa}	2.99±0.63^{Bb}	2.37±0.51^{Bb}
	3	4.89±0.77^{Bc}	6.73±2.60^{Bb}	1.52±0.60^{Ab}	2.92±0.68^{Dc}	2.31±0.55^{Cc}
April	1	4.83±0.80^{Da}	6.65±2.55^{Da}	1.50±0.59^{Ca}	2.88±0.68^{Ea}	2.27±0.56^{Da}
	2	4.73±0.87^{Cb}	6.52±2.54^{Db}	1.47±0.58^{Ba}	2.79±0.77^{Db}	2.20±0.63^{Cb}
	3	4.66±0.90^{Cc}	6.40±2.53^{Dc}	1.45±0.58^{Ba}	2.71±0.82^{Fc}	2.14±0.66^{Dc}
May	1	4.63±0.89^{Fa}	6.33±2.49^{Fa}	1.43±0.57^{Da}	2.67±0.82^{Ga}	2.11±0.66^{Ea}
	2	4.58±0.90^{Db}	6.27±2.44^{Fb}	1.42±0.56^{Ba}	2.65±0.81^{Fb}	2.09±0.65^{Da}
	3	4.54±0.90^{Db}	6.22±2.40^{Eb}	1.40±0.55^{Ca}	2.63±0.79^{Gc}	2.08±0.64^{Ea}
June	1	4.48±0.93^{Ha}	6.15±2.38^{Ga}	1.39±0.54^{Da}	2.60±0.80^{Ha}	2.05±0.65^{Fa}
	2	4.42±0.96^{Eb}	6.04±2.39^{Gb}	1.36±0.55^{Ca}	2.55±0.82^{Ga}	2.01±0.66^{Fa}
	3	4.37±0.97^{Fb}	5.94±2.39^{Fc}	1.34±0.55^{Da}	2.51±0.83^{Gb}	1.98±0.67^{Fb}
July	1	4.37±0.95^{Ia}	5.91±2.35^{Ha}	1.34±0.54^{Ea}	2.50±0.81^{Ia}	1.96±0.66^{Ga}
	2	4.36±0.94^{Fa}	5.81±2.38^{Hb}	1.31±0.54^{Ca}	2.53±0.81^{Ga}	1.99±0.66^{Fa}
	3	4.36±0.92^{Fa}	5.71±2.39^{Gc}	1.29±0.55^{Da}	2.54±0.80^{Ha}	2.00±0.65^{Fa}
August	1	4.39±0.93^{Ia}	5.64±2.38^{Ia}	1.27±0.54^{Fa}	2.61±0.87^{Hb}	2.05±0.70^{Fa}
	2	4.39±0.91^{Ea}	5.54±2.41^{Ib}	1.25±0.55^{Da}	2.64±0.88^{Fa}	2.07±0.70^{Ea}
	3	4.39±0.90^{Ea}	5.45±2.43^{Ic}	1.23±0.55^{Ea}	2.66±0.87^{Fa}	2.09±0.69^{Ea}
September	1	4.41±0.89^{Ia}	5.47±2.39^{Ja}	1.24±0.55^{Fa}	2.68±0.86^{Gb}	2.10±0.68^{Eb}
	2	4.42±0.88^{Ea}	5.38±2.43^{Jb}	1.21±0.55^{Da}	2.71±0.88^{Ea}	2.13±0.69^{Da}
	3	4.44±0.87^{Ea}	5.28±2.45^{İc}	1.19±0.56^{Fa}	2.75±0.89^{Fa}	2.16±0.71^{Da}

A, B, C, D, E, F, G, H, I, J, K, L Capital letters indicate the difference between months, and the difference between means having different letters in the same column is statistically significant ($p<0.05$).
a, b, c Lowercase letters indicate within the same month the difference between stations, and the difference between means having different letters the same column is statistically significant ($p<0.05$).

4. Discussion and Conclusion

Water samples were taken from the sections of Karasu River before and after the Erzurum Province Wastewater Treatment Plant, and nitrogen and nitrogen fractions were analysed. During our research, the *in situ* mean water temperature was 13.8 ˚C, the mean pH value was 7.7, the mean electrical conductivity was 403 µs/cm, and the mean dissolved oxygen value was 6 mg/l. The mean total suspended solids (TSS) amount was determined as 46.7 mg/l, the mean chemical oxygen demand (COD) concentration was 28.9 mg/l, the mean biological oxygen demand (BOD) concentration was 18.5 mg/l, the mean total nitrogen (TN) concentration was 4.47 mg/l, the mean nitrate (NO_3) concentration was 5.35 mg/l, the mean nitrate nitrogen (NO_3-N) concentration was 1.21 mg/l, the mean total hardness (TH) was 155 mg/l, the mean ammonium (NH_4) concentration was 2.75 mg/l, the mean ammonium nitrogen (NH_4-N) concentration was 2.16 mg/L.

When the data obtained in this research was evaluated according to the Intra-Continental Surface Water Standards (Orman ve Su İşleri Bakanlığı, 2015), it was determined that the Karasu River water was in Class I waters according to the water temperature and the nitrate nitrogen. pH, electrical conductivity and COD in the Class II waters according to the dissolved oxygen value, in the Class III waters according to the BOD and total nitrogen value and in the Class IV waters according to and ammonium nitrogen concentrations (Table 5).

Table 5. Intra-Continental Surface Water Standards (Orman ve Su İşleri Bakanlığı, 2015) and the Karasu River water class.

Parameter	I. Class	II. Class	III. Class	IV. Class	Water quality of Karasu River	Class
Water temperature (°C)	≤ 25	≤ 25	30 ≤	30 ≤	*13.8*	*I.*
pH	6.5-8.5	6.5-8.5	6.0-9.0	< 6.0 or > 9.0	*7.7*	*II.*
Electrical conductivity (µS/cm)	< 400	1000	3000	> 3000	*403*	*II.*
NH_4-N (mg/L)	< 0.2	1	2	> 2	*2.16*	*IV.*
NO_3-N (mg/L)	< 5	10	20	> 20	*1.21*	*I.*
TN (mg/L)	< 0.5	1.5	5	> 5	*4.47*	*III.*
BOD (mg/L)	< 4	8	20	> 20	*18.5*	*III.*
COD (mg/L)	< 25	50	70	> 70	*28.9*	*II.*

In a study investigating heavy metal pollution in the Karasu River, various heavy metals such as Copper (Cu), Zinc (Zn), Manganese (Mn), Lead (Pb), Nickel (Ni), Cadmium (Cd) and Iron (Fe) were assayed. Researchers reported that the water quality of the water samples was in classes IV, IV, IV, II and II, respectively (Sönmez et al., 2013). In another study evaluating the river according to its heavy metal concentrations, it was reported that the river faces intense pollution elements and that if the situation continued, the ecological balance could be negatively impacted (Sönmez et al., 2012). Similarly, in this research, it was determined that it is in the I, II, III and IV water classes.

In this study, when the differences between the stations were examined, it was determined that although higher values were measured from station 1 due to the season, the values at station 3 were measured lower. When we look at the values of TN, ammonia nitrogen and nitrate nitrogen in particular, the average values were found to be 0,96 mg/L, 2,61 mg/L, 4,17 mg/L at 1st station respectively, while these values 1,69 mg/L, 2,50 mg/L, 7,45 mg/L were found to be at 3rd station. However, the decrease in the concentration of ammonia nitrogen and the increase in the concentration of nitrate nitrogen, which is used by plants, may also affect the low plant biomass in the 3rd stations. Meanwhile, when COD and BOD values were compared, it was determined that the values at the 3rd station (28.78 mg/L and 18.42 mg/L) were lower than the values at the 1st station (29.77 mg/L and 19.38 mg/L). As a result of this indicates that the water quality of the Karasu River did not change or little different of water quality parameter, and some parameters were lower than 1st station after the treatment plant outlet water and that the treatment plant made a positive contribution. The Karasu River is not used source as a drinking water. In this research was measured the high levels of nitrogen and its fractions in the river, and its showed that there is an anthropogenic effect on there. For this reason, mass fish deaths, increased mosquito formation and diarrhea-like illnesses are observed, especially during periods when temperatures increase

In addition to this, in a study conducted between December 2015 and November 2016, it was determined that the water quality of the Karasu River exceeded the limit values for NH_4-N, BOD_5, Co, fecal coliform, Ba, Cu, Zn and dissolved oxygen parameters according to the regulation on the quality of surface waters and that they are the main parameters affecting the quality of the water. Alver and Baştürk (2019) reported that the water quality of the Karasu River was determined to be "moderate" according to the National Sanitation Foundation Water Quality Index (NSF WQI), "poor" according to the Canadian Council of Ministers of the Environment Water Quality Index (CCME WQI) and "poor" according to the Oregon Water Quality Index (OWQI). However, our current study shows that when all parameters are examined, a medium (III. Class) pollution level is generally determined.

In conclusion, according to some water quality parameter values, the Karasu River is in the I, II, III and IV water classes. It is thought that the river is under the influence of anthropogenic pollutants. Therefore, it is recommended to regularly monitor the river and take the necessary precautions to reduce its effects.

Conflict of Interest

The authors declare that they have no conflict of interest.

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http://agriprojournal.com
JOURNAL OF AGRICULTURAL PRODUCTION
ISSN: 2757-6620
PRENSIP
https://prensip.gen.tr

RESEARCH ARTICLE

Determination of Cytotoxic and Apoptotic Properties of Lobaric Acid, a Secondary Metabolite of Lichen and Investigation of Its Theoretical Potential

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ARTICLE INFO

Article History
Received: 22.07.2024
Accepted: 13.09.2024
First Published: 30.09.2024

Keywords
Apoptosis
Cytotoxicity
Lobaric acid
Molecular docking

ABSTRACT

In this study, we aimed to elucidate some of the mechanisms of cell death induced by lobaric acid in A549 (human lung cancer) cells. For this purpose, the effects of cytotoxic concentrations on p53 and caspase-3 gene expressions were investigated. A549 cells were treated with varying concentrations of lobaric acid (12.5, 25, 50, and 100 μg/ml) for 48 hours and then their viability was evaluated and p53 and caspase-3 mRNA expressions were determined at statistically cytotoxic concentrations of 12.5, 50, and 100 μg/ml. According to beta-actin, it was determined that the increase in lobaric acid concentration revealed an upward trend in p53 and caspase-3 mRNA expressions. Furthermore, quantum chemical parameters such as frontier molecular orbitals, band gap energy and ionization potential, electronic affinity, chemical softness, chemical potential, electrophilicity index and chemical hardness were analyzed. Furthermore, molecular docking was performed to identify the binding sites and the binding behavior of lobaric acid to some target proteins (P53, Caspase-3 and Bcl-2).

