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RESEARCH ARTICLE

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Impact of organic and inorganic fertilizers on the growth and yield of Beetroot (*Beta vulgaris* L.) in the hilly region of Nepal

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Abstract
The use of both organic and inorganic fertilizers plays a crucial role in farming practices in Nepal, depending on their availability. Applying fertilizers like compost, vermicompost, goat manure, and NPK has shown significant effects on various aspects of plant growth and yield. This study was conducted in Diktel, Khotang district of Nepal, from March 14th to June 7th, 2023, aiming to evaluate how different organic and inorganic sources affect the growth and yield of beetroot (Beta vulgaris L.). The experiment followed a one-factor randomized complete block design (RCBD) with four replications, involving five treatments: T1: 100% Compost manure, T2: 100% Vermicompost, T3: 100% Goat manure, T4: 100% NPK, and T5: Control. The results clearly showed that both vegetative and reproductive traits were significantly varies among several treatments utilized in the experiments at 0.1% level of significance. Compost application consistently showed better results across most growth and yield parameters assessed. Growth parameters included plant height, leaf count per plant, leaf length, and leaf width, while yield parameters included beetroot diameter, beetroot length, root yield, and leaf yield. Organic compost manure particularly stood out, displaying significantly larger beetroot diameter (4.85 cm) and greater beetroot length (9.3 cm). Additionally, compost manure led to notably increased root yield (13.95 t/ha) compared to the control treatment, which recorded a lower root yield (6.28 t/ha). Overall, all treatments outperformed the control in terms of growth and yield parameters. These findings suggest that organic compost manure is the most favorable choice for achieving high-quality beetroot production in the hilly regions of Nepal.
Keywords: Beetroot, Organic fertilizers, Synthetic fertilizers, Yield enhancement, Soil fertilit

INTRODUCTION

Beetroot (*Beta vulgaris* L.), also known as garden beet or table beet, is a prominent root vegetable that falls within the Chenopodiaceae family, sharing this botanical lineage with vegetables like spinach, Swiss chard, parsley, and celery. It has a chromosome count of 2n = 18. Beetroot's cultivation for human and animal consumption was first documented in Western Europe and North Africa (Kumar et al., 2022). This crop exhibits rapid growth, high productivity, and generally remains unscathed by pests and diseases. Although it is traditionally considered a cool-season crop, beetroot thrives in warmer climates, allowing for winter cultivation in the plains of Nepal. Nevertheless, the growth, development, and yield of beetroot are heavily influenced by soil conditions (Sapkota et al., 2021). The optimal growth temperature falls within the range of 12-19°C, with a soil temperature above 7°C required for germination. The ideal pH for successful beetroot cultivation ranges from 6.0 to 8.0. Beetroot thrives in deep, well-drained,

sandy loam to silt loam soils (Kumar et al., 2022).

Initially, *Beta vulgaris* was valued for its leaves and the fleshy elongated midribs that characterize chard (Nottingham, 2004). Beetroot is a nutritional powerhouse, rich in fiber, folate (vitamin B9), manganese, potassium, iron, and vitamin C (Kumar et al., 2022; Adaora et al., 2022). The application of organic manures, such as goat manure, vermicompost, farmyard manure (FYM), and compost, enhances soil water retention and supplies both macro and micro nutrients for improved crop yield (Biondo et al., 2014). In recent times, there has been an increasing inclination towards the use of natural fertilizers like Farmyard Manure (FYM), Vermicompost, Poultry manure, Neem cake, and Goat manure to enhance crop productivity and sustain soil health, as observed by Yadav et al. (2023a). Apart from nutrient requirements, the yield of beetroot is influenced by the genetic characteristics of the chosen variety. The selection of the variety should be tailored to the local growing conditions and the season (Kumar et al., 2022). Whereas, inorganic fertilizers release nutrients quickly, making them a popular choice among farmers to provide nutrients for vegetable crops and achieve high yields. However, excessive use of these fertilizers can pose risks to human health, lead to nutrient loss, contaminate groundwater, and reduce the effectiveness of microbial communities in the soil.

Research on beetroot cultivation has been conducted extensively in various parts of the world, including our own country. These studies have underscored the benefits of utilizing both organic and inorganic fertilizers in beetroot farming. Hussain and Kerketta (2023) have noted that organic fertilizers like compost and goat manure enhance soil fertility, boost nutrient availability, and stimulate microbial activity. Numerous greenhouse experiments have consistently shown that vermicompost can have positive effects on plant germination, growth, yield, and overall quality. Mbithi (2021) observed increased seedling emergence when vermicompost was used across a wide range of test plants, including pea, lettuce, wheat, cabbage, tomato, and radish. Addo (2021) found that vermicompost led to higher seedling emergence compared to control commercial plant growth media. Furthermore, Biondo et al. (2014) reported that the optimal use of fertilizers results in higher yields and improved crop quality. Among these fertilizers, nitrogen has emerged as a critical factor influencing vegetable yield and chemical composition, particularly in relation to nitrate content. Therefore, a judicious application of nitrogen can positively impact beetroot growth and yield characteristics. Rantao (2013) also noted that a balanced supply of phosphorus and potassium contributes to increased sugar and starch content in crops, while secondary and micronutrients play pivotal roles in enhancing crop quality.

Kumar et al. (2022) emphasized the need to fine-tune fertilizer application rates for beetroot across different environmental conditions. Furthermore, they noted that organic manure fertilizers typically lead to enhancements in soil physical and chemical properties, improved plant nutrition, better vegetative growth, and increased qualitative and quantitative attributes in vegetable crops. Shafeek et al. (2019) observed that elevating the levels of organic manure fertilizers results in improved plant growth characteristics, potentially leading to an increase in the nutritional elements available within the rooting zone of beetroot plants. This heightened availability of nutrients, particularly N (nitrogen), P (phosphorus), K (potassium), Zn (zinc), Fe (iron), and Mn (manganese), is notable even from the early stages of crop growth. Nitrogen, a key component of NPK, holds significant importance as a crucial nutrient for plant growth, exerting a substantial influence on crop development and yield, as emphasized by Mandal et al. (2023). Though, Katel et al. (2023) discovered that excessive application of NPK can result in a decline in crop productivity. Furthermore, Yadav et al. (2022a) indicated that the excessive utilization of manure such as poultry manure can lead to the contamination of crops, soil, or water sources. Devi et al. (2016) reported that the utilization of organic manures like farmyard manure (FYM), vermicompost, compost, and goat manure serves to enhance and ameliorate soil health, as well as positively impact the growth and yield of various crops. Moreover, as noted by Yadav et al. (2023b), the role of soil biota in enhancing soil quality, bolstering plant vitality, and fortifying soil resilience is paramount. Additionally, the existence of beneficial microorganisms is crucial for sustaining soil fertility, boosting plant resilience, and fostering overall crop well-being (Yadav et al., 2023c). This approach aligns with the broader objective of sustainable agricultural production and promotes the eco-friendly recycling of nutrients.

Generally, beetroot cultivation is done in open field in late winter spring in traditional manner. Nowadays, beetroots are gaining more importance due to their many positive nutritional and physiological properties. The total area occupied by beetroot cultivation is around 5.9 million hectares worldwide, resulting in an estimated total production of 240 million tons. On the other hand, fodder beet provides only 10 million tons for animal feeding. The major beetroot producing countries are Russia, the United States, Germany, France, Turkey and Poland.

In vegetable farming, especially in hilly areas like Khotang, numerous challenges exist. Primary concerns include the lack of irrigation, limited market access, climate change impacts, and outdated farming practices. In the case of beetroot cultivation in Khotang, specific issues include a lack of knowledge, inadequate fertilizers, low-yielding varieties, disease pests, storage problems, and traditional farming practices. In Nepal, only one variety, "Madhur," is officially registered, while other varieties like "Ruby Red" and "Ruby Queen" are imported from abroad and distributed

by private companies (Sapkota et al., 2021). To revitalize beetroot farming and enhance agricultural sustainability, addressing these challenges by improving irrigation, market access, farming techniques, knowledge dissemination, and promoting high-yielding varieties is crucial.

Therefore, this study aims to enhance beetroot crop productivity and quality through the effective use of organic and inorganic fertilizers. By promoting awareness among farmers and improving support systems, this research seeks to optimize beetroot production in Khotang district. The expected outcomes include increased economic returns for farmers and improved living standards. This study addresses various challenges in beetroot production and marketing, providing practical solutions and generating valuable evidence-based information. This data benefits not only researchers and students but also governmental bodies like PMAMP and AKC, contributing to agricultural advancement, livelihood improvement, and sustainable development in Khotang and beyond.

MATERIALS AND METHODS

Research site

The study site, located in Khotang district within the hilly terrain of Koshi Province, Eastern Nepal, possesses central geographic coordinates approximately at 27°11'60" N latitude and 86°46'59.99" E longitude, spanning an altitude gradient from 152 to 3652 meters above sea level (masl), as depicted in Figure 1. Majhuwagadhi, Diktel, was selected as the focal point of investigation owing to its favorable agroclimatic attributes and soil characteristics conducive to vegetable cultivation. The research site, located in Diktel Rupakot Majhuwagadhi Municipality Ward No. 1, was chosen for vegetable farming due to its favorable climate and soil conditions. It's within the vegetable zone designated by PMAMP and known for cooperative farmers. The district is renowned for tomato production, serving as a supplier to neighboring villages.

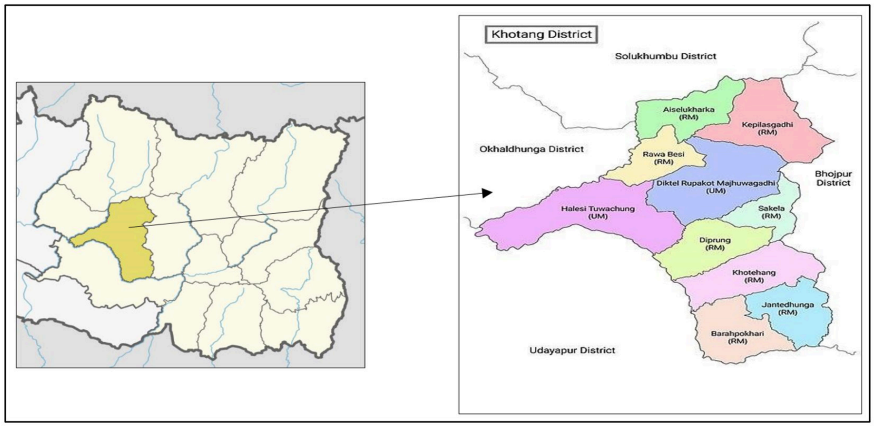

Figure 1. Map of Research site

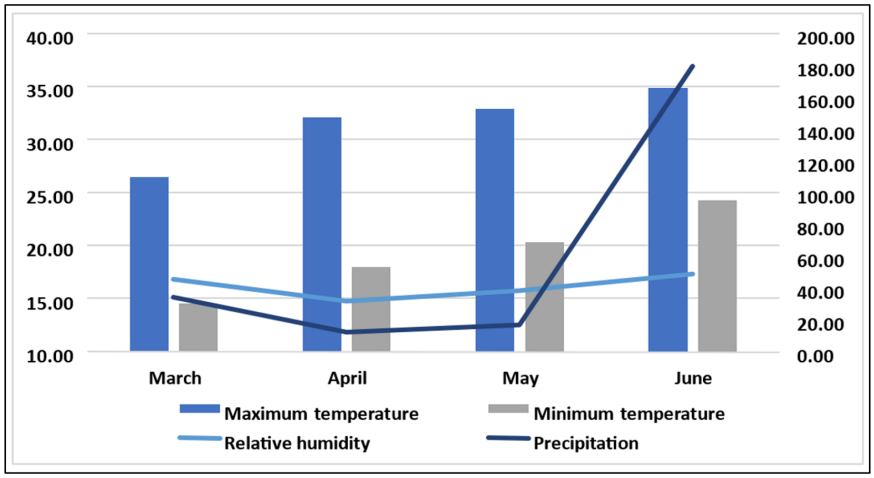

Figure 2. Meteorological data from sowing to harvesting

Research design

In this investigation, a randomized complete block design (RCBD) was utilized, comprising five distinct treatments replicated four times (Yadav et al., 2022b). The treatments included T1: 100% Compost at a rate of 20 tons per hectare, T2: 100% Vermicompost at a rate of 5 tons per hectare, T3: 100% Goat manure at a rate of 10 tons per hectare, T4: 100% NPK fertilizer at a rate of 120:80:40 kg per hectare, and T5: Control, where no recommended doses of organic manures or inorganic fertilizers were applied. The research area measured 12 meters in length and 5.90 meters in width, with each plot covering 1.53 square meters. A total of 20 plots were established with a spacing of 0.5 meters between treatments and replications. Plant-to-plant spacing was set at 10 centimeters, resulting in 42 plants per plot.

Varietal details

In Nepal, the only registered beetroot variety is Madhur, which was released in 2010 AD. This variety exhibits a broad adaptability range and demonstrates excellent field-holding capacity. The foliage is characterized by a medium, dull green color with a maroon tinge. Madhur boasts strong plant vigor, producing dark red and round roots, which maintain their proper shape even when plants are closely spaced. It is cultivated during the winter season in the plains and as a spring-summer crop in the hilly regions of Nepal. Madhur also thrives in warm weather conditions due to its adaptive capabilities.

Cultivation practices

The land preparation involved two ploughing sessions, with the initial ploughing taking place 15 days before sowing and the application of FYM at a rate of 20 tons/ha. Subsequently, one week prior to sowing, a mini-tiller machine was used to further till the soil, ensuring it was in good condition. The field was then leveled, and any stubbles, plant debris from previous crops, and weeds were removed. For manure and fertilizer application, FYM served as the primary source of organic fertilizer. Compost manure was applied at a rate of 20 tons/ha, vermicompost at 5 tons/ha, Goat manure at 10 tons/ha, and NPK at 120:80:40 kg/ha. These were uniformly incorporated into the soil after the final land preparation and before sowing to the deginated plots. Seed sowing was carried out manually, with germination observed seven days after sowing. Two seeds per hill were sown in rows on March 14, 2023, with a spacing of 20 × 10 cm. The soil was irrigated one day before sowing to loosen it for better germination. After germination, irrigation was provided once a week or at 3–4day intervals as required. Weeding was done manually by hand weeding at 20, 40, and 60 days after sowing, while thinning was performed manually when the seedlings reached a height of 7.5 cm. Harvesting took place manually through hand-picking, occurring at 85 days after sowing (DAS).