Please cite this paper as follows:
Kızıl, H. E., Ağar, G., & Ekincioğlu, Y. (2024). Determination of cytotoxic and apoptotic properties of lobaric acid, a secondary metabolite of lichen and investigation of its theoretical potential. *Journal of Agricultural Production*, *5*(3), 192-200. https://doi.org/10.56430/japro.1518450

1. Introduction

Health problems constitute an important part of the problems that arise in parallel with the continuous change in our world. The frequency and high mortality of cancer, one of the most important health problems of today, has caused the disease to become an important public health problem. The rate of spread of the first six types of cancer published by the International Agency for Research on Cancer in 2002 is as follows: lung, stomach, bladder, colorectal, laryngeal, larynx and prostate cancers in men, and breast, colorectal, stomach, ovarian, lung and leukemia in women (Çakabay, 2012). Cancer is a multistep and multifactorial disease caused by hereditary and environmental factors. Somatic and inherited mutations, diet and environmental factors are considered to be the causes of cancer (Lichtenstein et al., 2000). For years, scientists have been making intensive efforts for the diagnosis and treatment of cancer, which is a multistep and multifactorial disease caused by hereditary and environmental factors. Studies have been conducted on molecular changes that are effective in the emergence of cancer stages, and alternative treatment methods

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and active substance applications have been used for centuries in the treatment of the disease (Takayama et al., 2006). The use of natural complementary and alternative products has become widespread due to the fact that chemotherapy and radiotherapy methods and surgical operations used in the treatment process of cancer do not show enough benefit (Emsen, 2015). The most remarkable group of organisms used in these studies are lichens. It is reported that more than 17000 species of lichens have been identified since the 18th century and more than 2000 species are found in North America. Initially believed to be a plant, lichens were later understood to be symbiotic organisms consisting of a fungal partner called a mycobiont and a photosynthetic partner called a photobiont, which may be one or more algae or cyanobacteria, as a result of studies by Schwender and Trebox (Aprile et al., 2011). Lichens are completely separate from the fungi and algae that make up their structure (Nash, 1996). Lobaric acid, secondary metabolites isolated from the Antarctic lichen *Stereocaulon alpnum*, is characterized by its significant bioactivity, demonstrating a range of pharmacological properties and represents a unique class of bioactive molecules with substantial potential for therapeutic applications (Seo et al., 2009). Moreover, these compounds have been demonstrated to exhibit a spectrum of biological activities, encompassing anti-proliferative, anti-inflammatory, antioxidant, and antimicrobial effects. Their therapeutic potential extends to a variety of pathological conditions, highlighting their multifaceted bioactivity and the promise they hold for future pharmacological development (Ögmundsdóttir et al., 1998). Lobaric acid was reported to have high antimycobacterial activity (Ingólfsdóttir et al., 1998). Moreover, in another study, it was shown that lobaric acid had antiproliferative and cytotoxic effects on breast cancer cells T-47D and ZR-75-1 and leukemia cells K-562 according to 5-lipoxygenase inhibitory activity (Ögmundsdóttir et al., 1998). The cytotoxic and antioxidative properties of lobaric, atranorin, salazinic, barbatic, α-alectoronic, α-collatolic, cryptochlorofeic, caperatic and protolicesterinic acid obtained from 6 lichen species collected from Brazil and Antarctica were determined and certain NMR and cytotoxicity profiles were established (Ravaglia et al., 2014). Morita et al. (2009) emphasized that lobaric acid has an inhibitory effect on tubulin polymerization (Morita et al., 2009). Thadhani et al. (2012) found that lobaric acid showed high antimicrobial activity against *Escherichia coli* bacteria (Thadhani et al., 2012). In another study, it was reported that lobaric acid and lecanoric acid showed very strong antioxidant properties (Thadhani et al., 2011). Based on the findings of these studies, in this study, the cytotoxic and apoptotic properties of lobaric acid in A549 (human lung cancer) cell line was determined and molecular docking studies were performed with p53, Caspase-3 and Bcl-2 proteins.

2. Materials and Methods

2.1. Cell Culture

To observe the cytotoxic and apoptotic effect of lobaric acid, A549 (human lung cancer) cell line was purchased from Santa Cruz Biotech (Heidelberg-Germany). Stock cells stored at -196 °C were removed and thawed. Cells were centrifuged at 2000 rpm and the filtrate was discarded and transferred into 25 ml flasks with 10% FBS (Biochrome AG). Passaging was performed for 24 - 48 hours in a light-free environment with 5% CO_2 (CO_2 Incubator Thermo). After transfer to larger flasks (Greiner), dead and waste cells were removed by washing with 8 ml PBS. In order for the flasks with a polylysine layer to release the cells, 2-3 ml trypsin was added and left for 2-3 min. After the cells in the flask were examined under inverted microscope, 3 ml of FBS was added to eliminate the toxic effect of trypsin and the cells were transferred to a falcon tube. After centrifugation at 2000 rpm for 5 min, the filtrate was discarded and up to 1 ml of DMEM (Gibco) was added to the tube. A mixture of 20 µl of cells and 20 µl of trypan blue (Sigma) was loaded into a hemocytometer and cell count was performed. After cell counting, $1x10^6$ cells/ml cells were transferred to each well of 96-well plates. This number was incubated for 24 hours to reach 2-fold and growth was observed under the microscope (Zeiss-Germany). Lobaric acid was first dissolved in 5 ml DMSO (Merck) + 995 ml DMEM. Then the concentrations of the substances were adjusted by serial dilution. Different concentrations of lichen acids were added to the cultured cells in triplicate except for the control group. 100 µl of 10% FBS (9 ml DMEM 1 ml FBS) and 100 µl of 10% DMSO were used as control groups (Kapuci et al., 2014).

2.2. Wst-1 Analysis

The 96-well microplate with a cell density of $2x10^6$ cells/ml, incubated in an incubator at 37 °C and 5% CO_2 for 24 hours, was removed from the incubator and the supernatants in the wells were removed. Lobaric acid was added to the wells at the determined concentrations (12.5, 25, 50, 100 µg/ml) except for the negative control group. After 100 µl of 10% FBS (9 ml DMEM 1ml FBS) was added to the control wells, they were kept in incubation for 48 hours to see the effect of the substance on the cells. After 48 hours, the cells taken from the incubation were examined morphologically under an inverted microscope and the optimum time period for application was determined. At this stage, the yellow WST-1 (Roche-Germany) dye turned into blue purple formazone as a result of the breakdown of the tetrazolium ring. After removal from incubation, absorbance values at 490/690 nm wavelength were calculated on a microplate reader (Bio-Tek Powervawe-Winooski). Cell viability was calculated using the following formula: Cell Viability = (Sample Absorbance / Control Absorbance) X 100 (Kapuci et al., 2014).

2.3. Gene Expression Analysis

In the initial phase of the experiment, total RNA was meticulously extracted from cells treated with lobaric acid using the Qiagen RNeasy mini kit (Qiagen, Germany), adhering to established protocols for optimal yield and purity. Following RNA isolation, the synthesis of complementary DNAs (cDNAs) was performed using the Qiagen RT2 First Strand cDNA synthesis kit (Qiagen, Germany), ensuring the accurate transcription of mRNA into stable cDNA for subsequent analysis. In the final analytical phase, the relative mRNA transcript levels of the genes encoding cysteine aspartate-specific protease-3 (caspase-3) and tumor protein p53 were quantitatively assessed through the RT-PCR technique. This process involved preparing a reaction mixture comprising gene-specific primers, synthesized cDNAs, and SyberGreen Master Mix (Qiagen, Germany), followed by initiation of the reaction in the ROTORGENEQ system (Qiagen, Germany). The data obtained from qRT-PCR were rigorously analyzed using the comparative threshold cycle (Ct) method, as described by Livak and Schmittgen (2001). ACTB was employed as an internal control to normalize the expression levels and ensure data accuracy.

2.4. Computational Study

Density functional theory (DFT) calculations were carried out using Gaussian 09 package software and Gaussian files were displayed using the molecular visualization application Gauss-View (Frisch et al., 2009). The optimized structural geometry of lobaric acid was determined utilizing the B3LYP functional and 6-311++G (d, p) basis set. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energy research was performed by the same level. The Auto-Dock Vina software (Trott & Olson, 2010) application was used to calculate the binding affinities and types of interactions between the ligand (lobaric acid) and target proteins. In addition, Discovery Studio software (Biovia, 2019) was utilized to view the ligand and protein's expected docking poses.

2.5. Statistical Analyzes

The experiment was carried out in triplicate, and the results are reported as the mean ± standard deviation. Statistical analysis was conducted using SPSS 18.0 software, with a two-way analysis of variance (ANOVA). Duncan's multiple comparison test was applied to assess differences between the means, with a significance level of 5%.

3. Results and Discussion

3.1. Cytotoxicity Results

A549 cells were treated with 4 different concentrations of lobaric acid (12.5, 25, 50 and 100 µg/ml) for 48 hours and viability rates were determined (Table 1). Although all concentrations applied for treatment caused a decrease in viability rates, no concentration was found to be statistically significant ($p<0.05$).

Table 1. Cell viability results of 48^{th} hour lobaric acid treatment (SD±: standart deviation, % Viability: WST-1 assay results. Mean: the average of the test results performed in triplicate).

Concentrations	Mean±SD (% Viability)
Control	100±0
100 µg/ml	90.9±1.32
50 µg/ml	94.0±1.25
25 µg/ml	95.5±0.96
12.5 µg/ml	95.8±0.88

3.2. mRNA Expression Results

By using qRT-PCR, the expression profiles of the p53 and Caspase-3 genes in the A549 cells in the control and treatment groups were assessed. Since 25 and 12.5 µg/mL concentrations were similar according to the cytotoxicity results, 100, 50 and 12.5 µg/mL concentrations were used to determine mRNA expression levels. In the relative evaluation of both p53 and Caspase-3 mRNA expression levels compared to beta actin, it was determined that as the concentration increased, mRNA expression levels also increased. This means that the expression levels of apoptotic genes increased with lobaric acid treatment (Figure 1).

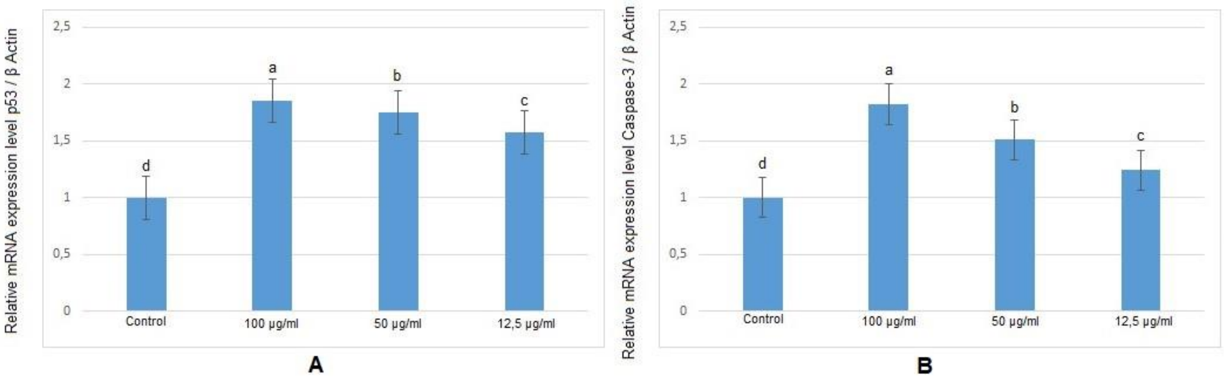

Figure 1. Effects of lobaric acid treatments on p53/Caspase-3 mRNA expression levels in A549 cells. **(A)** p53 mRNA transcript levels, **(B)** Caspase-3 mRNA transcript levels. Values are expressed as mean ± SD. Different letters (a–d) on the columns show a statistical difference ($p < 0.05$).

3.3. Frontier Molecular Orbitals

Frontier Molecular Orbitals (FMOs) are utilized to study the reactive regions in molecular systems. Additionally, they determine the chemical reactivity of the molecules. FMOs are known as HOMO and LUMO. HOMO is the highest occupied molecular orbital and also an electron-rich orbital while LUMO is the lowest unoccupied molecular orbital and also an electron-poor orbital. These two molecular orbitals are very important for understanding quantum chemistry, biological processes, electrical and optical characteristics, and medicinal research (Ekincioğlu, 2023; Srivastava, 2021). The energy difference between HOMO and LUMO orbitals is known as the band gap energy which indicates a molecule's stability and reactivity. If a molecule's band gap energy is large, it is more stable or has less chemical reactivity. The HOMO and LUMO molecular orbital distributions calculated are presented in Figure 2.

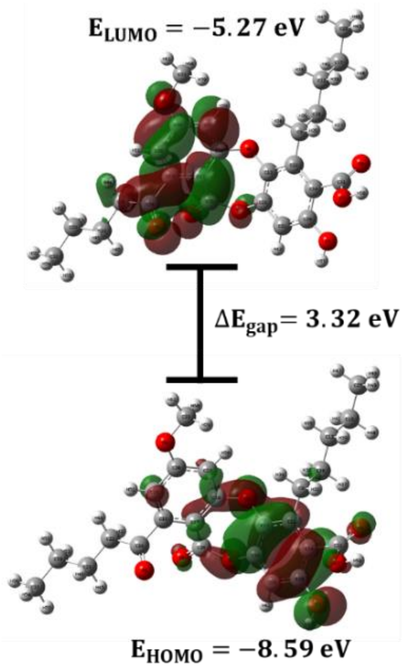

Figure 2. HOMO and LUMO patterns of lobaric acid.

FMOs analysis of the lobaric acid molecule showed that HOMO is located on the benzene ring region containing atoms C11 and C21, whereas LUMO is localized on the benzene ring region containing atoms C19 and C27. As a result of the calculations, the HOMO, LUMO, and band gap energy values were determined to be -8.59, -5.27, and 3.32 eV, respectively. Quantum chemical descriptors of a molecule can be determined utilizing FMOs, and they can be derived from Koopman's theorem which represents a theoretical method for connecting the chemical activities (ionization potential (IP), electronic affinity (EA), Chemical softness (S), Chemical potential (μ), electrophilicity index (ω) and chemical hardness (η)) of molecular structures to their electronic properties (Koopmans, 1934) and they may be utilized to study pharmacological characteristics and interactions with biological targets (González-González et al., 2023; Srivastava, 2021). The obtained quantum chemical descriptors values are presented Table 2. The quantum chemical descriptors parameters' mathematical definitions are as following equations:

$$IP = -E_{HOMO} \quad (1)$$

$$EA = -E_{LUMO} \quad (2)$$

$$S = \frac{1}{\eta} \quad (3)$$

$$\mu = -\frac{(IP+EA)}{2} \quad (4)$$

$$\omega = \frac{\mu^2}{2\eta} \quad (5)$$

$$\eta = \frac{(IP-EA)}{2} \quad (6)$$

Table 2. The HOMO, LUMO, Band gap energies and quantum chemical parameters.

Parameters	Values (eV)
E_{HOMO}	-8.59
E_{LUMO}	-5.27
Band gap energy (ΔE_{gap})	3.32
Ionization potential	8.59
Electron affinity	5.27
Chemical softness	0.60
Chemical potential	-6.93
Electrophilicity index	14.46
Chemical hardness	1.66

The IP and EA were calculated as 8.59 and 5.27 eV, respectively. The high IP values indicate a lower losing electron, whereas the high EA values indicate a greater accepting electron. The η and S of a molecule play a key role in defining its chemical reactions (Fahim & Farag, 2020). The obtained S and η values are 0.60 and 1.66 eV, respectively. To assess the chemical reactivity and bind to the molecule's biomolecules (Cárdenas et al., 2009; Mushtaq et al., 2024) the µ and ω values are essential. These values are obtained as -6.93 and 14.46 respectively. As a result, we can say that the molecule examined is hard and has good electrophilic behavior.

3.4. Molecular Docking

Molecular docking was used to investigate the binding mode, affinity, and potential interactions between P53, Caspase-3 and Bcl-2 genes and lobaric acid. the crystal structures of p53 (PDB: 6BSC), Caspase-3 (PDB: 3GJQ) and Bcl-2 (PDB:4MAN) were downloaded from the RCSB Protein Data Bank (https://www.rcsb.org) as the docking templates. We used Open Babel to get the ligand ready for docking (O'Boyle et al., 2011). We employed a docking approach that was previously reported (Ekincioğlu, 2023). For interaction between 6BSC (p53) and ligand, the coordinates for the center of the search space were −19.262, 11.658, and −4.339 Å along the x-, y-, and z-axis, respectively, for a cubic grid box with 90×90×90 Å sides. For interaction between 3GJQ (Caspase-3) and ligand, the coordinates for the center of the search space were 17.908, 14.402, and 17.76 Å along the x-, y-, and z-axis, respectively, for a cubic grid box with 90×90×90 Å sides. For interaction between 4MAN (Bcl-2) and ligand, the coordinates for the center of the search space were −12.102, -4.527, and −4.341 Å along the x-, y-, and z-axis, respectively, for a cubic grid box with 90×90×90 Å sides. Internal conformational search was conducted using the Lamarckian Genetic Algorithm. Hydrogen atoms and Kollman-type charges were added to the proteins by using AutoDock tools (Trott & Olson, 2010). Biovia discovery software was used to view the interactions between lobaric acid and proteins (Table 3) (Biovia, 2019).

Table 3. RMSD, Binding Energy an inhibition constant of different poses of Lobaric acid with 6BSC, 3GJQ and 4MAN as predicted by Auto dock Vina.

	Lobaric acid and 6BSC			Lobaric acid and 3GJQ			Lobaric acid and 4MAN		
Mode	RMDS	Binding Energy (kcal/mol)	Inhibition Constant (Ki) (mM)	RMDS	Binding Energy (kcal/mol)	Inhibition Constant (Ki) (mM)	RMDS	Binding Energy (kcal/mol)	Inhibition Constant (Ki) (mM)
1	34.73	-1.53	74.98	44.01	-1.50	79.29	21.41	-2.43	16.62
2	20.87	-1.50	80.17	43.47	-0.94	-	16.23	-1.69	57.96
3	30.05	-1.49	80.42	41.68	-1.29	113.22	24.08	-1.61	65.86
4	33.05	-1.31	109.08	35.52	-1.01	181.24	20.70	-1.59	68.28
5	30.76	-0.98	192.08	4023	-0.99	188.49	28.04	-1.34	104.99
6	21.39	-0.92	212.31	33.82	-0.80	259.11	19.62	-1.31	108.98
7	21.48	-0.81	254.89	37.79	-0.72	294.30	17.71	-1.25	120.35
8	36.22	-0.25	654.78	40.11	-0.56	386.52	15.07	-1.13	149.38
9	19.60	-0.13	800.13	38.38	-0.51	421.71	15.67	-0.74	288.13
10	26.36	-0.03	944.06	41.44	-0.40	509.60	19.96	-0.07	884.26

The obtained results between P53, Caspase-3 and Bcl-2 genes and lobaric acid are presented in Table 1 and Figure 3. We calculated root-mean-square deviation (RMSD), a commonly utilized measure of protein and ligand stability in complexes. The obtained results reveal that the RMDS values between proteins and ligand are 34.73, 44.01, and 21.41 for 6BSC, 3GJQ, and 4MAN, respectively. The binding affinities obtained as a result of interaction for 6BSC, 3GJQ, and 4MAN are -1.53, -1.50 and -2.43 kcal/mol-1, respectively. The calculated inhibition constant values as a result of the interaction between proteins and ligand were 74.98 (6BSC,), 79.29 (3GJQ,) and 16.62 (4MAN) mM. As a result, we can say that the best docking score is between 4MAN and lobaric acid.

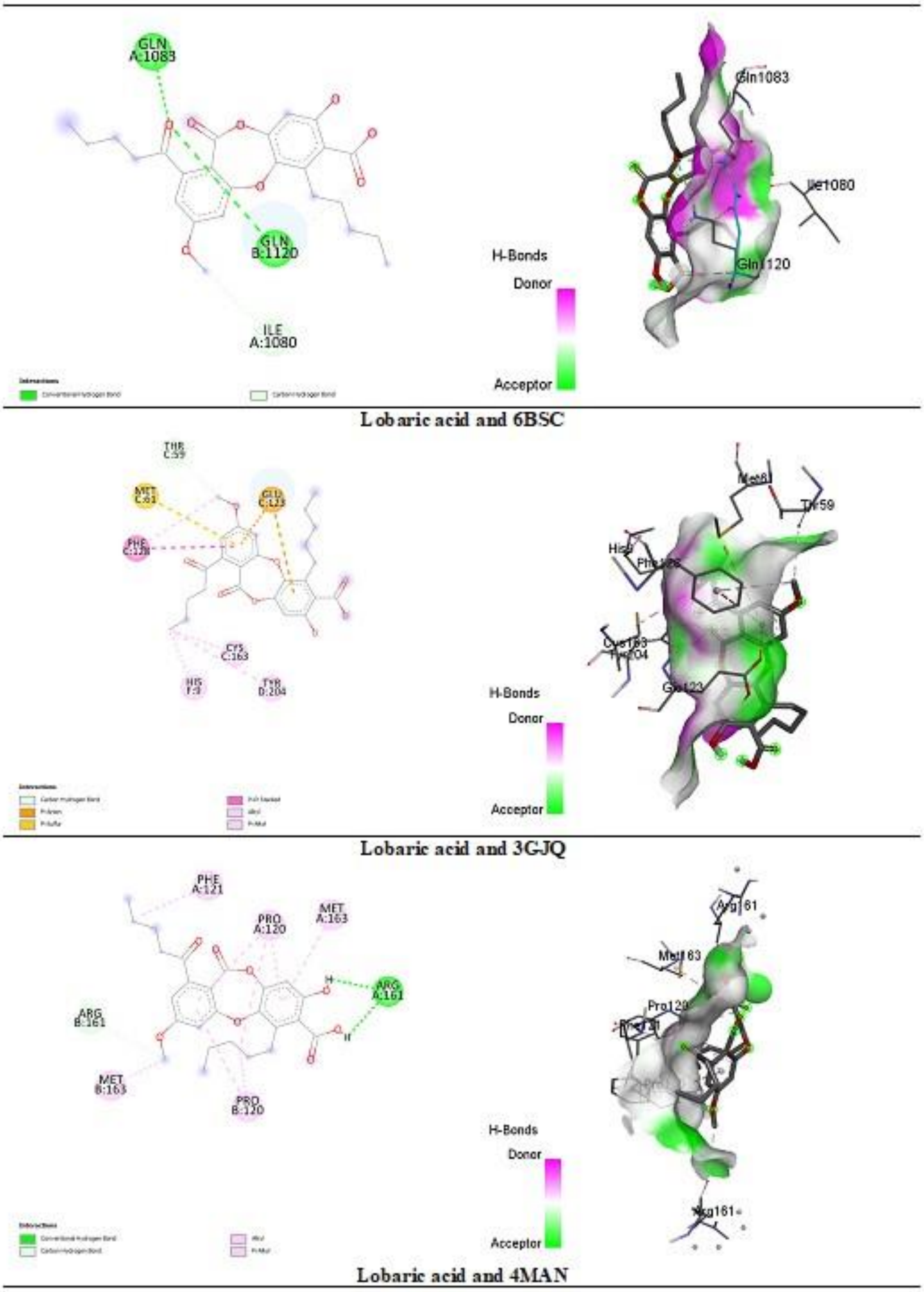

Figure 3. 2D molecular interaction and hydrogen binding surface of Lobaric acid with 6BSC, 3GJQ, and 4MAN.