Observation and data collection

For data collection, a set of eight random plants were selected for observation and measurement from each plot. It is important to note that the border plants were excluded to ensure that the data collected was representative of the interior of the plots and not influenced by border effect. During the crop growing period, several key parameters were recorded. This included plant height (cm), number of leaves per plant, leaf length (cm), and leaf breadth (cm). These measurements provided insights into the growth and development of the beetroot plants throughout their growth cycle. After the harvesting stage, additional parameters were measured to assess the yield parameters. These included root diameter (cm), root length (cm), root yield (t/ha), and leaf yield (t/ha).

Statistical analysis

The data collected from the experiment were inputted into Microsoft Excel (2019) and analyzed using R-studio software (4.2.2 Version). To compare the means of the parametric data, the Duncan Multiple Range Test (DMRT) was used as a statistical method (Gomez & Gomez, 1984).

RESULTS AND DISCUSSION

The ANOVA examination demonstrated a significant impact of organic fertilizers on beetroot plant height across various growth stages ($p \leq 0.05$) (Table 1 and Table 2). Particularly, compost consistently promoted the greatest plant height from 50 days post-sowing until the culmination of the growth period. At harvest, plants treated with compost displayed significantly greater height (38.30 cm) in comparison to those under the control treatment (30.26 cm) ($p \leq 0.01$). Moreover, at harvest, vermicompost application resulted in a significantly higher leaf count (12.27) compared to the NPK treatment, which yielded fewer leaves (10.20) ($p \leq 0.001$) (Table 1 and Table 2). These findings underscore agricultural practices conducive to augmenting crop productivity. Similarly, compost consistently induced significantly elongated leaves compared to other fertilization sources (Table 3 and Table 4). Conversely, the control treatment led to substantially shorter leaves, averaging 27.37 cm. Katel et al. (2021) discovered that super combined fertilizer releases its active components gradually, a trait that offers advantages in the agricultural domain. Furthermore, the ANOVA analysis unveiled that compost consistently induced broader beetroot leaves in comparison to alternative sources, with

statistically significant discrepancies ($p \leq 0.05$) (Table 3 and Table 4). This pattern persisted from 30 days post-sowing until the final harvest. Conversely, leaves from the control treatment exhibited significantly narrower dimensions, measuring merely 7.62 cm ($p \leq 0.05$). Likewise, the ANOVA analysis in Table 5 shows that the root diameter of beetroot was significantly influenced by organic and inorganic sources, especially at harvest. Compost had the largest average root diameter at 4.85 cm ($p \leq 0.05$), while the control treatment had the smallest at 2.35 cm. Table 5 also reveals that Beetroot length was notably affected by organic and inorganic sources, particularly at harvest. Compost resulted in the longest beetroot length, measuring an impressive 9.3 cm ($p \leq 0.001$), while the control treatment had the shortest length at 6.4 cm. Regarding root yield, Table 5 demonstrates that it was significantly affected by the choice of organic and inorganic sources. Compost led to the highest root yield at 13.95 tons per hectare (t/ha) ($p \leq 0.01$), while the control treatment had the lowest yield at 6.28 t/ha. Table 5 also details the impact of organic and inorganic sources on leaf yield. The ANOVA analysis shows that leaf yield was significantly affected by the choice of sources. NPK treatment resulted in the highest leaf yield at 11.09 t/ha ($p \leq 0.01$), while the control treatment had the lowest yield at 5.75 t/ha. Furthermore, both Adhikari et al. (2023) and Sangam et al. (2023) observed that organic fertilizers hold considerable potential for enhancing crop productivity. Hence, the utilization of Goat manure alongside NPK resulted in improved yield production, aligning partially with the aforementioned research findings.

The results unequivocally indicate that the application of organic fertilizers had a profoundly positive influence on various vegetative parameters of beetroot. Among the various organic fertilizers tested, plants that received vermicompost displayed the highest number of leaves per plant, followed by those treated with compost manure, while the control group exhibited the lowest leaf count (Rantao, 2013). This might be due to organic fertilizers, particularly vermicompost and compost manure, enhanced beetroot vegetative parameters, likely due to nutrient-rich soil enrichment. Similarly, the compost treatment surpassed the other methods in several aspects, leading to significantly greater plant height, longer leaves, and wider leaves when compared to alternative treatments. These findings align with the conclusions drawn by Ajari et al. (2003) and Kumari et al. (2022), highlighting the significant benefits of using organic fertilizers vermicompost and compost in particular to promote the vegetative growth and development of beetroot plants, which in turn improves crop quality and production. Moreover, the significant rise in root diameter and length noted in the group treated with compost was a direct cause of the increased root production attained in the same treatment. This phenomenon can be attributed to various factors associated with the utilization of compost manure. Primarily, the extended root length in plots treated with compost can be attributed to the presence of phosphorus, which was present at a substantial level (0.96%) in the compost manure. Phosphorus plays a pivotal role in stimulating root growth, facilitating enhanced nutrient absorption and translocation within the plant (Addo, 2021). It is also an essential component of various enzymes and energy-rich ATP, resulting in root growth (Kumar & Venkatasubbaiah, 2016). Clark et al. (1998) demonstrated that compost-amended soils exhibit elevated phosphorus levels, attributing this phenomenon to the organic matter's phosphorus enrichment. Concurrently, the application of organic manures like compost fosters a conducive microbial environment within the soil. These soil microorganisms play a pivotal role in synthesizing polysaccharides, thereby improving soil structure, which subsequently facilitates enhanced root growth (Balasubramanian et al., 1972). Moreover, the discernible augmentation in root diameter observed in compost-treated plots can be elucidated by the abundant availability of phosphorus from compost manure. Phosphorus, recognized for its pivotal role in facilitating metabolic processes within plants, exerts a pronounced influence on root diameter, particularly in root crops. This aligns with the findings of Kanauija (2013) and Jagadeesh et al. (2018), who documented increased root diameter in carrot crops following the application of a blend of urea and organic manure. The consequential increase in root diameter under compost treatment directly translates to amplified root yield within the same treatment. This cumulative effect underscores the interplay between improved growth and yield characteristics. Additionally, the heightened root yield can be ascribed to the utilization of organic fertilizers, which mitigate nutrient losses, enhance nutrient utilization efficiency, and augment soil nutrient availability, thereby culminating in amplified root yields (Rantao, 2013). Overall, this mechanistic understanding highlights the intricate interactions between soil enrichment, microbial activity, nutrient availability, and crop yield in the context of organic fertilizer application. It's imperative to acknowledge that the biological yield of plants is influenced by multiple factors, including leaf characteristics and root parameters. The combined use of inorganic fertilizers and organic manure enhances soil nutrient availability, particularly nitrogen, thus augmenting plant biological yield. These findings corroborate similar research outcomes, such as those presented by Subedi et al. (2018) & Sintayehu et al. (2022) in radish cultivation. Our study revealed that plots treated with organic fertilizers exhibited superior yield and growth metrics compared to those treated solely with inorganic counterparts. Thus, the notion that organic farming yields are inherently lower is unfounded based on our empirical evidence (Avery, 1995) & Pokharel et al. (2023). Our results unequivocally demonstrate that organic fertilizers can improve soil biological, chemical, and physical properties relative to inorganic alternatives, leading to enhanced crop productivity.

Table 1. Effect of organic and inorganic fertilizers on plant height and leaves per plant at different stages of Beetroot.

Treatments	Plant height (cm)					
	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS	85 DAS
Compost	7.37^{a}	12.95^{a}	23.10^{a}	28.55^{a}	33.37^{a}	38.30^{a}
Vermicompost	7.25^{a}	12.47^{ab}	21.30^{ab}	27.70^{a}	31.25^{a}	32.72^{b}
Goat manure	6.67^{a}	11.50^{abc}	19.57^{abc}	26.92^{a}	31.12^{a}	32.70^{b}
NPK	6.00^{a}	9.30^{c}	16.25^{c}	21.75^{b}	28.67^{a}	30.82^{b}
Control	6.52^{a}	10.22^{bc}	17.95^{bc}	23.62^{ab}	28.52^{a}	30.26^{b}
Grand mean	6.765	11.29	19.635	25.71	30.59	32.963
CV (%)	15.77	13.10	11.76	12.37	10.79	7.10
SEM (±)	0.55	1.04	1.72	1.91	1.63	1.82
F test	NS	*	**	*	NS	**

NPK: Nitrogen, Phosphorus, and Potassium; FYM: Farm Yard Manure; CV: Coefficient of Variation; SEM: Significant error of Mean; *Significant at 5% level of significance, **Significant at 1% level of significance, ***Significant at 0.1% level of significance, ^{NS}Non-significant

Table 2. Effect of organic and inorganic fertilizers on plant height and leaves per plant at different stages of Beetroot.

Treatments	Number of leaves per plant					
	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS	85 DAS
Compost	5.82^{ab}	7.20^{ab}	8.87^{ab}	10.07^{a}	11.05^{a}	11.80^{ab}
Vermicompost	6.35^{a}	7.60^{a}	9.57^{a}	10.77^{a}	11.62^{a}	12.27^{a}
Goat manure	5.27^{b}	6.40^{b}	8.35^{b}	9.55^{ab}	10.47^{a}	11.02^{bc}
NPK	3.57^{c}	4.90^{c}	6.97^{c}	8.52^{bc}	10.37^{a}	10.20^{c}
Control	3.40^{c}	4.92^{c}	6.82^{c}	7.65^{c}	8.70^{b}	11.30^{ab}
Grand mean	4.88	6.20	8.12	9.31	10.44	11.32
CV (%)	12.87	9.71	8.02	9.72	10.06	5.86
SEM (±)	0.67	0.64	0.61	0.69	0.68	0.50
F test	***	***	***	**	*	**

NPK: Nitrogen, Phosphorus, and Potassium; FYM: Farm Yard Manure; CV: Coefficient of Variation; SEM: Significant error of Mean; *Significant at 5% level of significance, **Significant at 1% level of significance, ***Significant at 0.1% level of significance, ^{NS}Non-significant

Table 3. Effect of organic and inorganic fertilizers on leaf length and leaf breadth at different stages of Beetroot.

Treatments	Leaf length (cm)					
	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS	85 DAS
Compost	7.35^{a}	12.45^{a}	21.67^{a}	28.02^{a}	32.80^{a}	34.77^{a}
Vermicompost	6.77^{a}	12.00^{a}	20.80^{ab}	28.07^{a}	30.72^{a}	31.55^{ab}
Goat manure	6.67^{a}	11.30^{a}	19.75^{ab}	26.37^{ab}	30.40^{a}	31.62^{ab}
NPK	5.05^{a}	9.22^{a}	15.62^{b}	21.22^{b}	28.12^{a}	30.42^{ab}
Control	5.02^{a}	8.65^{a}	16.02^{b}	22.02^{b}	26.57^{a}	27.37^{b}
Grand mean	6.175	10.725	18.775	25.145	29.725	31.15
CV (%)	23.07	21.98	17.05	14.34	12.74	10.75
SEM (±)	0.86	1.33	1.95	2.19	1.92	1.94
F test	NS	NS	*	*	NS	*

NPK: Nitrogen, Phosphorus, and Potassium; FYM: Farm Yard Manure; CV: Coefficient of Variation; SEM: Significant error of Mean; *Significant at 5% level of significance, ^{NS}Non-significant

Table 4. Effect of organic and inorganic fertilizers on leaf length and leaf breadth at different stages of Beetroot.

Treatments	Leaf breadth (cm)					
	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS	85 DAS
Compost	2.00^{a}	4.05^{a}	5.95^{a}	8.40^{a}	8.12^{a}	10.35^{a}
Vermicompost	1.97^{a}	3.87^{a}	5.45^{ab}	8.45^{a}	9.15^{a}	9.46^{ab}
Goat manure	1.82^{a}	3.67^{a}	5.10^{ab}	8.20^{a}	9.40^{a}	9.45^{ab}
NPK	1.52^{a}	2.72^{a}	4.05^{b}	6.72^{a}	7.92^{a}	8.87^{ab}
Control	1.42^{a}	2.62^{a}	3.80^{b}	6.17^{a}	7.42^{a}	7.62^{b}
Grand mean	1.75	3.39	4.87	7.59	8.40	9.15
CV (%)	24.86	25.32	22.88	21.41	22.59	15.57
SEM (±)	0.25	0.50	0.66	0.84	0.90	0.77
F test	NS	NS	*	NS	NS	NS

NPK: Nitrogen, Phosphorus, and Potassium; FYM: Farm Yard Manure; CV: Coefficient of Variation; SEM: Significant error of Mean; *Significant at 5% level of significance, ^{NS}Non-significant

Table 5. Effect of organic and inorganic fertilizers on Beetroot diameter, Beetroot length, root yield and leaf yield.

Treatments	Root diameter (cm)	Root length (cm)	Root yield (t/ha)	Leaf yield (t/ha)
Compost	4.85^{a}	9.3^{a}	13.95^{a}	7.28^{b}
Vermicompost	3.85^{b}	7.2^{c}	9.16^{c}	7.24^{b}
Goat manure	4.02^{b}	8.0^{b}	10.80^{b}	8.13^{b}
NPK	3.37^{b}	7.2^{c}	8.13^{d}	11.09^{a}
Control	2.35^{c}	6.4^{c}	6.28^{e}	5.75^{c}
Grand mean	3.69	7.62	9.66	7.90
CV (%)	12.92	6.57	5.70	10.44
SEM (±)	0.47	0.54	1.36	0.98
F test	***	***	***	***

NPK: Nitrogen, Phosphorus, and Potassium; FYM: Farm Yard Manure; CV: Coefficient of Variation; SEM: Significant error of Mean; ***Significant at 0.1% level of significance

CONCLUSION

The experiment conducted in Khotang district provides valuable insights into the impact of various organic and inorganic fertilizers on different parameters related to beetroot cultivation. Based on our study, it is evident that organic manure consistently outperformed other treatments in terms of overall growth and yield attributes. Our findings indicated that the application of organic manure alone can yield superior results compared to other approaches. This suggests that for optimal beetroot production in the hilly regions of Nepal, the use of organic sources, particularly compost, is highly recommended. It is important to recognize that the applicability of these findings may vary in different agroclimatic locations and with different beetroot varieties. Therefore, further researches are warranted across various regions of the country, encompassing different varieties and additional attributing characteristics, before widespread adoption of these recommendations. This study serves as a valuable contribution to the knowledge base of beetroot cultivation in Nepal, offering insights that can potentially enhance agricultural practices and contribute to food security and livelihood improvement in that region.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors state there is no competing interest.