The 2D molecular interaction and the hydrogen surface of lobaric acid with 6BSC, 3GJQ, and 4MAN is presented in figure 3. The active sites for 6BSC were determined as GLN 1083, GLN 1120, and ILE 1080, wherein green and light green balls represent conventional hydrogen bonds and carbon hydrogen bonds, respectively. The active sites for 3GJQ were found as GLU 123, MET 61, THR 59, PHE 128, CYS 163, HIS 9, and THR 204, wherein light green balls, gold balls, light gold balls, pink balls, and light pink balls represent carbon hydrogen bonds, Pi-Anion, Pi-Sulphur, Pi-Pi stacked, alkyl, and Pi-alkyl interactions, respectively. The active sites for 4MAN were determined as ARG A: 161, ARG B: 161, PHE A: 121 PRO A-B:120, and MET A-B: 163, wherein green, light green balls, light pink balls, and light pink balls represent conventional hydrogen bond and carbon hydrogen bonds, alkyl and pi-alkyl interactions, respectively.

Lichens, a group of symbiotic organisms dating back 400 million years and different from plant species, have attracted researchers due to their ability to synthesize a high amount and variety of metabolites at once and their biological activities have been the subject of various studies for years (Spribille et al., 2022). Especially considering the inadequacy of various chemotherapeutics and radiotherapy agents used in cancer treatment studies and their failure to obtain a definite response to treatment, interest in lichens with antimicrobial, antitumoral, antifungal and antioxidant properties has increased and many successful studies have been carried out by using both lichen extracts and lichen active ingredients (Aydın, 2012; Çapık, 2014). Hong et al. (2018) assessed how lobaric acid and lobarstin affect human cervix adenocarcinoma HeLa cells and colon carcinoma HCT116 cells, demonstrating that treatment with these compounds significantly reduced the proliferation of both HeLa and HCT116 cells in a manner dependent on both dosage and duration. In another study conducted in the same year, Emsen et al. (2018) showed that LA was highly toxic on Glioblastoma multiforme (GBM) and primary rat cerebral cortex (PRCC) cells. In another study on lobaric acid, lobaric acid was found to have a high inhibitory effect on 12(S)-lipoxygenase platelets (Bucar et al., 2004). Andania et al. (2019) conducted a study highlighting the potent activity of the methanolic extract derived from *A. submutica* rhizomes against the MCF-7 breast adenocarcinoma cell line, achieving an IC50 value of 70.95 µg/mL. Their investigation further revealed that while most isolated compounds showed minimal effectiveness against both MCF-7 and HSC-3 cell lines, atranorin (1), lobaric acid (6), and methyl-β-orsinol carboxylate (9) exhibited notable anticancer properties. Specifically, atranorin displayed an IC50 value of 208.20 µM against MCF-7, while lobaric acid and methyl-β-orsinol carboxylate showed IC50 values of 172.05 µM and 382.60 µM, respectively, against the same cell line. In contrast, lobaric acid demonstrated an IC50 of 260.09 µM against HSC-3, whereas methyl-β-orsinol carboxylate exhibited a more potent effect with an IC50 of 88.92 µM. In our study, lobaric acid was found to reduce the cell viability of A549 cancer cells at concentrations of 12.5, 25, 50 and 100 µg/ml according to WST-1 results. Caspase-3 and p53 gene expressions of A549 cancer cells treated with lobaric acid also increased at concentrations of 12.5, 50 and 100 µg/ml. In addition, molecular docking of lobaric acid was investigated to give an insight of its activity against of p53 (PDB: 6BSC), Caspase-3 (PDB: 3GJQ) and Bcl-2 (PDB:4MAN) were downloaded from the RCSB Protein Data Bank (https://www.rcsb.org) were investigated.

4. Conclusion

It is predicted that some concentrations of lobaric acid may show antiproliferative and apoptotic properties in A549 (human lung cancer) cell line. However, further molecular, cytological and in vivo studies are needed to determine the mechanism of apoptosis and the antiproliferative and apoptotic properties of lobaric acid in more detail and to elucidate the mechanism of action. These results give a clue that cell death may occur apoptotically. However, examination of the mRNA expressions of many genes in the apoptotic pathway will lead us to a clearer conclusion. Quantum chemical calculation of lobaric acid were carried out using DFT/B3LYP/6–311++G(d,p) level of theory. The frontier molecular orbital research demonstrated that the energy HOMO and LUMO, respectively, was -8.59 eV and -5.27eV. The value of the Band gap energy (ΔE_gap) has been calculated to be 3.32 eV. Quantum chemical parameters reveal that the molecule examined is hard and has good electrophilic behavior. The findings of molecular docking work reveal that lobaric acid indicated the highest binding affinity to the 4MAN. moreover, the target with a higher binding energy is shown to have a lower inhibition constant. Despite all these data, the biological activity of lobaric acid needs to be investigated by testing different molecular pathways. In addition, *in vivo* studies will help to remove many obstacles to the use of the molecule as a pharmacological agent.

Compliance with Ethical Standards

The authors declare that this study does not require ethical committee approval or any legal permission. This study includes data obtained from a doctoral dissertation completed in 2016 (YÖK ID: 426087), and in line with the decisions taken by Ulakbim TR Dizin, Ethics Committee Permission is required for studies to be published since 2020. Other data obtained are web-based, virtual data.

Acknowledgment

Some of the data in this study were obtained from Dr. Hamit Emre KIZIL's PhD thesis (YÖK ID: 426087) completed in 2016. We are pleased to extend our deepest gratitude to the entire laboratory team for their unwavering moral support throughout the course of this doctoral thesis.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Journal of Agricultural Production (2024), *5*(3), 201-207 https://doi.org/10.56430/japro.1535196

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JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

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RESEARCH ARTICLE

Effects of Drought Stress on Germination and Seedling Growth of Seed Primed with Boron in Spinach

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ARTICLE INFO

Article History
Received: 18.08.2024
Accepted: 19.09.2024
First Published: 30.09.2024

Keywords
Boron
Drought stress
Germination
Seed priming
Spinacia oleracea L

ABSTRACT

This study aimed to examine the effects of seed priming with different boron concentrations on the germination and seedling growth of spinach under drought-stress conditions. Seeds of the spinach cultivar Matador and sodium borate ($Na_2B_8O_{13}.4H_2O$) were used as materials. The seeds were primed with 0 (distilled water), 1, 10, 100, 500, and 1000 ppm B for 24 hours, with unprimed seeds serving as a control. Drought stress was induced by polyethylene glycol (PEG 6000) solution at a water potential of -3 bar and distilled water denoted as control conditions. A standard germination test was performed between papers at 20°C for 14 days. The germination percentage, mean germination time, germination index, seedling growth parameters, and root/shoot length ratio were investigated. The findings revealed that drought stress reduced germination percentage, germination index, and seedling growth of unprimed seeds of spinach. However, boron priming improved these parameters while mitigating the negative effects of drought stress. Under drought conditions, seed priming with 1 ppm B shortened mean germination time. Similarly, seedling fresh and dry weight of spinach were improved by seed priming with 100-1000 ppm B, whereas root growth was stimulated by 10 ppm B. The highest root/shoot ratio was found at 10 ppm B. Boron priming was more efficient in promoting seedling growth than germination in spinach. As a result, seed priming with 10-100 ppm B should be recommended to improve the germination and seedling growth performance of spinach in the event of drought stress after planting.

Please cite this paper as follows:

Kaya, G. (2024). Effects of drought stress on germination and seedling growth of seed primed with boron in spinach. *Journal of Agricultural Production*, *5*(3), 201-207. https://doi.org/10.56430/japro.1535196

1. Introduction

Spinach (*Spinacia oleracea* L.) is a cool-season annual crop grown for its leaves, which are consumed fresh and frozen. Its leaves are rich in minerals, antioxidants, and vitamins such as A, B, and C (Bunea et al., 2008). In Türkiye, its production is about 232.699 tons, with an area of 15.447 ha in 2023 (TÜİK, 2024). Spinach is grown from late summer to early winter in temperate regions and from late winter to spring in cold climates. Under these conditions, drought stress occurs during the life cycle of the spinach plant and has adverse effects on germination, emergence, and plant growth, such as short and small leaves that turn green to yellow (Vural et al., 2000).

Drought inhibits and delays seed germination by preventing water uptake and radicle emergence. Inadequate soil moisture can greatly hinder successful seed germination and emergence (Saha et al., 2022). Moreover, seeds germinating under drought stress often resulted in reduced vigor, and a lower germination index (Tang et al., 2019). To mitigate the adverse effects of drought stress, seed priming is a promising technique that enhances early mobilization of seed reserves, embryo elongation, endosperm weakening, etc. to increase and accelerate seed germination under various abiotic stresses (Chen & Arora, 2011; Kumar et al., 2020).

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Seed priming is a pre-sowing process in which seeds are soaked in water or various solutions containing different natural or synthetic priming agents (gibberellic acids, IBA, BA, glycine betaine, etc.) and dried again for storage (Bradford, 1986; Jisha et al., 2013; McDonald, 2000; Pallaoro et al., 2016; Waqas et al., 2019). In nutrient seed priming, seeds are soaked in a nutrient solution instead of pure water to improve the nutrient content of the seed in combination with the priming effect, which improves germination and seedling establishment (Imran et al., 2013, 2021; Iqbal et al., 2017). Priming solutions containing nutrients, such as nitrogen, calcium, manganese, zinc, and boron, called nutripriming, have been successful and responsive in vegetables (Kaur et al., 2002). In recent studies, it was discovered that nutripriming with different micronutrients enhanced priming efficiency in wheat (Iqbal et al., 2017), maize (Muhammad et al., 2015; Nciizah et al., 2020; Rasool et al., 2019), dill (Mirshekari, 2012), broccoli (Memon et al., 2013), rice (Farooq et al., 2011), bean (Majda et al., 2019), and rice (Ancy et al., 2022).

Boron (B), one of the most widely used micronutrients for seed priming, is important for cell division elongation, translocation, and membrane integrity (Iqbal et al., 2012). It has been extensively studied to improve the germination potential in several crops. For example, Farooq et al. (2011) reported that seed priming with B enhanced germination energy, percentage, and index, while shortening mean germination time in rice seeds. Bonilla et al. (2004) demonstrated that the application of B priming improved the germination characteristics and salt tolerance of developing pea plants. Kaya and Ergin (2023) reported that a lower infection rate was observed in safflower seeds primed with boron. Moreover, it was reported that the seedlings produced from B primed seeds grew better and were more resistant to heat stress due to lower ROS production, along with strong membrane stability and antioxidant defense system (Chakraborty & Dwivedi, 2023). Increases in antioxidant activities such as CAT, SOD, and hydrogen peroxidases were also observed in B-primed alfalfa seeds (Xia et al., 2020). Chakraborty and Bose (2020) found that seed priming with boron resulted in uniform and vigorous seedling establishment because they had much higher α-amylase activity compared to unprimed seeds. However, overdose and prolonged priming time with B resulted in a reduction in germination and a depression in seedling growth (Shahverdi et al., 2017; Xia et al., 2019). In this study, the efficiency of different levels of boron as a priming agent on germination and seedling growth of spinach under drought stress was investigated.

2. Materials and Methods

A laboratory experiment was conducted at the Seed Science and Technology Laboratory of Eskişehir Osmangazi University in 2023. Commercially available spinach (*Spinacia oleracea* L.) cultivar Matador seeds from the Arzuman Seed Company, Türkiye, and sodium borate (20.9% $Na_2B_8O_{13}\,4H_2O$, Etidot-67) were used as materials.

2.1. Seed Treatments

The spinach seeds were immersed in the solutions with different boron concentrations (1, 10, 100, 500, and 1000 ppm B) using sodium borate in an incubator at 20 °C for 24 hours in the dark. Seeds were also soaked in distilled water (hydration), and unprimed seeds were used as a control. Following the incubation period, excess water on the seed surface was directly removed with paper towels, and they were led to dry up to their initial seed weight at room temperature.

2.2. Germination Test

ISTA (2018) rules were followed for the germination test, with four replications and fifty seeds in each. The unprimed and primed seeds were spread on two layers of filter paper, with one paper covering them. Each paper was moistened either with 7 mL of a solution prepared with polyethylene glycol (PEG 6000 m.w.) at a water potential of -3 bar as drought stress or with distilled water as control conditions (Michel & Kaufmann, 1973). To minimize water evaporation, the rolled papers were placed inside a plastic ziplock bag, and then the packages were incubated at 20 °C in the dark and checked every 24 hours. Seeds with a radicle length of 2 mm were considered to have germinated. At the end of the experiment (14th day), mean germination time (MGT), as determined by ISTA (2018), was computed to determine germination speed as follows.

$$MGT = \frac{\Sigma(\mathrm{Dn})}{\Sigma \mathrm{n}} \tag{1}$$

where n is the number of seeds that germinated on day D, and D is the number of days since the germination test started.

Also, the germination index (GI) was calculated with the formula (Salehzade et al., 2009).

$$\mathrm{GI} = \frac{\text{Number of germinated seeds}}{\text{Days of first count}} + \ldots + \frac{\text{Number of germinated seeds}}{\text{Days of final count}} \tag{2}$$

Root and shoot length, seedling fresh and dry weight of seedlings after 14 days of incubation were measured from ten seedlings randomly selected from each treatment. Just after weighing the fresh seedlings, they were transferred to an oven at 80 °C for 24 h to determine the dry weight.

2.3. Statistical Analysis

The experiment was set up as a factorial experiment in randomized plots design with 4 replications, and all data collected were statistically analyzed using the JMP 14.0 software. The percentage data were subjected to an arcsine transformation before an analysis of variance was performed. The LSD test was used to assess the differences between the means (Düzgüneş et al., 1983).

3. Results and Discussion

Mean values of germination characteristics showed that drought had no effect on germination percentage, however, it did result in longer mean germination time and a lower germination index (Table 1). Boron levels also significantly influenced the germination characteristics. Seed priming with a boron level of 1 ppm resulted in the highest germination percentage and the shortest mean germination time. Germination index reached the highest level with 17.33 in hydration and 17.20 in 1 ppm B.

Table 1. Main effects of seed priming with different levels of boron and drought stress on germination parameters of spinach.

Factors	Germination percentage (%)	Mean germination time (day)	Germination index
Stress			
Control	93.9	3.02^{b}	18.76^{a}†
Drought	92.3	4.00^{a}	13.09^{b}
B priming			
Unprimed	90.0^{d}	4.75^{a}	10.91^{f}
Hydration	95.3^{ab}	3.12^{c}	17.33^{a}
1 ppm B	95.8^{a}	3.10^{c}	17.20^{ab}
10 ppm B	92.5^{bcd}	3.23^{c}	16.44^{bc}
100 ppm B	92.0^{cd}	3.34^{c}	16.11^{cd}
500 ppm B	91.5^{d}	3.31^{c}	15.50^{d}
1000 ppm B	94.8^{abc}	3.75^{b}	14.50^{e}
Analysis of Variance			
Stress (A)	NS	**	**
Treatment (B)	**	**	**
A×B	**	**	NS

†: Means followed by the same letter(s) in each column did not significant at p<0.05. **: significant at 1%. NS: not significant.

All the seedling growth parameters were significantly influenced by the interaction of drought x boron priming (Table 2). As expected, lower seedling parameters were obtained from drought stress than the control, with the exception of seedling dry weight. Seed priming with different boron levels enhanced the seedling growth of spinach. Hydrated seeds produced the longest root length, while the highest seedling fresh at 1000 ppm B and dry weight at 100 ppm B were determined.

Table 2. Main effects of seed priming with different levels of boron and drought stress on seedling growth parameters of spinach.

Factors	Shoot length (cm)	Root length (cm)	Seedling fresh weight (mg/plant)	Seedling dry weight (mg/plant)	Root/Shoot ratio
Stress					
Control	3.67^{a}	8.54^{a}	75.7^{a}	4.83^{b}†	2.35
Drought	1.04^{b}	2.71^{b}	48.1^{b}	15.80^{a}	2.29
Treatment					
Unprimed	2.48	3.75^{d}	43.8^{d}	7.21^{d}	1.67^{c}
Hydration	2.41	6.72^{a}	64.3^{b}	10.25^{c}	2.50^{b}
1 ppm B	2.32	5.77^{b}	64.4^{b}	10.85^{abc}	2.39^{b}
10 ppm B	2.20	5.51^{b}	59.9^{c}	10.50^{bc}	2.96^{a}
100 ppm B	2.30	4.43^{c}	64.4^{b}	11.48^{a}	1.83^{c}
500 ppm B	2.33	5.39^{b}	66.8^{ab}	10.63^{bc}	2.54^{ab}
1000 ppm B	2.42	6.51^{a}	69.5^{a}	11.23^{ab}	2.35^{b}
Analysis of Variance					
Stress (A)	**	**	**	**	NS
Treatment (B)	NS	**	**	**	**
A×B	**	**	**	**	**

†: Means followed by the same letter(s) in each column did not significant at p<0.05. **: significant at 1%. NS: not significant.

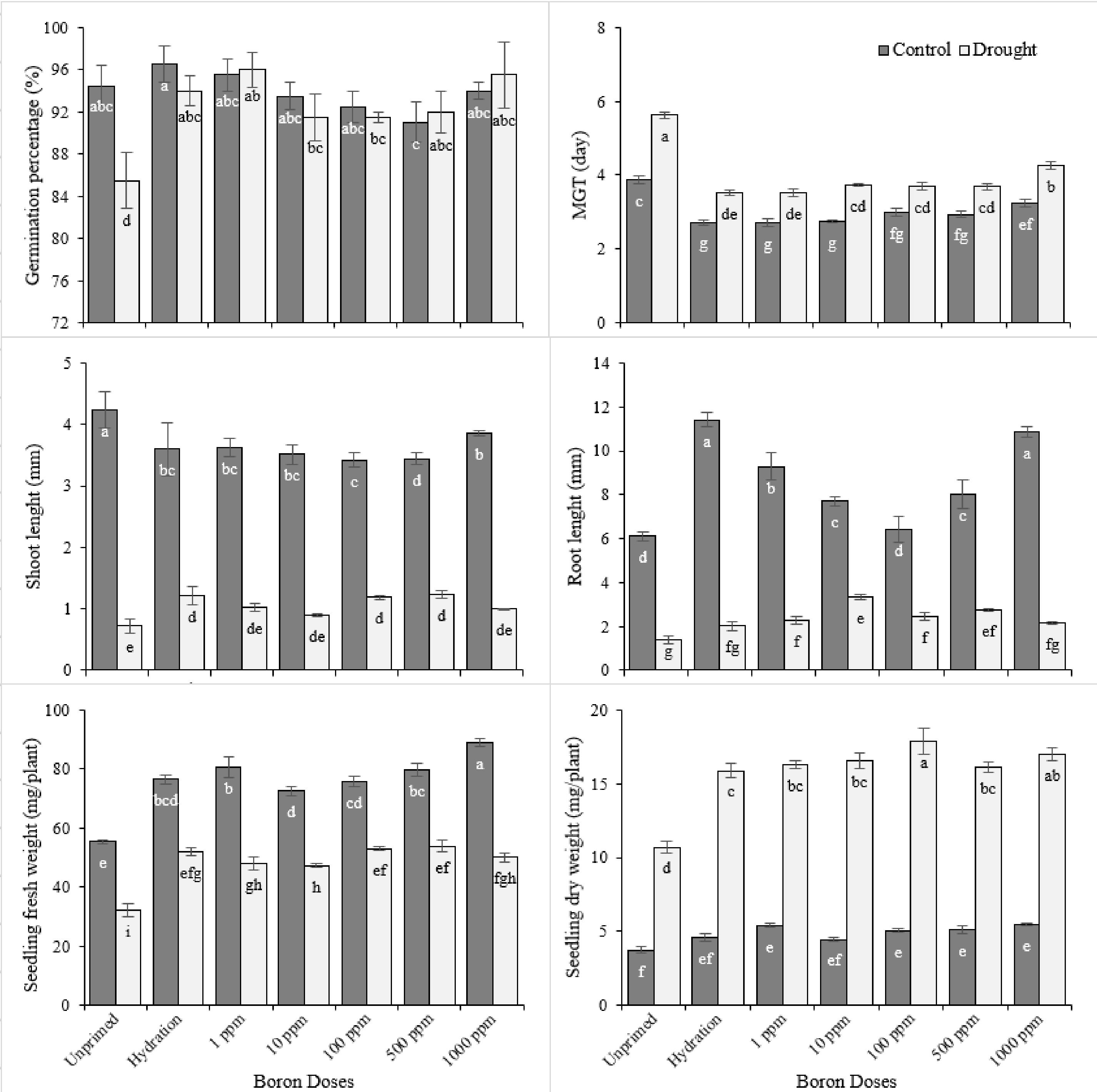

Figure 1. Interaction effects between seed priming with different boron levels and drought stress on germination percentage, mean germination time, shoot length, root length, seedling fresh weight, and seedling dry weight of spinach under control and drought stress.

A two-way interaction showed that priming treatments considerably enhanced the germination percentage of spinach seeds regardless of whether they were subjected to control conditions or drought stress. Unprimed seeds had a lower germination percentage when exposed to drought stress. Memon et al. (2013) found a clear increase in germination percentage of broccoli when its seeds were primed with hydration or 0.01%-0.5% B. Similarly, the beneficial effects of boron priming on germination were reported by Farooq et al. (2011) in rice at a concentration of 0.001-0.01% B and Kaya and Ergin (2023) in safflower at 5 ppm B. However, seeds primed with 1, 500, and 1000 ppm B exhibited a slightly higher germination rate under drought conditions compared to the control group. Drought stress resulted in a longer mean germination time. The highest difference between control and drought was observed in unprimed seeds (Figure 1). Spinach seeds primed with hydration and 1 ppm B had a shorter mean germination time under both control and drought conditions.