Author contribution

Conceived & designed the experiment, Soni Kumari Majhi, Sunny Kumar Shah & Umesh Timilsina; Performed the

experiment, Soni Kumari Majhi, Dipika Kumari Shah, Pratima Chaudhary & Prakash Rijal; Writing- original draft, Soni Kumari Majhi & Dipesh Kumar Mehata; Writing- review & editing, Umesh Timilsina, Dipesh Kumar Mehata & Nand Kishor Yadav; Data curation, Formal data analysis & Visualization of the data, Dipesh Kumar Mehata; Supervision, Sunny Kumar Shah & Umesh Timilsina.

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Data availability

Data will be made availabale on request.

Consent to participate

The authors consent to participate.

Consent for publication

The authors consent for publication.

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RESEARCH ARTICLE

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Efficacy of different pesticides in suppressing yellow stem borer in spring rice (*Oryza sativa*) in Ratuwamai, Morang, Nepal

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Abstract

Rice, a staple food for over half the global population, is crucial for food security, economic stability, and cultural significance. Its production, however, is threatened by pests like the Yellow Stem Borer (YSB), which causes substantial yield losses, especially in rice-dominant regions like Nepal. This study focuses on evaluating the effectiveness of biological, botanical, and chemical pesticides against YSB in spring rice crops of Ratuwamai, Morang, Nepal. For this, we apply biological methods/pathogens like *Bacillus thuringiensis*, to target YSB; botanical pesticides, derived from plants like Azadirachtin and Mugworth, act as repellents; while chemical pesticides such as Cypermethrin, Chlorpyriphos and Cartap Hydrochloride offer rapid control but come with environmental risks. Among those six pesticides used, the present findings revealed that chlorpyriphos 20% EC have minimum mean dead heart with 4.92% and cypermethrin 10% EC have minimum mean white ear head with 2.44% respectively after application of first and second dose of treatments respectively. Likewise, most plant yield attributes were superior where chlorpyriphos was used. Though bacillus and azadirachtin reduced the dead heart and white ear head symptoms, they couldn't give good yield than that of chemical pesticides. Thence, through our research we investigated the impacts of different biological, botanical and chemical pesticides in controlling YSB population densities, and influencing yield and yield attributing characters from the field experiment.

Keywords: Dead heart, Pesticides, Spring rice, White ear head, Yellow stem borer

INTRODUCTION

Rice is a crucial source of food for over half of the world's population for about 3.5 billion people, predominantly in Asia and Africa. Its global context is shaped by its significance to food security, economic livelihoods, and cultural importance (Roopwan et al., 2023; Rajput et al., 2020; Fukagawa & Ziska, 2019). Historically rooted in the fertile deltas of Asia, rice production has grown substantially to meet rising demands, making it the second most produced grain after maize. Nations like China and India dominate in rice cultivation, influencing international prices and trade policies (Schneider & Asch, 2020). Climate plays a pivotal role, with monsoons dictating yields in many regions. Excessive water usage, deforestation, and chemical inputs have posed sustainability challenges. As urbanization and climate change threaten traditional rice farming landscapes, there's an urgent need for innovative, sustainable practices (Eliazer Nelson et al., 2019). Innovations such as the System of Rice Intensification (SRI) are emerging to address these challenges. Globally, the rice trade is highly politicized, with countries holding

reserves to prevent food shortages. The balance between ensuring food security, maintaining economic stability, and upholding ecological sustainability is central to the global discourse on rice production (Glover, 2011).

Rice holds a significant place in Nepal's agricultural landscape, contributing to both food security and cultural practices. As a staple diet for a majority of its population, rice is cultivated across the Terai plains, hills, and even some mountain regions (Kakshapati et al., 2022; Gadal et al., 2019). A report published by MoALD in 2023 revealed the cultivation of rice on 1.48 million hectares of land, resulting in a total production of 5.13 million tons, with an average yield of 3.47 tons per hectare in Nepal (MoALD, 2023). The diverse topography and varying climate conditions result in a variety of rice types, tailored to specific altitude and rainfall conditions. While the Terai belt, with its flatlands and ample water sources, produces the bulk of Nepal's rice, hill and mountain terraces demonstrate the resilience of farmers in adapting to challenging terrains. However, Nepal's rice production faces multiple challenges (Chandio et al., 2021). Despite the potential of the Terai region, outdated farming practices and lack of access to modern technology limit yield enhancements. Water scarcity, exacerbated by changing monsoon patterns due to climate change, further impacts yields (Karki et al., 2021). Additionally, while the government has introduced subsidies and support for rice farmers, infrastructure challenges and market inefficiencies hamper growth. Still, there's an increased emphasis on organic and traditional rice varieties, attracting niche markets and promoting sustainability (Gadal et al., 2019). Furthermore, community-based approaches and indigenous knowledge play a crucial role in preserving and enhancing rice cultivation in Nepal amidst changing global and environmental dynamics (Chandio et al., 2021).

Further, Yadav et al. (2023a) indicated that 52.4% of rice crop losses result from damage caused by various pathogens, animal pests, and weeds. Yellow stem borer (YSB) is also a significant pest that affects rice cultivation, causing substantial yield losses globally and in Nepal (Nyaupane, 2022). Internationally, YSB has been a concern in many rice-growing countries of Asia, where traditional and high-yielding rice varieties are equally vulnerable. Infestation leads to "dead hearts" in young plants and "whiteheads" in older ones, both resulting in reduced grain production. Global trade, climate change, and monoculture practices have inadvertently facilitated the spread and intensity of this pest (Kattupalli et al., 2021). In the Nepalese context, the challenge is magnified due to the country's reliance on rice as a staple and its central role in agricultural livelihoods. The diverse topography of Nepal, spanning the Terai plains to hilly terraces, offers varied habitats for YSB (Choudhary et al., 2022). While modern pesticides and control measures are available, many farmers, especially in remote areas, rely on traditional methods, which might not be as effective against severe infestations. The limited resources and lack of access to advanced agricultural practices exacerbate the problem (Chandio et al., 2021).

Both globally and in Nepal, integrated pest management (IPM) practices, which combine cultural biological, botanical, and chemical methods, are being promoted as sustainable solutions (Roopwan et al., 2023; Kakshapati et al., 2022; Kafle et al., 2014). Further, Sharma et al. (2022) and Yadav et al. (2023b) stated that effective screening of invasive pests can greatly assist in identifying and controlling these organisms. Continued research, farmer education, and international cooperation are vital to tackle the YSB challenge effectively. Farmers employ a multi-faceted approach to combat the Yellow Stem Borer (YSB) menace in rice fields. One prevalent method is the use of resistant rice varieties, bred specifically to reduce susceptibility to YSB. Alongside, cultural practices like adjusting planting dates can disrupt the life cycle of the borer, thereby reducing its impact. Yadav et al. (2022a) and Yadav et al. (2022b) reported that the proper understanding of lifecycle and behavior of pests is crucial for effective pest management; the more information available about their lifecycle and behavior, the greater the likelihood of successful management. Furthermore, pheromone traps are used to monitor and reduce adult YSB populations (Katti, 2021). Biological control, involving the introduction of natural predators like *Bacillus*, has gained traction as an eco-friendly alternative to control YSB populations. This environmentally friendly method minimizes harm to beneficial insects and reduces the need for chemical interventions. However, its effectiveness can be influenced by factors like local biodiversity and climate conditions (Estiati, 2020). Derived from plants, botanical pesticides are naturally occurring insecticides. Neem, for instance, acts as a repellent and antifeedant against YSB. While botanical pesticides are biodegradable and less toxic to non-target organisms, their efficacy can sometimes be lower than chemical counterparts, requiring frequent applications (Adhikari et al., 2020). Chemical Pesticides remain a common choice for rapid and effective control. Chemical formulations target various YSB life stages, ensuring reduced infestation. However, their overuse can lead to resistance in pest populations. Additionally, non-judicious application poses environmental risks, potentially harming beneficial organisms and contaminating water sources. While chemical pesticides are available, their use is approached with caution due to environmental and health concerns, pushing for an increased focus on integrated pest management (Sah & Sharma, 2023; Mishra et al., 2021). Further, Yadav et al. (2023c) reported a rising trend among farmers towards adopting integrated pest management approaches for controlling pests in their crops. Thus, by exploring the most effective measures against YSB, this study addresses potential yield losses which in turn, aids for economic security of the community. Given these precedents, it's paramount to evaluate alternative methods, such as biological and botanical pesticides, in the Ratuwamai context. This research will offer insights into sustainable

pest control measures that uphold ecological balance, benefiting both current and future generation.

MATERIALS AND METHODS

Experimental Location and Design

The research was meticulously conducted in a farmer's field from February to June 2023 at Ratuwamai municipality in Morang district of Nepal. Using a sophisticated Randomized Complete Block Design (RCBD), seven distinct treatments were introduced. These treatments underwent three replicates, culminating in a comprehensive 21 individual plots.

Treatments

Six rigorously selected pesticides and a control without any treatment were subjected to testing. The experiment involved seven different treatment groups namely; T1 involved the use of *Bacillus thuringiensis* var *krustaki* at a rate of 2ml/lit, T2 and T3 involved the application of Azadirachtin and Mugworth leaf extract at a rate of 2ml/lit and 15ml/lit, respectively, T4 used Cypermethrin at a rate of 1.5ml/lit, T5 involved the use of Chloropyriphos at a rate of 2ml/l, T6 utilized Cartap Hydrochloride at a rate of 20kg/ha, while T7 was an untreated control plot.

Table 1. Lists of pesticides applied in research plots.

SN	Generic Name	Trade Name	Notation	Dose
1	*Bacillus thuringiensis var krustaki* 15% SC	Minchu+	T1	2ml/lit
2	Azadirachtin 0.03%	Multineem	T2	2ml/lit
3	Mugworth leaf extract	-	T3	15ml/lit
4	Cypermethrin 10% EC	Cyper-10	T4	1.5ml/lit
5	Chloropyriphos 20% EC	Dhanvan-20	T5	2ml/lit
6	Cartap Hydrochloride 4% G	Cartap	T6	20kg/ha
7	Control	-	T7	-

Plot Dimensions and Planting

A precision layout was employed. Each plot was exactly 2×2 meters squared. To prevent cross-contamination and allow for unhindered maintenance, a buffer zone of 0.5 meters was established between plots. Given the 20 cm spacing both between plants as well as between rows, each plot perfectly accommodated 100 rice plants.

Cultivation Practices

The study focused on "Chaite Dhan-4" variety of spring rice, which is commonly cultivated by farmers in the research location. The Chaite Dhan-4 variety was transplanted from nursery beds to main research field after extensive soil preparation. All plots adhered to regional cultivation practices, which included irrigation frequency, soil fertility management, and weed control. Each pesticide was systematically applied using knapsack sprayer at two pivotal growth stages of the rice plants, timed with the pest population reaching its economic threshold level. The first treatment aimed at the vegetative phase, while the second targeted the reproductive phase, with a strict 20-day interval to ensure consistent growth response. The crop was harvested when most of the crops had reached 80% maturity stage, and crop cutting was conducted manually using sickles.

Data Collection and Observation

Dead Hearts % & White ear Heads %

Initial data for dead hearts were taken one day prior to the first spray of pesticides from ten randomly selected hills of each individual plots. Subsequent observations were then noted on 5, 10, and 15 days after first treatment application. For white ear heads, similar post-second application counts were taken. White ear heads were initially observed one day before the second application of pesticides. Further observations were made on 5, 10, and 15 days after the second spray. The percentage of dead hearts and white heads was calculated, and the mean was determined:

Dead Hearts % = (Number of dead hearts / Total number of tillers) x 100.

White ear Head % = (Number of white ear head / Total number of tillers with panicle) x 100. (Chatterjee & Mondal, 2014)

Plant Height and Number of tillers

Once the rice plants reached their full length in terms of growth, their height was measured from the base to the tip, and the number of tillers or side shoots branching from the main plant was counted.

Filled grains % and Unfilled grains %

At the pinnacle of their maturity, rice plants were gently subjected to panicle cutting, allowing for an accurate count of both filled and unfilled grains.

Filled Grains % = (Number of filled grains / Total number of grains) * 100

Unfilled Grains % = 100 – filled grains %

Test Weight and Grain Yield

Ensuring minimal grain loss, the harvested rice was subjected to assessments. Moisture content of the harvested crops was measured using a moisture meter, and the yield was calculated from the 1 m^2 sections of each plot. The test weight was carefully documented, ensuring a consistent moisture content of 13% across samples.

Test Weight = Weight of 1000 Grains

Statistical Analysis

The data collected over the span of the research months was diligently entered into MS Excel for initial scrutiny. Parameters such as dead hearts %, natural enemies/predators count, white ear heads %, plant height, filled grains % and unfilled grains %, test weight, and grain yield per hectare formed the foundation for analysis. To meet the assumptions of the statistical tests, data underwent necessary transformations: square root for dead hearts % and white ear heads %, and arc sine for filled and unfilled grain percentages as given by Gomez & Gomez (1984). The final analysis was executed in R-Studio, using relevant statistical tests to determine significant differences among treatments.

RESULTS

Impact of Pesticides on Dead Hearts Percentage Following the First Spray

Before the application of pesticides at 1 day before 1st spray, the Economic Threshold Level for dead hearts had exceed (mean DH %= 7.61%). Upon the application of the seven different treatments to the spring rice fields, variations were observed in the outcomes concerning dead heart percentages. For *Bacillus* treatment, a day prior to the first spray, we observed a dead heart percentage of approximately 7.89%. This percentage somewhat increased noticeably to 8% just five days after the treatment. By the 10th and 15th day post-spray, the dead heart percentage stabilized at 6.65% and 5.87% respectively, indicating the effectiveness of the treatment in the initial days. Remarkably, the mean percentage of dead hearts after the first spray stood at 6.84%. The Azadirachtin treatment, began with a higher initial dead heart percentage of 7.4%. However, by the 15th day, it had reduced to 6.34%, averaging at 6.68% after the first spray. On the other hand, the Mugwort treatment commenced with a dead heart percentage of 8.98%, which saw a little drop to 8.39% by the fifteenth day. It's evident that while treatments like Cypermethrin, Chloropyriphos and Cartap Hydrochloride had varying levels of impact on dead heart percentages, there was a more pronounced reduction in dead hearts number, especially with Chloropyriphos, which saw a highest drop in DH% from 7.11% before spray to 4.18% by the 15th day. The overall effectiveness of different treatments following the first spray, as indicated by the mean percentage of dead hearts after spraying, showed that Chloropyriphos 20% EC was the most efficient and significantly superior (4.92%) among all treatments in reducing dead hearts. It was followed by Cartap Hydrochloride 4% G and Cypermethrin 10% EC, with percentages of 5.23% and 6.01%, respectively. Bacillus thuringiensis var kurstaki 15% SC showed similar efficacy to Azadirachtin 0.03%. Mugwort demonstrated the least effectiveness, with the highest percentage of dead hearts recorded at 8.58%. The untreated control exhibited 7.69% dead hearts. The mean percentage of dead hearts at 1 day before spraying (dbs) and at 5, 10, and 15 days after spraying (das) is detailed in Table 2 .

Impact of Pesticides on White ear Heads Percentage Following the Second Spray

Before applying pesticides at 1 day before 2nd spray, the Economic Threshold Level for white ear head had neared (mean WH %= 4.93%). Post the second spray, the Bacillus treatment showcased a consistent reduction in white ear heads percentage, from an initial 3.71% a day before the spray to 2.78% by the fifteenth day. Azadirachtin again displayed similar patterns as observed post the first spray, with a continuous decline in both white ear heads percentage from 5.06% to 4.11%. Mugwort recorded much smaller change in white ear head from 6.64% before spray to 6.19% at 15 days after spray. Chemical pesticides such as Cypermethrin, Chloropyriphos and Cartap Hydrochloride were better pesticides in reducing white ear head in spring rice in our study. Among six pesticides used, Cypermethrin was found to have best performances (low mean WH = 2.44%) in terms of declining the white ear head in rice experimental plots after 15 days of spray. This was followed by Cartap Hydrochloride (WH = 2.88%) and Chloropyriphos (WH = 3.39%) which were in par with each other for efficacy. Likewise, biological pesticides like as *Bacillus thuringiensis var*

krustaki was found to be less effective (WH = 3.39%) than that of other chemical pesticides applied but was in par with Chloropyriphos and Cartap Hydrochloride. However, botanical pesticides such as Azadirachtin and Mugwort were found to have least performances in suppressing pest number with white ear head of 4.74% and 6.69% respectively. The control group have highest level of infestation of 7.78%. The mean white ear head percentage (WH%) at 1 day before spray (dbs) followed by 5, 10 and 15 days after spray (das) is illustrated in Table 3.

Influence of Pesticides on Yield and Yield Attributing Characters

Finally, assessing the impact on yield and its attributing characters, the *Bacillus* treatment resulted in an average plant height (PH) of 88.23 cm. The filled grain percentage (FG%) stood at a promising 78.59%, while the unfilled grains (UG%) were at 21.41%. The test weight (TW) was recorded at 23 gm, with an overall yield per hectare (YH) of 5.11 tons. Similar observations were made for other treatments, with each showcasing unique patterns in terms of yield and its attributing characteristics. There was no notable difference in plant height among the treatments, which could be attributed to genetic characteristics and variations in fertilizer dosages reaching the rice roots. Nonetheless, Cypermethrin 10% EC exhibited superior performance in terms of plant height at 89.15 cm, followed by Azadirachtin 0.03% and Chloropyriphos 20% EC at 88.42 cm and 88.33 cm, respectively. The number of filled grains of rice showed statistically significant differences across the various pesticide treatments (see Table 4). Chloropyriphos 20% EC resulted in the highest number of filled grains at 82.48%, followed closely by Cartap Hydrochloride 4% G at 81.70% and Cypermethrin 10% EC at 80.53%. Bacillus thuringiensis var kurstaki 15% SC, Azadirachtin 0.03%, and Mugwort leaf extract followed suit with 78.59%, 76.57%, and 74.54% filled grains, respectively, while the control exhibited the lowest number of filled grains at 68.11%. Significant variations were also observed in the number of unfilled grains of rice due to different pesticides (see Table 4). Chloropyriphos 20% EC had the lowest number of unfilled grains at 17.52%, followed by Cartap Hydrochloride 4% G at 18.30% and Cypermethrin 10% EC at 19.47%. Bacillus thuringiensis var kurstaki 15% SC, Azadirachtin 0.03%, and Mugwort leaf extract showed 21.41%, 23.43%, and 25.46% unfilled grains, respectively, while the control had the highest number of unfilled grains at 31.89%. Similarly, there were no significant variations in test weight among the six treatments. However, chemical treatments such as Chloropyriphos 20% EC, Cartap Hydrochloride 4% G, and Cypermethrin 10% EC exhibited the highest test weights at 25.67 gm, 24.33 gm, and 23.67 gm, respectively, while the control plots had the least test weight at 20.67 gm. The grain yield was found to be significantly different due to those applied pesticides in our research study. The maximum yield was obtained from Chloropyriphos 20% EC (6.71 ton/ha) in our field which was succeeded by Cartap Hydrochloride 4% G (5.96 ton/ha), Cypermethrin 10% EC (5.59 ton/ha), *Bacillus thuringiensis var krustaki* 15% SC (5.11 ton/ha), Azadirachtin 0.03% (4.79 ton/ha) and Mugworth leaf extract (4.39 ton/ha). The minimum grain yield was recorded in control individual units (4.01 ton/ha). The above description is represented in Table 4:

Table 2. Incidence of dead hearts (DH) before and after first spray of pesticides.

Treatments	1 DAS	5 DAS	10 DAS	15 DAS	Pooled
Bacillus thuringiensis var krustaki	7.89^{ab}	8.00^{ab}	6.65^{ab}	5.87^{b}	6.84^{abc}
	(2.81)	(2.83)	(2.58)	(2.42)	(2.61)
Azadirachtin	7.40^{ab}	7.53^{ab}	6.16^{ab}	6.34^{ab}	6.68^{abc}
	(2.71)	(2.73)	(2.47)	(2.51)	(2.57)
Mugworth leaf extract	8.98^{a}	9.13^{a}	8.22^{a}	8.39^{a}	8.58^{a}
	(2.97)	(3.00)	(2.84)	(2.87)	(2.90)
Cypermethrin	8.38^{ab}	6.86^{ab}	5.97^{ab}	5.19^{b}	6.01^{bc}
	(2.89)	(2.62)	(2.44)	(2.28)	(2.45)
Chloropyriphos	7.11^{ab}	5.69^{b}	4.88^{b}	4.18^{b}	4.92^{c}
	(2.66)	(2.38)	(2.21)	(2.04)	(2.22)
Cartap Hydrochloride	7.32^{ab}	5.97^{b}	5.21^{b}	4.52^{b}	5.23^{c}
	(2.70)	(2.44)	(2.28)	(2.12)	(2.28)
Control	6.21^{b}	7.00^{ab}	7.71^{a}	8.39^{a}	7.69^{ab}
	(2.49)	(2.64)	(2.77)	(2.89)	(2.77)
Mean	7.61	7.17	6.40	6.13	6.56
CV	8.287	8.307	9.059	9.141	8.791
SEM	0.051840	0.04893	0.051792	0.05003	0.050018
F-test	ns	ns	*	**	*

Note: Values are the mean of three replications at different days of observation; DAS: Days after spray; CV: Coefficient of variation; ns: non-significant; **: Significant at 1% level of significance; *: Significant at 5% level of significance; SEM: Standard error of mean; Values with the same letters in a column are not significantly different at 5% level of significance by DMRT test and parenthesized values indicate square root transformation values.

Table 3. Incidence of white earheads (WH) before and after second spray of pesticides.

Treatments	1 DAS	5 DAS	10 DAS	15 DAS	Pooled
Bacillus thuringiensis var krustaki	3.71^{c} (1.93)	4.17^{bc} (2.04)	3.24^{bc} (1.80)	2.78^{cd} (1.67)	3.39^{bc} (1.84)
Azadirachtin	5.06^{abc} (2.24)	5.53^{ab} (2.35)	4.58^{b} (2.13)	4.11^{c} (2.02)	4.74^{b} (2.17)
Mugworth leaf extract	6.64^{a} (2.55)	7.16^{a} (2.65)	6.71^{a} (2.56)	6.19^{b} (2.45)	6.69^{a} (2.55)
Cypermethrin	3.91^{c} (1.98)	2.93^{c} (1.71)	2.44^{c} (1.56)	1.96^{d} (1.40)	2.44^{c} (1.56)
Chloropyriphos	4.83^{abc} (2.20)	3.88^{bc} (1.97)	3.38^{bc} (1.84)	2.91^{cd} (1.70)	3.39^{bc} (1.84)
Cartap Hydrochloride	4.24^{bc} (2.05)	3.34^{c} (1.82)	2.88^{bc} (1.69)	2.43^{cd} (1.55)	2.88^{bc} (1.69)
Control	6.11^{ab} (2.47)	7.07^{a} (2.66)	8.04^{a} (2.83)	8.23^{a} (2.87)	7.78^{a} (1.79)
Mean	4.93	4.87	4.47	4.09	4.47
CV	9.733	9.693	10.571	11.531	10.483
SEM	0.045931	0.04426	0.04741	0.05064	0.04683
F-test	*	***	***	***	***

Note: Values are the mean of three replications at different days of observation; DAS: Days after spray; CV: Coefficient of variation; ***: Significant at 0.1% level of significance; *: Significant at 5% level of significance; SEM: Standard error of mean; Values with the same letters in a column are not significantly different at 5% level of significance by DMRT test and parenthesized values indicate square root transformation values.

Table 4. Effect of pesticides on yield and other plant characters.

Treatments	PH (cm)	FG%	UG%	TW (gm)	Yield (ton/ha)
Bacillus thuringiensis var krustaki	88.23^{a}	78.59^{d} (62.44)	21.41^{d} (27.56)	23.00^{a}	5.11^{cd}
Azadirachtin	88.42^{a}	76.57^{e} (61.05)	23.43^{c} (28.95)	22.67^{a}	4.79^{d}
Mugworth leaf extract	85.41^{a}	74.54^{f} (59.69)	25.46^{b} (30.31)	21.33^{a}	4.39^{de}
Cypermethrin	89.15^{a}	80.53^{c} (63.81)	19.47^{e} (26.19)	23.67^{a}	5.59^{bc}
Chloropyriphos	88.33^{a}	82.48^{a} (65.25)	17.52^{g} (24.75)	25.67^{a}	6.71^{a}
Cartap Hydrochloride	85.83^{a}	81.70^{b} (64.67)	18.30^{f} (25.33)	24.33^{a}	5.96^{b}
Control	85.00^{a}	68.11^{g} (55.62)	31.89^{a} (34.38)	20.67^{a}	4.01^{e}
Mean	87.19	77.50	22.49	23.05	5.22
CV	2.79	0.2454	0.5376	13.89	7.82
SEM	5.922	0.023	0.023	10.254	0.166
F-test	ns	***	***	ns	***

Note: Values are the mean of three replications at different days of observation; PH: Plant height; FG: Filled grains; UG: Unfilled grains; TW: Test weight; CV: Coefficient of variation; ns: non-significant; ***: Significant at 0.1% level of significance; SEM: Standard error of mean; Values with the same letters in a column are not significantly different at 5% level of significance by DMRT test and parenthesized values indicate arc sine transformation values.

The data suggests that while certain treatments like Chloropyriphos and Cypermethrin demonstrate a substantial reduction in pest impact, but it might require a more balanced approach, considering both pest control and ecological impact. The efficacy of treatments also has a pronounced influence on the yield and its attributing characters.

DISCUSSION

The varying degree of pest incidence and their responses to different six pesticides in spring rice plants highlight the delicate balance between pest management and environmental sustainability in our experiment. Of those six treatments plus a control applied, all three chemical pesticides were dominant in controlling pest population indicated by minimum dead hearts percentage (4.92%) in chloropyriphos 20% EC and minimum white ear head percentage (2.44%) in cypermethrin 10% EC. Our findings were in consistent with that of Roopwan et al. (2023) and Kakshapati et al. (2022) which pointed chemical treatments to be most effective in suppressing yellow stem borer other than biological and botanical treatments in short term totally can be attributed to active nature and rapid mode of action of pesticides used as well with better yields. However, Yadav et al. (2022c) highlighted that botanical pesticides play a significant role in reducing pest infestations sustainably. Furthermore, chlorpyriphos is a common organophosphate pesticide employed in controlling a variety of pests in different crops.

In a field experiment by Karki et al. (2023), Chlorpyriphos 20 EC @ 2ml/litre was identified as more effective in reducing yellow stem borer incidence in spring rice compared to other treatments. This was further supported by Sawant et al. (2019) and Karki et al. (2023) in line with our experiment. While chemical pesticides have shown significant reductions in borer pests, biological agents like Bacillus and botanical pesticides such as azadirachtin have also been effective in the long term. Commercial formulations containing Bacillus thuringiensis have proven to be a viable alternative for controlling various insect pests as mentioned in different papers (Sah & Sharma, 2023; Estiati, 2020; Balasubramamiam and Kumar, 2019; Kumari et al., 2019). The treatment involving Bacillus demonstrated a gradual decrease in dead heart and white earhead occurrences throughout the observation period in our experiment. The toxins produced by this bacterium can damage the gut tissues of the larvae, causing gut paralysis, which leads to cessation of feeding and ultimately the death of the larvae due to starvation and damage to the mid-gut epithelium (Chatterjee & Mondal, 2014).