The results of Memon et al. (2013) in broccoli, Farooq et al. (2011) in rice, Iqbal et al. (2017) in wheat, and Kaya and Ergin (2023) in safflower support the findings of this study.

Drought stress caused a depression in shoot length of spinach. It was emphasized by the findings of Zargar et al. (2021). Shoot length was statistically decreased by priming treatments in control conditions, but all seed primings showed better shoot growth than unprimed seeds under drought stress (Figure 1). Hydration, 100 ppm B, and 500 ppm B resulted in longer shoots than the others. Similar results were reported by Farooq et al. (2011), Memon et al. (2013), and Kaya and Ergin (2023).

Boron primings improved the root length of spinach under control and drought stress conditions. The longest root length was determined in seeds treated with 10 ppm B under drought, while 500 ppm B produced longer roots than the others. This result was confirmed by the findings of Farooq et al. (2011), Xia et al. (2019), and Kaya and Ergin (2023), who found that root growth of the investigated plants was promoted by boron priming.

Seedling fresh weight of spinach was stimulated by seed priming treatments under both control and drought stress conditions. Seeds primed with 1000 ppm B produced the heaviest seedlings under control, whereas it was the highest at 100 ppm B and 500 ppm B under drought. This result showed similarity with the findings of Memon et al. (2013) in broccoli, Farooq et al. (2011) in rice, and Kaya and Ergin (2023) in safflower, who determined that boron priming led to an increase in seedling fresh weight.

Seed priming promoted seedling dry weight in spinach, but the superiority of priming was evident under drought stress. Under drought conditions, 100 ppm B produced the highest dry weight. All seed priming treatments had heavier dry weight than unprimed seeds. Farooq et al. (2011) reported that boron priming with 0.001-0.01% B increased the dry weight of rice seedlings. Iqbal et al. (2017) found a significant improvement in seedling dry weight of wheat with a low boron dose (0.5 M) seed priming.

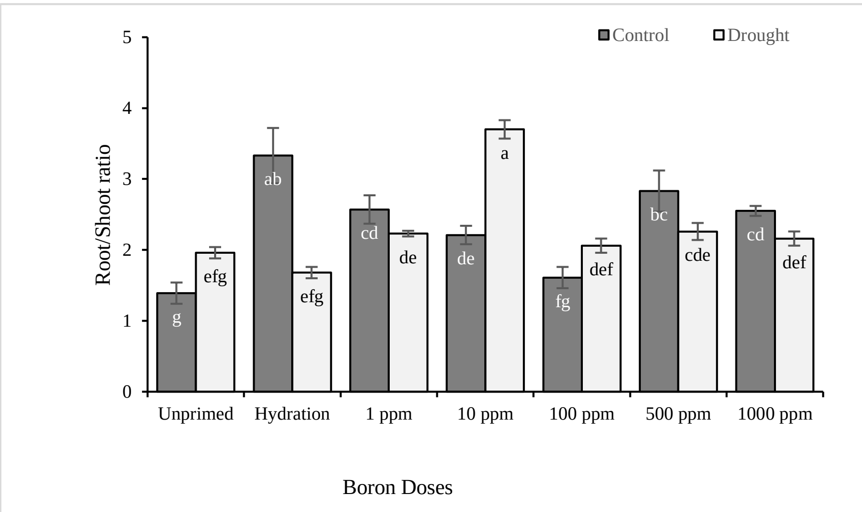

Figure 2. Interaction effects between seed priming with different boron levels and drought stress on root/shoot ratio of spinach.

The root/shoot ratio was changed by boron priming (Figure 2). The maximum ratio was obtained in the seeds primed with 10 ppm B under drought stress. However, the highest ratio in control conditions was recorded in hydration. This finding should be evaluated as boron priming stimulated root growth rather than shoot growth in spinach.

4. Conclusion

Drought is a global phenomenon that adversely affects crop production, and several strategies have been developed to mitigate its negative effects on food supply. Seed priming is a valuable method to improve germination and seedling growth under drought stress after planting. In this study, seed priming with different doses of boron was tested under drought stress in spinach. Boron priming was effective in stimulating germination and seedling growth of spinach. Moreover, seedling growth was induced much more than germination, and root growth was enhanced more than shoot growth by boron priming. This achievement may be due to a reduction in seed-borne pathogens reported by Kaya and Ergin (2023) in safflower. It was concluded that 10-100 ppm B priming should be recommended to increase germination and seedling growth of spinach under both control and drought conditions.

Acknowledgment

The author would like to thank PhD student Pınar Harmancı, and MSc student Elif Yaman for their assistance in counting germination scores.

Conflict of Interest

The author has no conflict of interest to declare.

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Journal of Agricultural Production (2024), *5*(3), 208-216 https://doi.org/10.56430/japro.1515747

http://agriprojournal.com JOURNAL OF AGRICULTURAL PRODUCTION ISSN: 2757-6620 PRENSIP https://prensip.gen.tr

SHORT COMMUNICATION

Effects of Phosphorus Fertilizer, Poultry Manure Applications with *Bacillus megaterium* M-3 Inoculation on Yield and Yield Components of Common Vetch (*Vicia sativa*)

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ARTICLE INFO

Article History
Received: 17.07.2024
Accepted: 13.09.2024
First Published: 30.09.2024

Keywords
Fertilizer
Hay yield
Phosphorus
Phosphorus-solubilizing bacteria

ABSTRACT

Phosphorus is considered one of the essential elements for legumes as it increases nitrogen fixation due to nodulation. Hence, for high productivity in legumes, it should be added as inorganic or organic fertilizer to soils containing insufficient phosphorus. Especially in recent years, using inorganic and organic fertilizers together has gained importance in terms of sustainable agriculture, considering plant growth and soil and environmental health together. The effects of three different doses of phosphorus fertilizer (0, 50, 100 kg P_2O_5 ha^{-1}), and two different doses of poultry manure (0, 3 t ha^{-1}) applications with two different doses (B0 or B1) of phosphorus solubilizing bacteria (*Bacillus megaterium* M-3) inoculation on hay yield and yield components of common vetch were examined in this study. In fact, while the highest hay yield and crude protein rate were obtained by bacterial inoculation together with phosphorus fertilizer application, the effect of poultry manure application on yield and yield parameters was variable. Instead, the applications had no significant effect on the number of main branches, ADF and NDF rates. Consequently, the application of 100 kg P_2O_5 ha^{-1} together with *Bacillus megaterium* inoculation can be recommended for high hay yield in common vetch under irrigated conditions in areas with poor or medium soils in terms of phosphorus.

Please cite this paper as follows:

Kaynar, D., & Çomaklı, B. (2024). Effects of phosphorus fertilizer, poultry manure applications with *Bacillus megaterium* M-3 inoculation on yield and yield components of common vetch (*Vicia sativa*). *Journal of Agricultural Production*, *5*(3), 208-216. https://doi.org/10.56430/japro.1515747

1. Introduction

Common vetch is one of the critical leguminous forage crops in crop rotation systems due to its high dry matter yield, nutritional value, production of grain forage, and being an annual plant. Like other leguminous forage crops, common vetch requires additional phosphorus fertilization to achieve optimal yield (Mitran et al., 2018; Tan, 2018). Although inorganic fertilizers, among factors affecting agricultural productivity, are vital, their unconscious use can deteriorate the soils physical, chemical, and biological characteristics (TEMA, 2018). Furthermore, they are toxic element source for human, animal, and environmental health since the cadmium they contain accumulates in plants to which they are applied (Chukwu et al., 2014; Debele, 2021; Roba, 2018).

In recent years, a prominent approach in terms of sustainable agriculture is to alleviate the adverse effects of

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chemical fertilizers by incorporating natural sources such as organic fertilizers like poultry and cattle manures, along with microbial fertilizers to enhance the effectiveness of phosphorus (P) utilization for plant growth (Chauhan et al., 2022; Meraklı & Memon, 2020; Solmaz et al., 2022). Organic fertilizers increase organic matter, nutrient content, and aggregate stability in the soil and improve the quality and yield of crops without causing environmental pollution (Kocagöz et al., 2022). Poultry manure is a significant source of organic plant nutrients since it increases the soil organic matter, nutrient element content, and aggregate stability (Adeyemo et al., 2019; Masocha & Dikinya, 2022). On the other hand, the inoculation of soils with rhizobacteria, called as Plant Growth-Promoting Rhizobacteria (PGPR), including phosphorus-solubilizing bacteria, promotes plant growth by converting phosphorus in the soil into a form available to plants, and that this process promotes plant growth by making phosphorus available for plants by altering the activity of metabolic enzymes, synthesis of certain hormones and suppressing the pathogen development (Chen & Liu, 2019; Meena et al., 2017). Since it is not feasible to give up the use of inorganic fertilizers completely, which play a crucial role in agricultural production today, supporting fertilizer use with organic fertilizers is essential to alleviate their adverse effects (Cüre, 2022). Numerous studies investigated the effects of inorganic fertilizers on common vetch cultivation (Rafaat, 2022; Türk & Yıldız, 2016). Nevertheless, the number of studies studying the combined use of organic fertilizers and the increasingly popular bacterial inoculation with inorganic fertilizers is very limited. It is essential to investigate the impacts of these applications on the sustainability of agriculture and the health of soil. The principal objective of this study is to evaluate the individual and combined effects of phosphorus fertilizer, poultry manure treatments, and the phosphorus solubilizing bacteria (*Bacillus megaterium* M-3) inoculation on the achievement of maximum yields in common vetch.

2. Materials and Methods

The research was conducted in the experimental field of the Faculty of Agriculture, Atatürk University in Erzurum. The study was established with three replications according to the factorial arrangement in the randomized complete blocks trial design. The experiment included three different doses (0, 50 and 100 kg P_2O_5 ha^{-1}) of chemical phosphorus fertilizer (Triple superphosphate), two different doses (0 and 3 t ha^{-1}) of poultry manure (composition of poultry manure was given in Table 1) and two different doses (B0 and B1) of phosphorus solubilizing bacteria inoculation.

Table 1. Chemical composition of poultry manure.

Parameter	Value
pH (1:5.0)	6.5
Organic matter (%)	22.0
Al (mg kg^{-1})	315.3
B (mg kg^{-1})	20.4
Ca (mg kg^{-1})	18546.0
Cd (mg kg^{-1})	0.09
Cr (mg kg^{-1})	1.23
Cu (mg kg^{-1})	36.1
Fe (mg kg^{-1})	352.0
K (mg kg^{-1})	12312.0
Mg (mg kg^{-1})	3451.0
Mn (mg kg^{-1})	172.4
Na (mg kg^{-1})	956.0
Ni (mg kg^{-1})	2136.0
P (mg kg^{-1})	4845.0
Pb (mg kg^{-1})	0.049
S (mg kg^{-1})	452.1
Zn (mg kg^{-1})	185.6

The seeds used in the experiments were subjected to inoculation with *Rhizobium leguminosarum* bacteria, which were obtained from the Ankara Soil and Fertilizer Research Institute. A combination of phosphorous fertilizer and poultry manure was added into the soil using a harrow. A suspension of cultures of *Bacillus megaterium* was prepared at a density of 10^8 CFU mL^{-1} and his suspension was then used to inoculate the seeds intended for sowing in the plots. The Karaelçi variety of common vetch (*Vicia sativa*) (12 kg da^{-1}) were planted in pre-prepared seed beds at a depth of 4-6 cm (Tan, 2018).