In our recent research, Bacillus thuringiensis var kurstaki was found to be less effective against rice yellow stem borer, resulting in a gradual reduction in insect incidence and a decrease in the occurrences of dead heart and white ear heads in the field, albeit with mild yield impacts compared to other chemical pesticides used. However, its efficacy may be compromised in populations of yellow stem borers that have developed resistance to the bacterium's insecticidal proteins (Rajput et al., 2020). Research by Roopwan et al. (2023), Adhikari et al. (2020), Madhu et al. (2020) and Ogah et al. (2011) supported neem oil/azadirachtin as an effective alternative remedy against yellow stem borers. In contrast, Azadirachtin, though less proficient against dead hearts, exhibited a modest reduction in pest population. Dougoud et al. (2019) and Hashemitassuji et al. (2014) highlighted the effectiveness of neem-based pesticide due to its disruption on insect metabolism, causing female infertility, hindering molting, and possessing antifeedant properties. Mugwort's performance aligns with recent studies by Kakshapati et al. (2022) and Gao et al. (2020), underscoring its natural insect-repelling qualities, albeit necessitating careful monitoring of broader ecological implications.

However, Hashemitassuji et al. (2014) concluded that environmental factors such as temperature, humidity, and precipitation affected the efficacy of the pesticides, resulting in an increase in pest populations even after treatment in line with our research. Even though the outcomes of various treatments varied, Kaur and Singh (2021) demonstrate how important it is to evaluate the short-term yield benefits of each treatment against any potential long-term ecological implications. It's crucial to take into account the fact that pests will eventually become resistant to particular chemical treatments. For instance, despite having demonstrated that cypermethrin showed notable immediate effectiveness, as has been shown for other pesticides used in rice farming, its long-term performance may be called into question if pests become resistant (Norton et al., 2010). In addition to rendering treatments ineffective, this kind of resistance can cause pests to resurface in worse forms. Although our findings shed light on the treatments' immediate efficacy, more research must take a comprehensive approach that takes into account cultural dynamics, economic viability, resistance patterns, and wider environmental and health impacts (Yadav et al., 2024). Such extensive research in the future guarantees that the solutions are not only useful in the long run but also effective in the immediate term.

CONCLUSION

Rice cultivation in Ratuwamai, Morang, Nepal, faces the persistent challenge of the yellow stem borer (YSB). Our study evaluates biological, botanical, and chemical pesticides, revealing Bacillus and Azadirachtin as potent and ecologically sensitive options. These alternatives reduce pest impact while preserving beneficial predator populations, marking a sustainable approach crucial for optimal yields and ecological balance. Despite immediate benefits, chemical pesticides like Cypermethrin and Chloropyriphos pose ecological risks, emphasizing the need for a nuanced

perspective. Beyond field boundaries, considerations extend to environmental and health impacts. Balancing short-term gains with long-term sustainability is vital. This study offers a blueprint for rice cultivation—harmonizing productivity with sustainability through continuous research, community collaboration, and integrating modern science with traditional wisdom. The goal: productive and sustainable fields, fostering harmonious coexistence with nature.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest. Author contribution All authors contributed equally in the paper formation. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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RESEARCH ARTICLE

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Production of a novel biodegradable film made from chitosan and pomegranate (*Punica granatum* L.) seed essential oil

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Abstract

This study evaluated the effects of pomegranate (*Punica granatum* L.) seed essential oil (PSO) on chitosan-based films. The results showed that the addition of PSO slightly increased thickness, while significantly decreasing the moisture content, and solubility properties. The color values of the composite films containing PSO changed noticeably, with a tendency toward light brown, which was beneficial in resisting food decomposition caused by ultraviolet light. X-Ray diffraction analysis (XRD) and Fourier-transform Infrared (FT-IR) results indicated that the addition of PSO affected the structure of the chitosan films, while the interaction between chitosan and polyphenols in PSO established new hydrogen bonds. Scanning electron microscopy (SEM) showed that the surface of the PSO-containing blend films was rougher compared to the chitosan control film, and the roughness increased as the PSO content in the blend films increased. Additionally, composite films that contained PSO had substantial antibacterial action, particularly against pathogenic *E. coli*. In short, the novel active chitosan-based films with incorporated PSO present broad application prospects in the packaging of fresh-cut meat or vegetables. Therefore, this study will also be beneficial in these areas.

Keywords: Chitosan film, Pomegranate seed essential oil, Edible film, Antimicrobial, Blend films

Type: Research Article

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INTRODUCTION

In response to growing ecological concerns over plastic waste and depleting fossil fuel reserves, the shift towards biopolymer-based packaging, particularly in food packaging, has gained momentum (Kanmani & Rhim, 2014). Biopolymers offer a sustainable, biodegradable, and non-toxic alternative for food packaging, enabling the extension of food shelf life without direct contact with the food. The interest in natural additives for edible coatings further highlights this trend (Wang & Rhim, 2015).

The shelf life of food products is influenced by factors such as the food's nature, chemical composition, processing, packaging materials, and storage conditions (Pacheco et al., 2019). Edible films, a key research area, contribute to food safety and sustainability but face challenges due to their inferior water vapor barrier properties compared to synthetic films. To improve this, lipids are added to edible films, though this can reduce the film's strength (Xue et al., 2021). Chitosan, known for its antimicrobial properties, faces limitations in physical properties, which can be enhanced by incorporating natural antioxidants and antibacterial agents like essential oils (EOs). Essential oils, derived from natural plant sources, are celebrated for their pronounced antibacterial and antioxidant properties.

Their inclusion in chitosan films markedly improves the antibacterial, antioxidant capabilities, water solubility, and vapor-permeability of the films (T. Liu, Wang, Chi, Tan, & Liu, 2020).

Pomegranate (*Punica granatum* L.), a fruit with a rich historical consumption for its nutritional and medicinal value, continues to draw scientific interest due to its bioactive components and health-promoting benefits. The seed oil of pomegranate, a by-product of the juice manufacturing process, is a significant source of punicic acid, a fatty acid with known health benefits, including antimicrobial, antioxidant, and anticancer properties (Paul & Radhakrishnan, 2020).

Pomegranate seed oil (PSO), rich in beneficial fatty acids and antioxidants, is underutilized in chitosan films. Despite the extensive investigation into the chemical, physical, structural, and biological properties of chitosan films infused with EOs, studies focusing on chitosan composite films containing PSO are sparse. This research utilized cold-pressed PSO, reputed for its superior fatty acid profile and physicochemical properties, such as low peroxide value, high phenolic content, and enhanced aroma profile (Khoddami, Man, & Roberts, 2014). The study aimed to evaluate the antibacterial efficacy of PSO and hypothesized that its addition to chitosan films would improve their antimicrobial properties. The primary objective was to characterize the physicochemical properties of the CH-control and CHPS films using SEM, FTIR, XRD, and UV-visible analyses while examining changes in their mechanical characteristics and antimicrobial activity.

MATERIALS AND METHODS

Materials Acquisition

Methanol, ethanol, and acetic acid were obtained from Carlo Erba. Chitosan with a low molecular weight and 75% deacetylation level were procured from Sigma-Aldrich and used as received. Pomegranate seed oil was purchased from Dnl, a local company in Turkey, that produces oil using the cold pressing method. All experimental procedures were conducted in an oxygen-free nitrogen environment, employing solvents that were not subjected to any purification process.

Film Characterization Techniques

For film analysis, an Agilent 630 device was utilized to capture FT-IR spectra, while the phenolic composition of pomegranate seed essential oil was determined using an Agilent 6890N GC (GC-MS). The surface morphology of the CH and CHPS films and their crystalline structures were examined using an SEM FEI-Quanta-250-FEG and a Bruker-AXS-D8 Advance X-ray diffractometer, respectively. Film thickness was measured in micrometers using an Insize digital micrometer, whereas thermal properties and elemental composition were assessed with a TGA Shimadzu DSC-60 and through elemental analysis, respectively.

Essential Oils Volatile Components Analysis via GC-MS

The analysis of the volatile components in the essential oils (EOs) incorporated into CH-PSO films was performed using GC–MS. The system consisted of an Agilent 6890N GC paired with a 5973 Network mass selective detector, equipped with a 30m, 0.25mm i.d., 0.25 µm film-coated Stabilwax®-DA capillary column by Restek. The EOs were diluted in acetone, with 1 µL injected at a 250 °C injector temperature with a 10:1 split ratio. The temperature program started at 60 °C, increased to 200 °C at 8 °C/min, held for 1 min, then increased to 240 °C at 20 °C/min, and held for 3.5 min. Helium served as the carrier gas at a flow rate of 1.0 mL/min. Mass spectrometry settings included an ion source temperature of 230 °C, electron energy of 70 eV, multiplier voltage of 1447 V, GC/MS transfer line temperature of 250 °C, and a scan range from 33 to 650 mass units. Compound identification was based on comparisons with existing spectral libraries.

Preparation of Chitosan-Based Films

The film formation process was executed using the solvent casting technique as outlined in prior studies (Mouhoub et al., 2022). In summary, chitosan at a concentration of 2% (w/v) was dissolved in 1% acetic acid with continuous agitation. Following the integration of glycerol (as a plasticizer) and Tween 80 (as an emulsifier), essential oils were added at a 2% (v/v) concentration. These components were then uniformly blended at 40 °C for 10 minutes utilizing an ultrasonic cleaner (Wisd WiseClean WUC A22H). Afterward, 3 mL of this blended mixture was evenly spread over a 5-cm diameter plastic mold and subjected to drying at 40 °C for 48 hours within an oven. The resultant films were then prepared for subsequent evaluations. A film devoid of essential oil served as the control group, whereas a film embedded with a mixture of essential oil (0.2% v/v for each type) was employed to assess the synergistic effects of three films with essential oil under investigation. Various concentrations of pomegranate seed oil (2.5%, 5.0%, and 7.5%) were also integrated into the film solutions. A formulation excluding both pomegranate seed oil and Tween 80 was designated as the baseline control. The produced chitosan films were stored in Petri dishes at 4 °C until further examination (Figure 1).

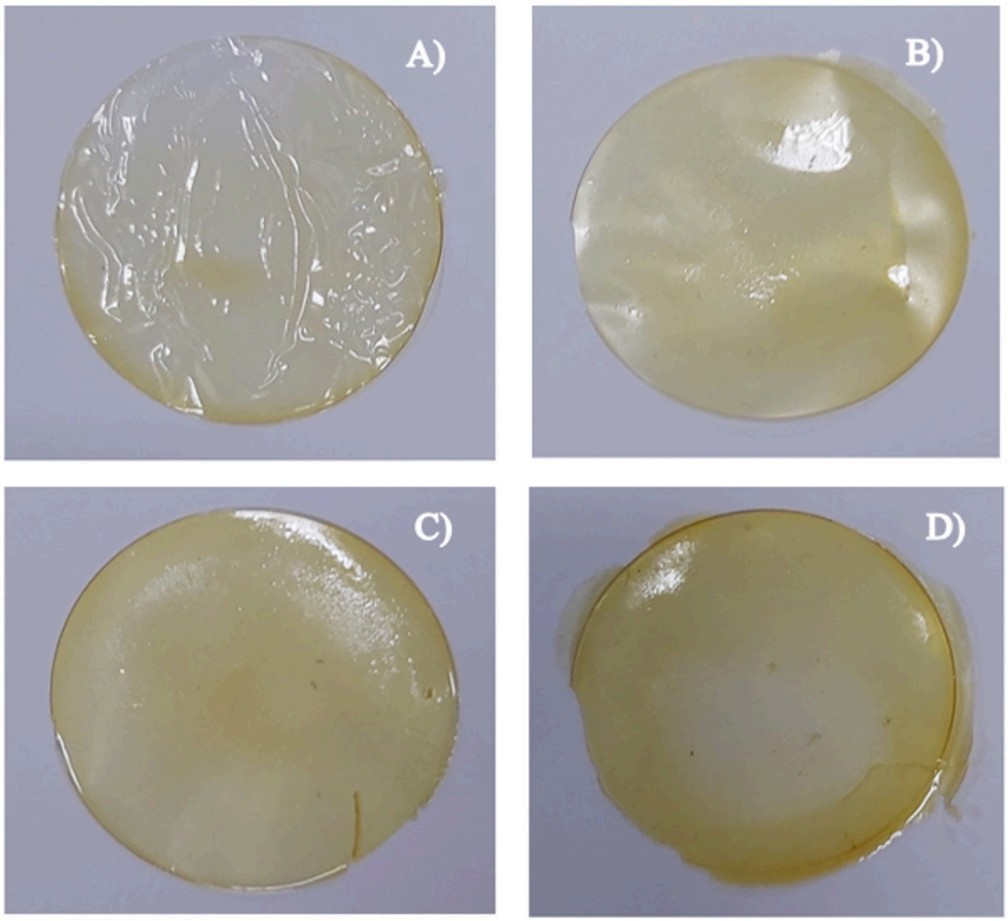

Figure 1. Photographs of CH control (A), CHPS-2.5% (B) , CHPS-5.0% (C), CHPS-7.5% (D).

Physical properties of films

Thickness of the Films

The thickness of films was measured with a micrometer with an ac-curacy of 1 µm and thickness. Measurements were taken at five distinct points for each film using a digital micrometer (Insize, China). The average value was then calculated and used for characterization.

Moisture Content (MC)

Moisture content of film samples (2.4x2.4 cm^2) was determined by measuring their mass loss after oven drying at 105 °C for 24 h. The moisture content was calculated as follows (Eq. 1):

Moisture content: $(W_1\text{-}W_2)/W_1$ x 100 (1)

where W_1 is the initial weight of the film and W_2 is its final weight post-drying.

Solubility of Films in Water

Pieces of film 2.4 x 2.4 cm^2, were cut from each film and weighed. The solubility in water of the different blend films was measured from immersion assays under constant agitation in 50 mL of distilled water for 24 h at 25 °C. The remaining pieces of film after immersion were dried at 105 °C in oven (a Pol-Eko-Aparture son 53,115) to constant weight (final dry weight). The initial dry weight was determined by thermal processing at 105 °C to constant weight. Solubility in water (%) was calculated as follows (Eq. 2):

Water solubility (%) = $((W_0\text{-}W_f)/W_0) \times 100$ (2)

Where W_0 is the initial dry weight of the film, and W_f represents the weight of the residual, non-dissolved film after immersion.

Optical properties

Optical transmittance

The transparency of prepared films was measured at selected wavelengths (250–500 nm) using a Lambda 40 UV/Vis spectrophotometer (Shimadzu-1900). Filmsamples was cut into a rectangle piece and attached to a quartz cell and placed in a spectrophotometer test cell directly, and air was used as reference. Measurements were performed at least in three replicates.Tthe results were reported as the samples' percent transmittance (%T), calculated as follows (Eq. 3):

$$\%T=10^{-A} \times 100. \quad (3)$$

As a reference, an empty cuvette, or air, was utilized. Optical transmittance examination of the films was performed with a UV spectrophotometer (Shimadzu -1900) in the 250–500 nm range (Bajić et al., 2019).

Scanning electron microscopy (SEM)

Surface as well as cross-sectional morphologies of the CH-control and CHPSs films were investigated using FEI Quanta 250 FEG model SEM. The film samples were cut into small pieces, then mounted on aluminum stubs with adhesive tape.

X-ray diffraction (XRD)

The crystalline characteristics of CHPSs were assessed using XRD with Ni-filtered Cu Kα radiation, scanning in the 2θ range from 0° to 60°. The X-ray analyses were carried out on CH powder, CH control and CHPS-7.5% films.

Antimicrobial Properties

The antimicrobial efficacy of CHPS films containing 2.5% to 7.5% concentrations was assessed against foodborne pathogens, including the Gram-negative bacteria *Escherichia coli* ATCC 25922 (*E. coli*) and the Gram-positive bacteria *Staphylococcus aureus* ATCC 25923 (*S. aureus*), along with the fungus *Candida albicans*, using the disc diffusion method. Cultivation of these microorganisms was performed under optimal conditions in suitable media at 37°C. Mueller-Hinton agar, provided by Merck, served as the testing medium for the antimicrobial analysis. Microbial solutions were standardized to match the McFarland 0.5 turbidity standard, equating to 1.5x108 CFU/mL. Sterile discs were saturated with the CHPS films at varying concentrations (2.5%, 5.0%, and 7.5%) and pure chitosan for comparison. These discs were then methodically placed on Mueller-Hinton agar plates inoculated with each microbe. The agar plates were incubated at 37°C for 24 hours to allow for interaction between the microbes and the films' active components. Following the incubation, the zones of inhibition around each disc were observed and measured in millimeters using a digital caliper to quantify the antimicrobial effect.

RESULTS AND DISCUSSION

Gas Chromatography-Mass Spectrometer (GC-MS) analysis of Pomegranate Seed Essential Oil profiles

The volatile profiles of pomegranate seed essential oil were ascertained using Agilent 6890N GC (GC-MS). Table 1 presents the results of compound determinations from GC-MS analyses.

Table 1. Chemical Constituents of Pomegranate (*Punica granatum* L.) Seed Essential Oil

Chemical constituents	Result (%)	Chemical constituents	Result (%)
Butyric acid (C4:0)	0,11	Linoleic acid (C18:2)	18,72
Caproic acid (C6:0)	0,12	Linolenic acid (C18:3)	0,36
Caprylic acid (C8:0)	-	Arachidic acid (C20:0)	0,23
Capric acid (C10:0)	-	Eicosenoic acid (C20:1)	0,25
Lauric acid (C12:9)	-	Eicosadienoic acid (C20:2)	-
Myristic acid (C14:0)	0,03	Behenic acid (C22:0)	0,29
Palmitic acid (C16:0)	8,40	Erucic acid (C22:1)	-
Palmitoleic acid (C16:1)	0,06	docosadienoic acid (C22:2)	-
Margaric acid (C17:0)	-	Tricosanoic acid (C23:0)	15,93
Heptadecenoic acid (C17:1)	0,09	Lignoceric acid (C24:0)	2,55
Stearic acid (C18:0)	2,98	Nervonic acid (C24:1)	-
Oleic acid (C18:1)	49,88		

Analysis of Fourier-Transform Infrared Spectroscopy

FT-IR analysis was utilized to elucidate the molecular interactions between chitosan and pomegranate seed oil (PSO) within the films. The FTIR spectra depicted in Figure 2 highlight the differences between chitosan films with and without PSO incorporation. For the chitosan control film, notable features include a wide band spanning from 4000 to 3000 cm^{-1}indicative of O–H bond stretching; peaks at 2929 cm^{-1} and 2858 cm^{-1} representing symmetric and asymmetric CH2 vibrations, respectively; and a peak at 1546 cm^{-1} associated with N–H bending of amide II. The presence of residual N-acetyl groups in chitosan is confirmed by bands around 1643 cm^{-1}, attributable to C=O stretching of amide I, while the band at 1148 cm^{1} suggests asymmetric stretching of the C–O–C bridge.

In films containing PSO, the FTIR spectrum reveals distinctive bands: a peak at 3499 cm^{-1} is linked to NH stretching, and the peak at 3011 cm^{-1} to C-H vibrations of cis-alkene. The band at 1744 cm^{-1} corresponds to C-O stretching vibrations of carbonyl groups in triacylglycerols. The spectrum also shows a band at 2922 cm^{-1} for C-H stretching of methylene and -CH2 groups in lipids, and a sharp peak at 2851 cm^{-1}, typically associated with terminal -CH3 groups. Notably, the spectrum features a strong absorption band at 1635 cm^{-1}, characteristic of amide I β-sheet structures, along with bands at 984 $cm^{-1}$and 760 cm^{-1}indicative of amide III β-sheets and CH bending, respectively (Adiba et al., 2023).

With the addition of PSO to chitosan films, shifts in spectral peaks were observed, including from 3313 cm^{-1} to 3373 cm^{-1} for NH and OH stretching vibrations, and from 1546 cm^{-1} to 1554 cm^{-1} indicating changes in NH bending. A shift from 1379 cm^{-1} to 1364 cm^{-1} in CN stretching vibration was noted, alongside a significant reduction in peak intensity. The peak at 2858 cm^{-1} divided into two at 2851 cm^{-1} and 2918 cm^{-1}, reflecting symmetric and asymmetric CH vibrations (T. Liu, Liu, Gong, Chi, & Ma, 2021). Additionally, the intensity of the peak at 1010 cm^{-1}, corresponding to C–O–C stretching, decreased significantly upon PSO addition, likely due to hydrogen bonding interactions between polyphenols' hydroxyl groups and chitosan's hydroxyl and amino groups (T. Liu et al., 2021; Nguyen & Bui, 2020).

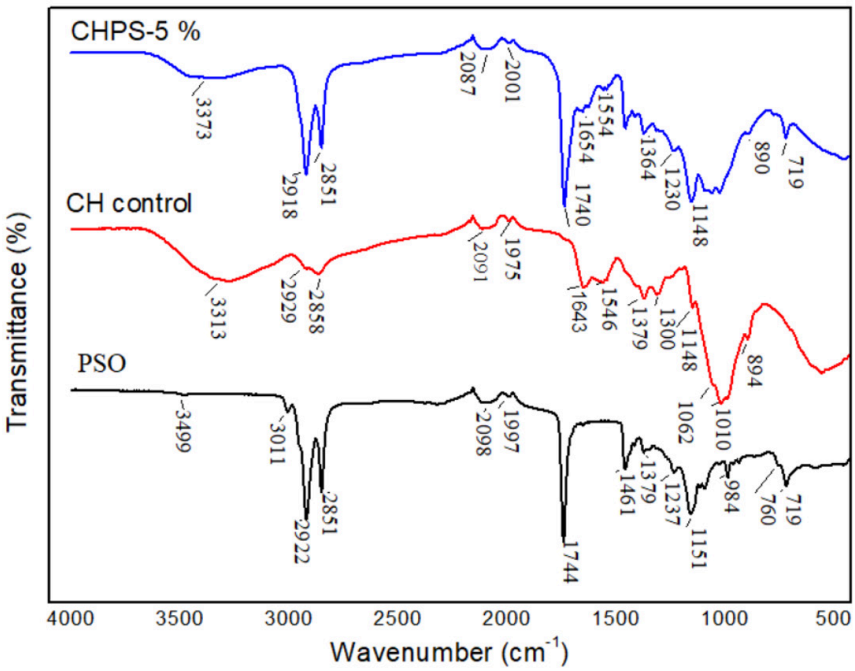

Figure 2. FT-IR Spectra of CH, CH control and CHPS

Scanning Electron Microscopy Analysis

Scanning Electron Microscopy (SEM) analysis provided insights into the surface morphology of CHPS films with PSO concentrations ranging from 2.5% to 7.5% (v/v), as illustrated in Figure 3. The SEM images revealed that the CHPS films exhibited a rough and non-uniform surface texture. Specifically, the chitosan films integrated with PSO displayed significant variations in microstructure, particularly at higher PSO concentrations, leading to a heterogeneous appearance due to the incorporation of oil droplets within the polysaccharide matrix. Notably, these oil droplets deviated from the typical spherical shape found in oil/water emulsions, possibly due to the chitosan matrix's tensile forces during the drying process.

Comparatively, chitosan films without PSO addition were characterized by a smooth, flat surface without any visible cracks, indicating uniformity. The introduction of PSO, however, altered the film's microstructure markedly, embedding oil droplets unevenly throughout the chitosan network. This disruption increased with the PSO concentration, making the droplets more prominent and less spherical, suggesting an interaction with the chitosan framework.

Reference to a study by Hafsa, J., et al.(2015) (Hafsa et al., 2016) highlighted that chitosan films impregnated with Eucalyptus globulus essential oil nanoemulsion at varying concentrations (0-4% v/v) maintained a smooth and continuous structure at lower oil concentrations. Yet, as the oil nanoemulsion concentration escalated, the films exhibited an increase in oil droplet presence, closely integrated within the polymer matrix. The phenomenon of oil droplet enlargement with rising PSO content was also observed, with the largest droplets becoming visible at higher magnifications in films with the highest PSO levels. This trend was attributed to the increased likelihood of droplet collisions and subsequent coalescence in higher lipid-content emulsions (McClements, 2004).

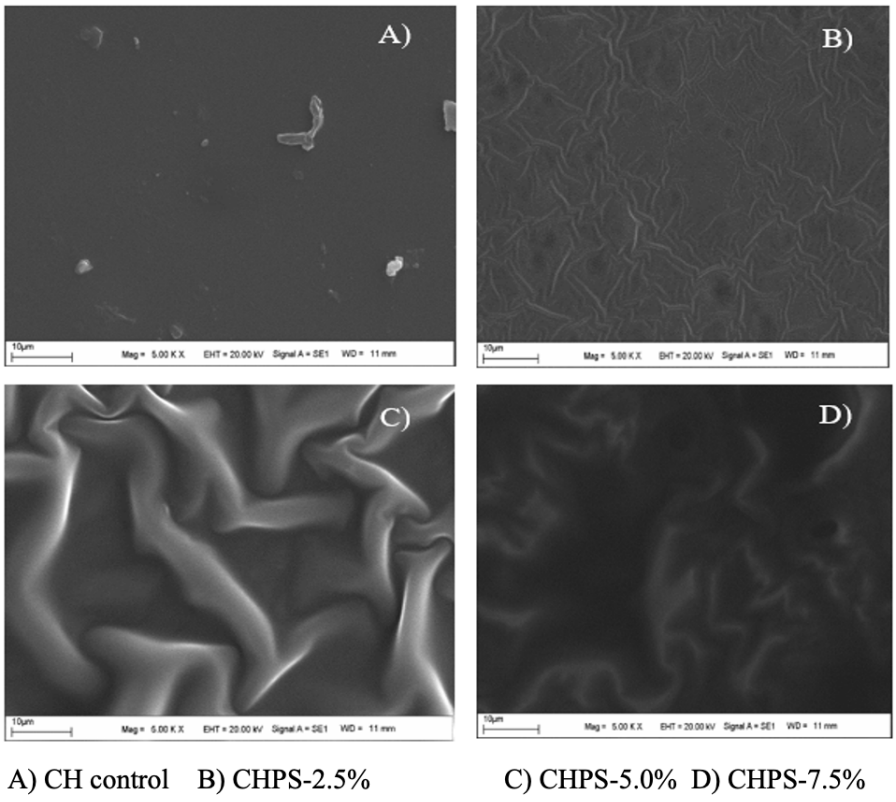

Figure 3. SEM Micrographs of CH control and CHPS-2.5-7.5 % films.

X-Ray diffraction analysis (XRD) patterns of CHPSs

XRD analysis was conducted to investigate the crystallinity within the film components. The XRD patterns for CH powder, the CH control film (CH/glycerol), and CHPS-7.5% films are shown in Figure 4. CH powder is observed to be semi-crystalline, displaying two primary peaks at 2θ=14° and 20° (Evcil & Karakaplan, 2022; J. Liu et al., 2016), which are indicative of inter- and intra-molecular hydrogen bonding facilitated by the presence of free amino groups.

The XRD pattern for the mixture without PSO reveals its crystallinity with a distinctive peak at 20.88°, suggesting that the CH/Gly films have a quasi-amorphous form with limited crystallization, likely reflecting the semi-crystalline nature of chitosan. This result implies that the strong interactions, such as hydrogen bonding and ionic interaction between glycerol and chitosan, might be disrupted in the blend films' matrix, leading to a more organized packing of chitosan molecules and formation of a regular crystalline structure (Ghaffari, Navaee, Oskoui, Bayati, & Rafiee-Tehrani, 2007).

When PSO is added to the CH/Gly blend films, there is a slight shift in the peak position to 20.22° in the XRD pattern, but the peak intensity decreases compared to the CH/Gly films. This suggests that PSO disperses at the molecular level within the biopolymer matrix, likely forming interactions with glycerol and chitosan's functional groups. Therefore, it can be concluded that PSO incorporation does not significantly alter the amorphous structure of the blend films.