Planting was conducted manually using a hand drill, with the first year planting took place on April 20 and the second year on April 29. To prevent contamination, different drills were used to sow seeds. The crops were irrigated three times over a two-year period when they turned dark green due to deficiency of moisture in the soil during the growing season.

Table 2. Total precipitation, temperature average and relative humidity average values of 2009, 2010 and long-term averages in Erzurum province.

	Total Precipitation (mm)			**Average Temperature (ºC)**			**Average Relative Humidity (%)**		
	2009	2010	LYA	2009	2010	LYA	2009	2010	LYA
J	2.3	52.2	19.8	-12.1	-4.3	-9.7	82.4	84.0	77.0
F	18.8	14.8	24.8	-3.1	-1.8	-8.6	84.7	82.3	77.0
M	51.1	82.2	31.0	-0.7	3.1	-2.8	73.8	69.1	75.0
A	42.7	54.2	58.4	4.3	5.6	5.4	64.6	71.3	66.0
M	43.2	63.6	70.0	10.0	10.4	10.5	61.0	69.6	63.0
J	76.2	50.5	41.6	14.7	15.9	14.9	65.0	60.1	58.0
J	29.2	55.5	26.2	17.2	19.5	19.3	60.7	56.0	52.0
A	22.8	9.0	15.1	17.1	20.3	19.4	50.6	44.8	49.0
S	43.7	8.8	20.0	12.4	17.0	14.3	53.1	48.1	52.0
O	51.0	72.2	47.9	8.7	9.2	7.6	62.4	70.2	65.0
N	41.4	0.0	32.9	1.8	1.8	-0.1	75.7	66.1	73.0
D	15.4	12.9	22.5	-1.1	-1.9	-6.6	84.7	76.6	78.0
Total/Average	437.8	475.9	410.2	5.8	7.9	5.3	68.2	66.5	65.4

LYA: Long year average.

The dimensions of each plot were 5 × 3 m, with a distance of 0.5 m along each edge and a buffer zone of 2 m around the perimeter of the plot. A total of six rows of plants were sowed, with a spacing of 30 cm between each row (Tan, 2018).

The long year annual precipitation was recorded 410.2 mm, which is lower than the values observed in the first year (437.8 mm) and the second year (475.9 mm). It is worth noting that all three values above the long-term average precipitation. Table 2 indicates that a higher amount of precipitation was recorded in June of the first year and May of the second year.

The average temperature throughout the period from 1929 to 2009 was recorded as 5.3 °C. Furthermore, the annual mean temperature for the first year was 5.8 °C, while for the second year it was 7.9 °C, both of which were above the long-term average. In the first year, the maximum temperature recorded in July was 17.2 °C, but in the second year, the highest temperature recorded in July was 19.5 °C.

The plants were harvested by using a scythe when the lower pods started to fill with seeds (Tan, 2018). Harvesting was carried out after removal of one row from each side of the plots and a 0.5m area from the beginning or end of each plot.

Before harvest, the plant height of 10 randomly selected plants from each plot was measured, and the average number of main branches per plant was determined by counting the number of primary branches (Sümerli et al., 2002). Harvested plants were oven dried at 68 °C to constant weight and weighed to determine dry matter yield. Plant samples were then ground, passed through a 2 mm sieve and prepared for chemical analysis. The total nitrogen content of the plants was determined by the Kjeldhal method and the crude protein ratio was calculated by multiplying by 6.25 (Jones, 1991). The acid detergent fiber (ADF) and neutral detergent fiber (NDF) ratios were determined using the Van Soest analysis method (Goering & Van Soest, 1970) (ANKOM Technology, Fairport, NY). The organic matter contents of the soils were determined by the Smith-Weldon method (D. W. Nelson & Sommers, 1982) and were evaluated in the low-level class (1.40% in the first year and 1.80% in the second year).

The textures of the soils were determined by the Bouyoucus hydrometer method (Gee & Bauder, 1986) and were in the loam class in both years. The lime content of the soil was determined volumetrically by Scheibler calcimeter (R. E. Nelson, 1982) as medium level (0.82% in the first year and 0.85% in the second year). In addition, the pH levels of soils were potentiometrically determined to be neutral by utilizing a pH meter (McLean, 1982) (7.45 in the first year and 7.65 in the second year).

Soil phosphorus contents were determined according to the phosphomolybdic acid method (Olsen & Summers, 1982), indicating insufficient (27.5 kg ha^{-1} in the first year and 62 kg ha^{-1} in the second year), and potassium content was determined by the flame photometry method (Thomas, 1982) were determined high (118 kg ha^{-1} in the first year and 158 kg ha^{-1}) in the second year).

Analysis of variance in the JMP package software (SAS, 2002) was used to analyze the data. Means were compared using the LSD test (Yıldız & Bircan, 1994).

3. Results and Discussion

The application of phosphorus fertilizer resulted in a significant increase in plant height ($p<0.01$), as given in Table 3. Plant height showed a positive correlation with increasing phosphorus levels, reaching the highest value of 63.8 cm with

100 kg ha^{-1} P_2O_5 application. Legumes demonstrate a greater sensitivity to phosphorus in comparison to other plant species (Mitran et al., 2018). In a previous study, Cömert (2014) reported that increased phosphorus fertilizer application increased the height of the common vetch. Similarly, poultry manure application also significantly increased plant height ($p<0.01$). Poultry manure is a valuable source of nitrogen and readily available phosphorus for soil enrichment. Moreover, its exceptional fertilizer efficiency has been found to significantly boost yields of crops in plant production (Kurt, 2019).

PGPR inoculation statistically significantly increased the plant height ($p<0.05$). Previous researches have indicated that bacteria inoculation increased plant growth (İmriz et al., 2014; Kaynar & Çomaklı, 2023). In addition, the values for plant height in the second year were higher than in the first year and a statistically significant difference was also found between plant height over the years ($p<0.01$). This situation might be linked to the more favorable precipitation and temperature conditions in the second year. PGPR inoculation increased the plant height under the PM0 conditions, but it decreased the plant height when inoculated under the PM1 condition. This response resulted in a significant B X PM interaction ($p<0.01$) (Figure 1a).

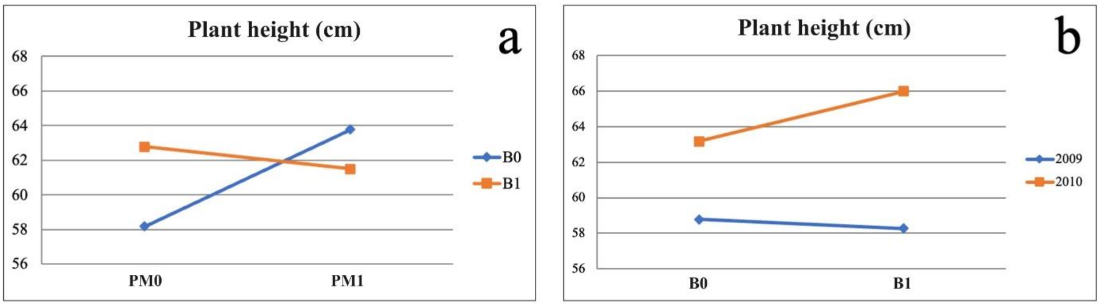

Figure 1. Effects of phosphorus solubilizing bacteria and poultry manure applications on plant height (cm) in common vetch. **(a)** Bacteria X Poultry manure interaction, **(b)** Bacteria X Year interaction.

This result could be attributed to the phosphorus concentration in the poultry manure, and the phosphorus resulting from the inoculation of the bacteria reached the maximum level that could be potentially toxic for the nitrogen-fixing bacteria (Amba et al., 2011; Truu et al., 2017) In addition, PGPR inoculation reduced plant height in the first year but caused a significant increase in the second year. It resulted in a significant B X Y ($p<0.01$) (Figure 1b).

The effects of applications on the number of main branches of the plant were insignificant and no significant interactions were found between the treatments or between the years. Similarly, it was reported by Sawires (2011), number of main branches in plants was not influenced by varying dosages of phosphorus.

On the other hand, as the phosphorus doses were raised, there was a statistically significant increase ($p<0.01$) in the dry matter yield of common vetch. This increase was reached the highest level (6.30 t ha^{-1}) with P2 dose (Table 3). Phosphorus plays a significant role in the energy conversion process and nitrogen fixation within the nodules of leguminous forage crops, making it an essential element for their growth and development (Öncan Sümer & Erten, 2022; Uyanık et al., 2011). Consequently, it is essential to ensure an adequate supply of phosphorus in order to achieve optimal productivity in forage legumes (Mitran et al., 2018; Udvardi & Poole, 2013). Previous studies have reported that phosphorus fertilizer increases the yield of common vetch and forage pea (Erkovan et al., 2014; Rafaat, 2022; Yüksel & Türk, 2019). Poultry manure application significantly increased the dry matter yield ($p<0.01$). The application of organic fertilizers, such as poultry manure, has been found to positively impact soil structure, aeration, and water infiltration, hence leading to an elevation in crop yields (Azmi et al., 2019; Türkkan & Kibar, 2022). Inoculation of plant with growth-promoting rhizobacteria resulted in a statistically significant increase in dry matter yield ($p<0.01$). Dry matter yield, which was 5.54 t ha^{-1} without PGPR inoculation, increased to 5.75 t ha^{-1} with PGPR inoculation. Phosphorus-solubilizing bacteria can convert bound phosphorus into a form that is readily accessible to plants through the production of diverse organic acids within the soil. This process ultimately leads to an improvement in plant productivity, as demonstrated by Meena et al. (2017) and Chen and Liu (2019). The difference in dry matter yield over the years was statistically significant ($p<0.01$), with an increase in the dry matter yield from 5.28 t ha^{-1} in the first year to 6.01 t ha^{-1} in the second year. Considering the two-year average results, the application of poultry manure in common vetch under B0 conditions increased dry matter yield, whereas it decreased the yield under B1 conditions. This difference resulted in a significant ($p<0.01$) B X PM interaction (Figure 2a). The difference might be related to the chemical composition of poultry manure. Despite providing additional nutrient elements, poultry manure may negatively affect microbial activity in the soil and cause to a decrease in plant yield (Li et al., 2011). This reduction in microbial activity is significant as soil microorganisms play a crucial role in facilitating the uptake of phosphorus and other essential nutrients by plants (Chauhan et al., 2022; Matse et al., 2020; Öksel et al., 2022). Dry matter yield increased linearly with increasing P doses under PM0 conditions, whereas the increase in dry matter yield stopped after P1 doses under PM1 conditions. This reaction caused a significant P × PM interaction ($p<0.01$) (Figure 2b).