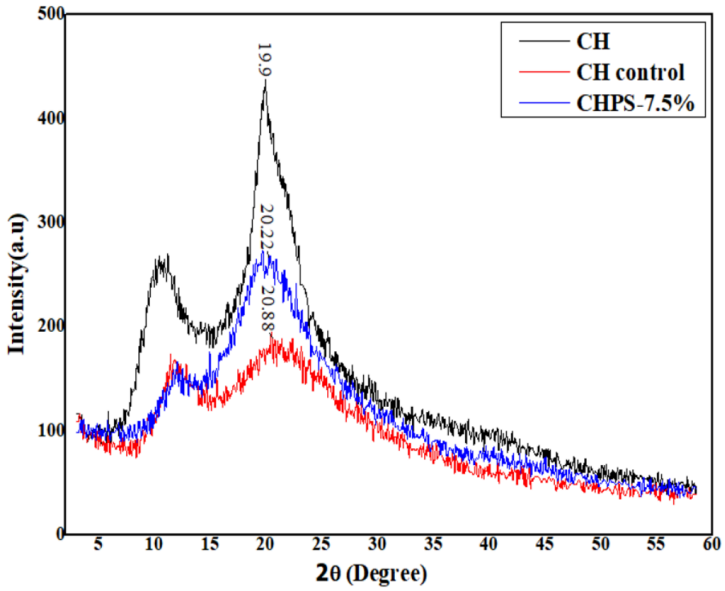

Figure 4. XRD of CH, CH control and CHPS-7.5%

Thickness

Film thickness is a crucial parameter affecting the film's physical and mechanical characteristics, such as its barrier properties against water, gases, and moisture, along with its tensile strength and resistance to impact (Hosseini, Ghaderi, & Gómez-Guillén, 2021). As presented in Table 2, the thickness measurements for films comprising chitosan-control (CH control), CHPS-2.5%, CHPS-5.0%, and CHPS-7.5% were recorded at 0.051 mm, 0.30mm, 0.60 mm, and 0.66 mm, respectively. These findings align with those from previous research (Haghighi et al., 2019;Li et al., 2019), which suggest that the observed increase in film thickness can be attributed to the addition of solid content within the film matrix. An observed increase in the film network's density is attributed to the integration of various chemical compounds found in essential oils, which in turn affects the arrangement of chitosan chains (Khezrian & Shahbazi, 2018).

Water Solubility

Table 2 displays the solubility in water for both the CH-control film and the chitosan films enhanced with PSO. A decline in the solubility of the films in water was observed, decreasing from 11.08% to 8.11%, as the inclusion of PSO was raised from 2.5% in the CHPS-2.5% film to 7.5% in the CHPS-7.5% film. The decrease in moisture content due to the non-polar nature of the essential oil used in our research parallels previous findings with lemon essential oil (Bof, Jiménez, Locaso, García, & Chiralt, 2016). This effect is likely attributable to the covalent bonding between the chitosan polymer and the functional groups within the oil as PSO is amalgamated into the chitosan films. Consequently, this diminishes the availability of chitosan's hydroxyl and free amine groups to interact with water, thereby reducing water interaction within the polysaccharide. The diminished water affinity with chitosan films further impacts their solubility in water significantly. The resilience of the chitosan film network was highlighted by their near intact state after 24 hours of immersion in water. A decrease in dry mass of the films indicates a degree of solubility. The solubility rate of the chitosan control film in water corroborates with prior studies (27.35%) (K., G., Banat, Show, & Cocoletzi, 2019), indicating a reduction from approximately 28.1% for the neat chitosan or CH-control film to about 8.11% for the CHPS-7.5% films, thereby marking a significant decrease as the proportion of PSO in chitosan escalates.

Table 2. Thickness, moisture content and water solubility of films

Samples	Thickness (mm)	Water Solubility (%)	Moisture Content (%)
CH control	0.051	28.1	21.25
CHPS-2.5%	0.30	11.08	21.78
CHPS-5.0%	0.60	10.03	20.72
CHPS-7.5%	0.66	8.11	16.76

Moisture Content (MC%)

The introduction of phenolic compounds resulted in a significant reduction ($p < 0.05$) in the moisture content of the films, as detailed in Table 2, when compared to the control sample. This reduction can be linked to enhanced molecular interactions and an increase in hydrophobic properties. Additionally, the moisture content in these edible films was considerably influenced by the specific type or characteristics of the phenolic compounds utilized, with the films infused with PSO exhibiting the lowest moisture content. This finding aligns with the results of previous studies, such as those conducted by Hafsa, J., et al., indicating similar outcomes (Hafsa et al., 2016).

Optical Transmittance

Characteristics related to optical transmittance, including color and clarity, are vital for determining the acceptability and visual appeal of the film to end-users. Transparency, an essential measure for evaluating the clarity of the film, indicates that a higher transparency value corresponds to reduced clarity of the film. The transparency levels of the films, both CH-control and those containing CHPS, are depicted in Figure 5. Among all films tested, the CH-control film exhibited the highest light transmittance, at 66.3%. The inclusion of plasticizers and PSO resulted in a notable decrease ($p < 0.05$) in optical transmittance from the CHPS-2.5% to the CHPS-7.5% films, with the CHPS-5.0% and CHPS-7.5% films demonstrating lower light transmission. The presence of Gly and PSO in the films leads to the creation of structures that contain oil or water droplets, which disrupt light transmittance and increase light scattering at the droplet boundaries. These observations indicate that PSO and Gly can effectively block the transmission of ultraviolet rays through these edible films. Furthermore, the films containing PSO showed reduced transparency due to the colorful constituents of PSO. From a commercial perspective, these composite films enriched with PSOs can obstruct the passage of UV rays, thereby providing enhanced protection for light-sensitive food products against oxidation and other external factors that could damage the food (Mohammadi, Mirabzadeh, Shahvalizadeh, & Hamishehkar, 2020).

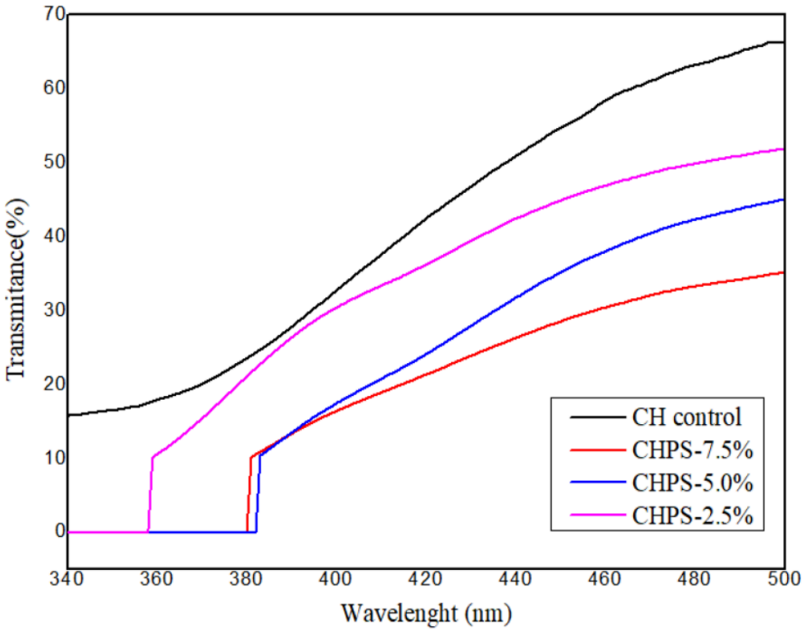

Figure 5. The light transmittance of CH control and CHPSs in the UV–vis light region

Antimicrobial Properties

The antimicrobial effects of CHPS-2.5-7.5% films prepared with three different concentrations were examined on food pathogens and the fungus *C. albicans* using the disc diffusion method. Measurements, revealed that the inhibition zones formed due to the antimicrobial effect of CHPS-2.5-7.5% films were significantly larger compared to chitosan. When it comes to inhibiting the growth of Gram-negative *E. coli*, it seems that various concentrations of CHPS-2.5-7.5 are more effective than the control. When compared to the control, the effect of the various concentrations of CHPS-2.5-7.5 on the other strains that were utilised in the application was found to be quite insignificant(Table 1). Among the microorganisms that were utilised in the application, gram-negative *E. coli* was one of the microorganisms that was

found to be considerably successful in reducing the growth of CHPS-2.5-7.5 percent films. The presence of inhibitory zones gives the impression that it is successful in suppressing the growth of other strains, despite the fact that its effect on other strains is insignificant in comparison to the control.Herbal extracts are compounds rich in phenols and flavonoids. These compounds exhibit bioactive properties, including antimicrobial and antioxidant activities (Rauf et al., 2019)"id":"ITEM-1","issued":{"date-parts":[["2019","8"]]},"page":"108999","title":"Proanthocyanidins: A comprehensive review","type":"article-journal","volume":"116"},"uris":["http://www.mendeley.com/documents/?uuid=8374df55-21c9-422c-9892-dde1e1ac2c8e"]}],"mendeley":{"formattedCitation":"(Rauf et al., 2019. In the (GC-MS) profile (Table 1), shows high concentrations of phenolic and flavonoid compounds, including protocatechuic acid, chlorogenic acid, vanillin, and isoquercitrin. The presence of these compounds in the structure of the films shows that they are effective in antimicrobial activity (Eroglu & Girgin, 2021). Phenolic and flavonoid compounds found in plant extracts increase the ROS level in microorganisms. These bioactive compounds act by negatively interfering with the synthesis of important molecules such as biofilm, glucosamine, and proteins. In addition, these compounds in the extract show antimicrobial effects by causing structural and functional disruption of cell membranes, inhibition of important enzymes such as DNA gyrase and protein kinase, and inhibition of dehydratase and type III secretion inactivation mechanisms (Rempe, Burris, Lenaghan, & Stewart, 2017;Silva et al., 2021; Wali et al., 2020;Yang et al., 2022).

Table 3. Measurement results of inhibition zones formed due to the antimicrobial effects of CHPS-2.5-7.5% and control (chitosan) films on food pathogens and fungus C. *albicans* for 24 hours using the Disc Diffusion method

			Films in different concentrations Measured Inhibition Zones (mm)			
			Control (Chitosan)	0.25 μg mL^{-1}	0.50 μg mL^{-1}	1.00 μg mL^{-1}
Microorganism	Bacteria	***E. coli***	7.00	8.00	8.00	8.00
		S. aureus	9.00	8.00	8.00	8.00
	Fungus	***C. albicans***	9.00	8.00	8.00	8.00

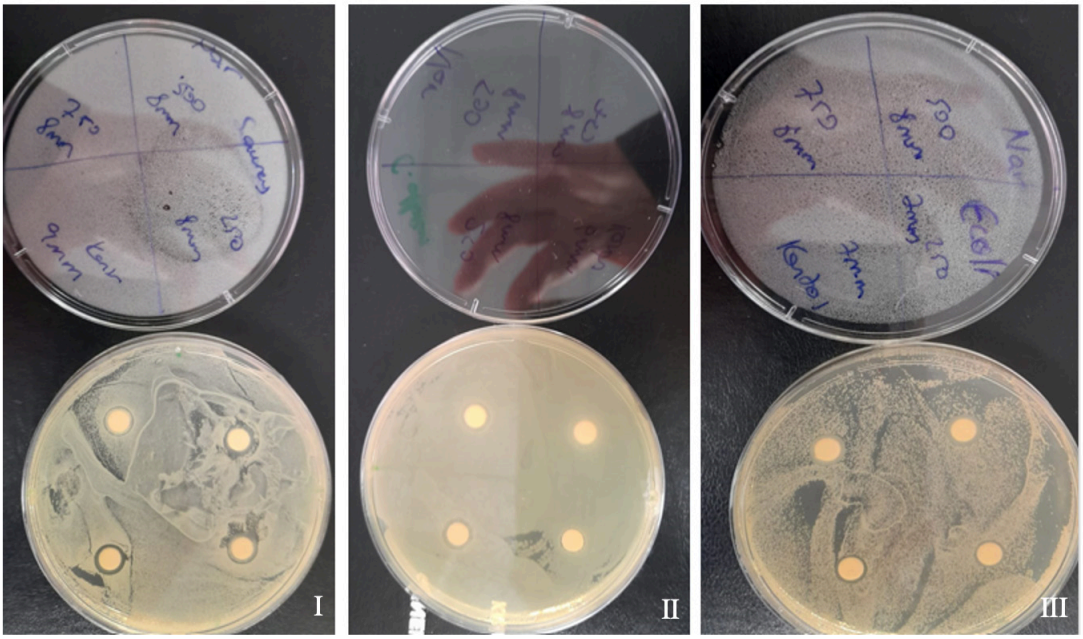

Figure 6. Inhibition zones formed as a result of the antimicrobial effects of CHPS-2.5-7.5% films on food pathogens and fungi through the Disc Diffusion method; Inhibition zones formed as a result of the antimicrobial effects of 0.25, 0.50, and 1.00 concentrations of CHPS-2.5-7.5% films on (I) *S. aureus*, (II) *C. albicans*, and (III) *E. coli*

CONCLUSION

In summary, this research has successfully integrated pomegranate seed essential oil into chitosan films and assessed their characteristics. X-ray diffraction analysis demonstrated the semi-crystalline nature of the films, with the inclusion of PSO enhancing their solubility. An increase in the concentration of PSO led to a reduction in the moisture content of the films. It was observed that with a higher binding ratio of PSO to chitosan, the thickness of the films increased, while their water solubility and moisture content decreased. The antimicrobial capabilities of the films were also investigated, revealing that chitosan films infused with PSO exhibited antimicrobial properties. These results indicate that chitosan films containing PSO could serve as a viable alternative to synthetic polymers in food coating and packaging sectors. Future research should focus on exploring the potential uses of these films in a variety of applications.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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In-vitro efficacy of different essential oils against *Sclerotium rolfsii* (Sacc.)