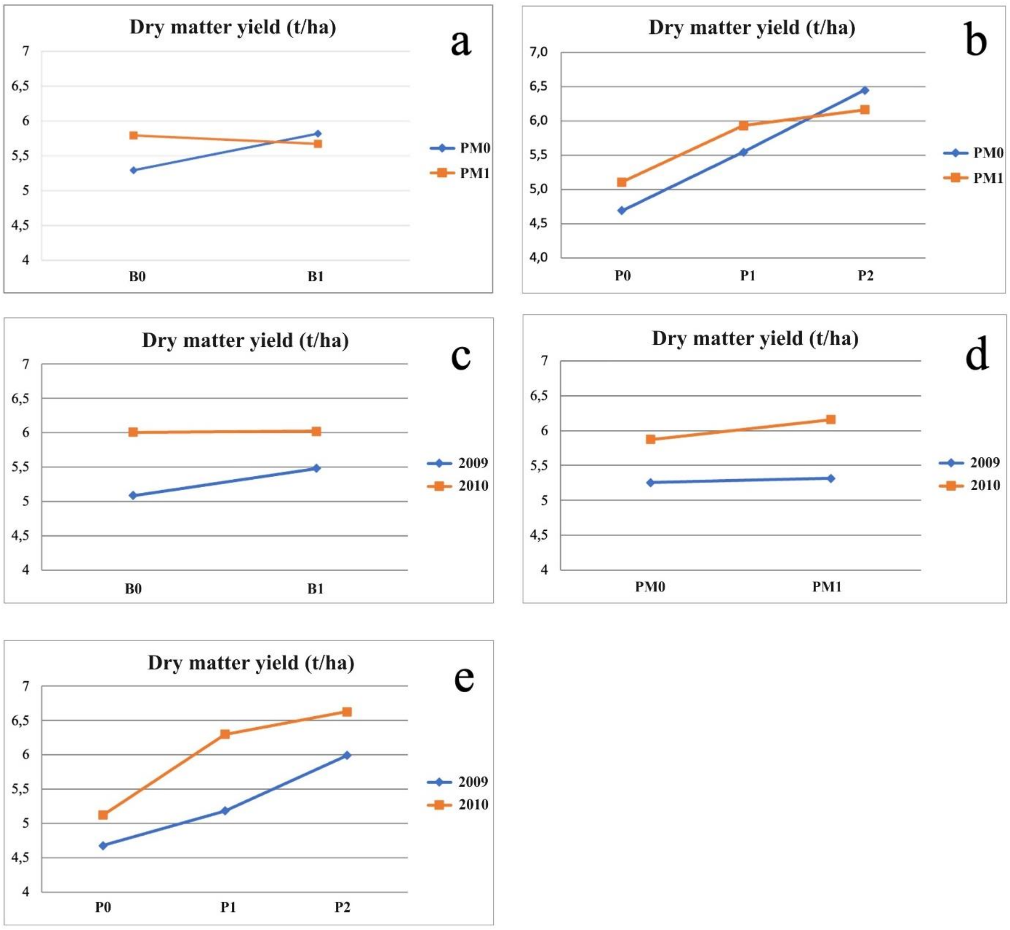

Figure 2. Effects of phosphorus solubilizing bacteria, poultry manure, and phosphorus fertilizer applications on dry matter yield (t/ha) in common vetch. **(a)** Bacteria X Poultry manure interaction, **(b)** Phosphorus X Poultry manure interaction, **(c)** Bacteria X Year interaction, **(d)** Poultry manure X Year interaction, **(e)** Phosphorus X Year interaction.

This suggests that the application of 50 kg P_2O_5 phosphorus, along with phosphorus provided by poultry manure, reached phosphorus saturation for common vetch. Indeed, poultry manure is known to be particularly rich in phosphorus for plants (Kurt, 2019). While dry matter yield increased with PGPR inoculation in the first year, it was ineffective in the second year. Because of these different effects, Y X B interaction was found to be significant ($p<0.01$) (Figure 2c). Previous studies indicated that factors such as irrigation, temperature, soil type, plant species, bacterial strain, and application method play a crucial role in the activity of bacteria (Gerçekçioğlu et al., 2018; Gupta et al., 2015). The application of poultry manure resulted in an increase in dry matter yield during the second year in comparison to the first year. This reaction caused a significant Y X PM interaction ($p<0.05$) (Figure 2d). The dry matter yield, which increased with the P1 dose in both years, further increased with the P2 dose in the first year but decreased with the P2 dose in the second year. This different response led to a significant ($p<0.01$) Y X P interaction (Figure 2e). These differences might be attributed to variations in temperature and precipitation values between the years. The application of poultry manure increased dry matter yield more in the second year when compared to the first year. This resulted in a significant ($p<0.05$) Y X PM interaction (Figure 2d). The dry matter yield, which increased with the P1 dose in both years, further increased with the P2 dose in the first year but decreased with the P2 dose in the second year. As a result of these different effects, Y X P interaction was found to be significant ($p<0.01$) (Figure 2e). These differences might be attributed to variations in temperature and precipitation values between the years.

The effects of phosphorus application on the crude protein ratio of the resulted in a significant increase in the crude protein content ($p<0.01$). Phosphorus has an influence on root development and the activity of *Rhizobium* bacteria, resulting in significant effect on nitrogen fixation (Yüksel & Türk, 2019). Moreover, the presence of phosphorus has been seen to enhance the crude protein content in legumes (Belete et al., 2019; Kaynar & Çomaklı, 2023).

Table 3. Effects of phosphorus fertilizer, poultry manure applications and PGPR (*Bacillus megaterium* M3) inoculation in common vetch (*Vicia sativa*) plant height (cm), number of main branches (pieces), hay yield (t ha^{-1}), crude protein ratio (%), NDF (%) and ADF (%) rates.

		PH	NMB	DHY	CPR	NDF	ADF
P	P_0	59.8^C±1.17	1.91±0.10	4.90^C±0.15	17.07^B±0.29	40.5±0.17	30.2±0.18
	P_{50}	61.1^B±0.99	2.00±0.10	5.74^B±0.14	17.87^A±0.25	40.5±0.15	30.0±0.16
	P_{100}	63.8^A±0.85	1.91±0.08	6.30^A±0.10	18.33^A±0.23	40.6±0.15	30.2±0.17
	Av.	61.5±0.52	1.95±0.10	5.64±4.78	17.75±0.19	40.5±0.15	30.1±0.16
PM	PM_0	60.4^B±0.90	1.94±0.07	5.56^B±0.15	18.22^A±0.20	40.4±0.14	29.9±0.13
	PM_1	62.6^A±0.78	1.97±0.08	5.73^A±0.13	17.29^B±0.22	40.6±0.11	30.3±0.14
	Av.	61.5±0.42	1.95±0.85	5.64±3.90	17.75±0.15	40.5±0.12	30.1±0.13
B	B_0	60.9^b±0,90	1.94±0.08	5.54^B±0.16	17.53^b±0.13	40.6±0.11	30.2±0.15
	B_1	62.1^a±0.82	1.94±0.08	5.75^A±0.13	17.98^a±0.21	40.5±0.14	30.1±0.13
	Av.	61.5±0.42	1.94±0.85	5.64±3.90	17.75±0.15	40.5±0.12	30.1±0.13
Years	2009	58.5^A±0.43	2.00±0.04	5.28^B±0.11	17.38^B±0.22	40.3^b±0.13	30.2±0.15
	2010	64.5^B±0.89	2.02±0.10	6.01^A±0.15	18.13^A±0.22	40.7^a±0.11	30.0±0.13
	Av.	61.5±0.42	2.01±0.85	5.65±3.90	17.75±0.15	40.5±0.12	30.1±0.13
	P	**	ns	**	**	ns	ns
	PM	**	ns	**	**	ns	ns
	B	*	ns	**	*	ns	ns
	Y	**	ns	**	**	*	ns
	BxP	ns	ns	ns	ns	ns	ns
	BxPM	**	ns	**	ns	ns	ns
	BxY	**	ns	**	ns	ns	ns
	PMx P	ns	ns	**	ns	ns	ns
	PMxY	ns	ns	*	ns	ns	ns
	PxY	ns	ns	**	ns	ns	ns
	BxPxPM	ns	ns	**	ns	ns	ns
	BxPxY	ns	ns	**	*	ns	ns
	BxPMxY	**	ns	**	**	ns	ns
	PxPMxY	ns	ns	ns	*	ns	ns

[#]Values shown in capital letters and ** sign are significant at 1% ($P<0.01$), small letters and * sign are significant at 5% ($p<0.05$).
PH: Plant height; NMB: Number of main branches; DMY: Dry matter yield; CPR: Crude protein rate; ADF: Acid detergent fiber; NDF: Neutral detergent fiber, B: Bacteria; P: Phosphorus; PM: Poultry manure; Y: Year; Ns: None significant.

The application of poultry manure resulted in a significant decrease in crude protein content ($p<0.01$). This effect could be attributed to the high nitrogen content present in poultry manure. The application of nitrogen fertilizers has been widely recognized as a factor that induces stress in legume plants, leading to a decrease in protein content (Bayram et al., 2009). The PGPR inoculation resulted in a statistically significant increase in the crude protein content ($p<0.05$). The observed results can be attributed to the positive impacts of bacterial inoculation on the growth of root systems, the metabolic activity of microorganisms involved in nitrogen fixation, and the development of complex root systems, hence facilitating the establishment of a mutually advantageous symbiotic association (Bhat et al., 2013). Previous research has also reported that the utilization of Plant Growth-Promoting Rhizobacteria (PGPR) has a positive impact on the chemical composition of plants, leading to elevated levels of crude protein and mineral content (Erkovan et al., 2014; Yüksel & Türk, 2019).

The applications were not affected the ADF and NDF rates. The ADF and NDF values obtained after analysis in common vetch were found to be below the recommended thresholds of

31% for ADF and 41% for NDF, which are considered indicative of acceptable fodder quality (Yavuz et al., 2009). A statistically significant change ($p<0.05$) was observed in the NDF ratio between the two years, with an increase in NDF from 40.3% in the first year to 40.7% in the second year. This difference could potentially be attributed to the variability in temperature experienced over the duration of plant growth, as the second year of the experiment exhibited higher temperatures compared to the first year. According to Osman et al. (2010), it has been observed that the cellulose content in forage tends to rise in response to warm weather conditions.

4. Conclusion

The use of inorganic fertilizers in combination with organic fertilizers as an alternative approach to reducing the use of inorganic fertilizers has become increasingly important in the context of sustainable agriculture. For this reason, this study investigated the effects of phosphorus fertilizer and poultry manure applications with bacterial inoculation on yield and yield components of common vetch. Based on the two-year average results, it was found that the plots treated with 100 kg P_2O_5 per hectare together with PGPR inoculation yielded the highest dry matter and crude protein yields. Therefore, it can be suggested to apply phosphorus fertilizer of 100 kg ha^{-1} P_2O_5 with PGPR inoculation to optimize forage production in common vetch grown in Erzurum and comparable environmental circumstances with low to moderate phosphorus levels. Additionally, given the results, poultry manure appears promising as an important organic fertilizer source for sustainable agriculture. For this reason, new studies are needed to investigate issues such as different doses and application methods of poultry manure.

Acknowledgment

This paper includes the results of the PhD thesis of the first author. The study was supported financially by Atatürk University Scientific Research Projects Coordination Unit (BAP 2010/75).

Conflict of Interest

The authors declare that they have no conflict of interest.

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