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Abstract
This experimental study evaluated the effectiveness of different essential oils against the in vitro growth of *Sclerotium rolfsii*. The experiment employed a completely randomized design (CRD) with three concentrations (500, 1000, and 1500 ppm) of each essential oil, including thyme oil (*Thymus vulgaris* L.), cinnamon oil (*Cinnamomum zeylanicum* Blume), juniper oil (*Juniperus horizontalis* L.), neem oil (*Azadirachta indica* A. Juss.), lemon grass oil (*Cymbopogon citratus* (DC.) Stapf), peppermint oil (*Mentha piperita* L.), and an unamended control medium. This setup aimed to evaluate their efficacy against the mycelial growth of *S.* rolfsii. The data were analyzed using R software in R-Studio, and means were compared using Duncan's Multiple Range Test (DMRT) at a 5% level of significance. Mycelium growth data were recorded at 24 hours, 48 hours, and 72 hours of incubation. All tested essential oils significantly inhibited the mycelial growth of the pathogen compared to the control ($p<0.05$). After 72 hours, thyme oil at all concentrations and lemongrass oil at 1500 ppm both achieved 100% growth inhibition. In contrast, neem oil at 500 and 1000 ppm showed the lowest inhibitory effects, with rates of 27.56% and 34.62%, respectively. Lemongrass oil at 500 ppm (75.39%) showed statistical similarity to cinnamon oil at 1000 ppm (79.12%). Peppermint oil at 1500 ppm resulted in 82.73% inhibition, and cinnamon oil at 1000 ppm (75.73%) showed comparable results to peppermint oil at 1000 ppm. Thus, the study highlights the superior performance of thyme oil among the tested essential oils. These effective essential oils can potentially be used at lower concentrations to minimize potential hazards. However, further research and field trials are essential to validate these findings for practical applications.
Keywords: Incubation, Inoculation, Mycelium, Poisoned Food Technique, Potato Dextrose Agar

INTRODUCTION

Sclerotium rolfsii (Sacc.) is a soil-borne necrotrophic pathogen commonly found in tropical, subtropical, and warm temperate regions worldwide (Mullen, 2001; Roberts et al., 2014). This genus is associated with economically significant diseases such as root rot, stem rot, wilt, foot rot, and collar rot, affecting over 500 plant species, including almost all agricultural and horticultural crops (Fernando et al., 2004; Clarkson et al., 2007; Del Río et al., 2007; Sten et al., 2017). The economic losses attributed to diseases caused by this pathogen are substantial due to its wide host range, prolific development, and the formation of persistent sclerotia (Kokub et al., 2007). In the United States alone, *Sclerotium rolfsii* has infected more than 270 host genera (Farr and Rossman, 2006). It has been reported that

growing legumes, cucurbits, and other vegetable crops in rotation with beans can increase the incidence and severity of *S. rolfsii* (IITA, 1996). According to Mayee and Datar (1988), typical yield losses due to this pathogen and associated diseases have been reported to exceed 25%, with the potential to reach up to 80% under severe conditions. Globally, it is estimated that losses attributed to *S. rolfsii* range from 10 to 20 million dollars, resulting in yield depletions ranging from 1% to 60% in fields (Liamngee et al., 2015).

S. rolfsii is primarily characterized as a polyphagous, non-selective, moisture-dependent, and widely distributed facultative parasitic basidiomycete fungus. It can cause damage to plant tissues before colonization through the production of oxalic acid, poly-galacturonase, and cell wall-degrading enzymes (cellulase) as integral components of its pathogenicity (Punja, 1985; Chen et al., 2020). This pathogen, *S. rolfsii*, persists in the soil as resistant structures called sclerotia, which can remain viable for extended periods even under adverse climatic conditions in the absence of a susceptible host. These sclerotia act as the primary source of disease inoculation (Aycock, 1966; Kokalis-Burelle et al., 1997; Wu et al., 2008; Kokub et al., 2007). The pathogen possesses the ability to infect crops at various growth stages, presenting a formidable challenge in terms of management.

Common management measures for controlling *S. rolfsii* include the removal and destruction of diseased plants, which serve as sources of inoculum; the treatment of plants and seeds with fungicides like metalaxyl; and the use of resistant plants in crop rotations (Paparu et al., 2020). Moreover, in addition to these practices, effective strategies for managing the pathogen include various soil management techniques. Soil solarization, as researched by Chellemi (2002) and Flores-Moctezuma et al. (2006), utilizes solar heat to control the pathogen. Researchers have explored the use of both inorganic and organic soil fertility amendments, as recommended by Bulluck and Ristaino (2002) and Bonanomi et al. (2007). Another successful approach involves the cultivation of host plants with resistance, as highlighted by Woodward et al. (2008). Furthermore, natural plant products, which do not exhibit phytotoxicity and have systemic action, have garnered significant attention from scientists worldwide (Fawcett and Spencer, 2003; Gilbert, 1977; Dubey et al., 2008). These products, derived from higher plants and microorganisms, are generally characterized by their broad-spectrum efficacy, cost-effectiveness, biodegradability, and ecological soundness, making them ideal for use as agrochemicals (Cutler and Cutler, 1999).

Plant extracts and essential oils have evinced antifungal properties against a wide range of plant pathogenic fungi (Davidson, 1989; Kurita et al., 1981; Rice, 1995). Essential oils are volatile and naturally fragrant compounds, primarily composed of functional groups such as terpenoids. They are produced as secondary metabolites by aromatic plants and offer multi-purpose functional usage potential and enhanced safety (Wilson et al., 1997; Bakkali et al., 2008). Numerous studies have highlighted the antifungal potential of essential oils against various fungal pathogens, including *S. rolfsii*. El-Wakil et al. (2011) evaluated seven essential oils against *S. rolfsii* growth and found that thyme and basil oils, when applied at a 2% concentration, completely suppressed the fungal infection. Thyme oil, in particular, had a detrimental effect on the mycelium and sclerotia structures of seed-borne *S. rolfsii*. Similarly, El-Mohamedy et al. (2013) observed significant antifungal properties in lemongrass, thyme, citral, and nerol essential oils against the tomato root rot pathogen *S. rolfsii* in vitro. These essential oils, at a concentration of 1.5%, completely inhibited fungal growth. Moreover, as the concentration increased, their inhibitory effects became more potent. Thyme oil and lemongrass oil, in particular, completely halted mycelial growth and spore germination at a concentration of 100 µl/L each. Another study by Ragab et al. (2012) outlined that thyme, lemongrass, peppermint, clove, and mint oils showed superior mycelial inhibition percentages compared to lemon, cinnamon, and mustard oils. The inhibitory effect on fungal mycelial growth was positively correlated with increasing essential oil concentrations. Osman Mohamed Ali et al. (2017) reported significant antifungal activity of Neem Nano Emulsion Oil 10 (NNE10) and Citronella Nano Emulsion Oil 10 (CNE10) against *S. rolfsii*, with ED50 values of 14.71 mg L^{-1} and 20.88 mg L^{-1}, respectively. Moreover, Abdel-Kader et al. (2011) observed that peppermint oil caused 48.8% inhibition at a 1% concentration (v/v), while thyme oil achieved 100% inhibition at the same concentration (v/v). In addition, Kumar et al. (2007) observed the effective inhibition of the radial mycelial growth of *S. rolfsii* by Mentha oil at a concentration of 0.10 mg mL^{-1}. Gairhe et al. (2021) observed the highest inhibition of mycelial growth at 1000 ppm using cinnamon oil (98.15%), followed by mustard oil (40.00%) and coconut oil (32.04%). Chandra Sekhar et al. (2020) evaluated palmarosa, karanja, menthol, thyme, and lemongrass oils at 2%, revealing inhibition rates of 72.76%, 38.21%, 34.15%, 42.7%, and 34.15%, respectively. Shervin et al. (2019) reported thyme oil (400 ppm) and aloe vera (400 ppm) as highly effective, with inhibition rates of 99.98% and 99.82%, followed by garlic oil (89.15%) and cumin oil (59.75%) at the same concentration. Salome and Zacharia (2021) found neem oil and neem seed cake (5%) to have the least inhibitory effects on *S. rolfsii* growth, with inhibition percentages of 37.71% and 47.80%, respectively. Nurmansyah et al. (2022) found Citronella grass to be the most effective (95.75%) among five essential oils tested at 1500 mg/L against S. rolfsii, followed by lemongrass oil (92.70%) and cinnamon oil (92.15%).

In recent years, Nepal has witnessed an increasing prevalence and severity of diseases caused by *S. rolfsii*, including southern blight in vegetables, seedling blight in rice, and collar rot in lentils. Prominent instances of *S. rolfsii* infections

include rice in Sunsari, Jhapa, Morang, and Udaypur districts in 2016/17 (RARS, 2017), onion in Dhading district in 2018/19 (PPD, 2018), lentil, rajma, chickpea, and mustard in Lumbini and Sudur Paschim provinces since 2015 (NGLRP, 2015), and chili in Chitwan since 2015. The excessive use of chemical fungicides by farmers to manage these diseases has raised concerns about food contamination and potential health risks, including toxicity, neurological effects, and reproductive health issues. In many regions, biological control methods utilizing antagonistic microbes have proven effective against various plant diseases (Sivan, 1987). Simultaneously, ongoing research seeks alternative and effective plant pathogen control compounds, aiming to reduce reliance on antimicrobial chemical fungicides either partially or completely and exploring their integration with biological compounds (Ons et al., 2020). Consequently, there is an urgent need to explore sustainable alternatives to control *S. rolfsii*. Essential oils emerge as promising candidates due to their inhibitory effects on pathogen proliferation. This study addresses this need by investigating the antifungal activity and efficacy of various essential oils against the in vitro growth of *S. rolfsii*. The findings of this study offer natural and effective alternatives like essential oils to address the rising challenges posed by *S. rolfsii*-induced diseases and the limitations of chemical fungicides in agricultural practices in Nepal and beyond.

MATERIALS AND METHODS

Experimental Site

The experimental study was conducted at the Central Laboratory of IAAS, Lamjung Campus, Sundarbazar, from August to September 2022, within a fully controlled laboratory environment.

Isolate Collection

The *S. rolfsii* isolate, collected from the Nepal Agricultural Research Council (NARC), Khumaltar, was cultured in the laboratory and incubated for one week at 27°C in a biological oxygen demand (BOD) incubator under complete darkness.

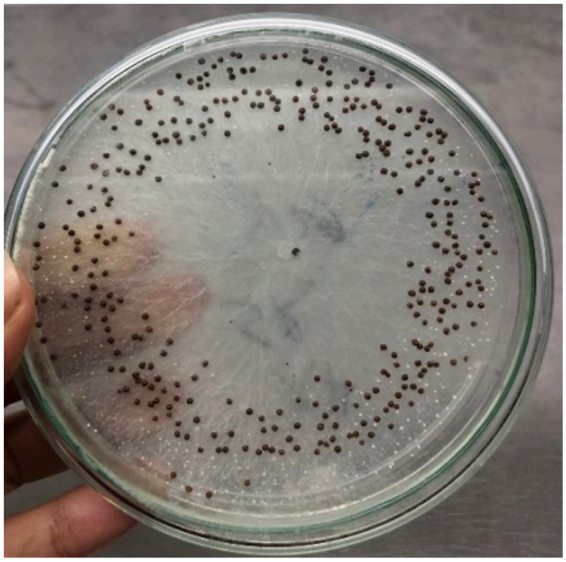

Figure 1. Mycelium and sclerotia of *S. rolfsii*

Design of the Experiments and Treatment Details

The experiment was conducted using a completely randomized design (CRD) with seven distinct treatments, as indicated in Table 1. Each treatment, except for the control, was subjected to three different concentrations (500 ppm, 1000 ppm, and 1500 ppm), and three replications were conducted for each concentration. The treatments included thyme oil (*Thymus vulgaris*) as T1, peppermint oil (*Mentha piperita*) as T2, juniper oil (*Juniperus horizontalis*) as T3, cinnamon oil (*Cinnamomum zeylanicum*) as T4, neem oil (*Azadirachta indica*) as T5, lemon grass oil (*Cymbopogon citratus*) as T6, and a control group as T7.

Table 1. Treatment details used in the experimental study

Treatments Symbol	Essential Oils	Composition	Manufacturer (Company)
T1	Thyme oil (*Thymus vulgaris* L.)	Thymol (44.7%), P-cymene (18.6%), g-terpinene (16.5%), terpinone (16.5%), B-caryophyllene (3.07%), carvacrol (3.04%)	Everest Aroma Pvt. Ltd.
T2	Peppermint oil (*Mentha piperita* L.)	Menthol (41%), Menthone (23.4%), menthyl acetate (4%)	Kanti Herbal Industries
T3	Juniper oil (*Juniperus horizontalis* L.)	α-pinene (50.5%), myrcene (7.5%), sabinene (5.2%), limonene (5.0%) and β-pinene (5.0%)	Everest Aroma Pvt. Ltd.
T4	Cinnamon oil (*Cinnamomum zeylanicum* Blume)	Linalool (52.5 %), a- pinene (8.7 %), p-cymene (5.4 %), B-pinene (4.5 %),	Kanti Herbal Industries
T5	Neem oil (*Azadirachta indica* A. Juss.)	Azadirachtin (65%)	Kanti Herbal Industries
T6	Lemon grass oil (*Cymbopogon citratus* (DC.) Stapf)	Geranial (30.6%), neral (29.7%) and myrcene (16.1%)	Sara Foods Nepal
T7	Control		

These essential oils were chosen due to their diverse chemical compositions and potential anti-microbial properties. Thyme oil (T1) is rich in hydrocarbons and phenolic compounds such as borneol, carvacrol, and thymol, which can disrupt the pathogen's cell membrane integrity (Porte and Godoy, 2008; Anìovar et al., 2014). Peppermint oil (T2) is characterized by its high menthol content, menthofuran, menthyl acetate, menthone, and 1,8-cineole, which contribute to its antimicrobial properties (Behnam et al., 2006; Saharkhiz et al., 2012; Marwa et al., 2017). Juniper oil (T3) is composed of monoterpene hydrocarbons like α-pinene, δ-3-carene, limonene, and myrcene, which enhance antioxidant-related enzyme activities while reducing pathogenicity-related enzymes (Höferl et al., 2014; Zheljazkov et al., 2021). Cinnamon oil (T4) contains E-cinnamaldehyde, linalool, β-caryophyllene, eucalyptol, and eugenol, which can inhibit the growth of pathogenic bacteria by disrupting cell envelopes (Alizadeh Behbahani et al., 2020). Neem oil (T5) comprises active ingredients like azadirachtin, nimbin, salannin, meliantriol, nimbolinin, and sodium nimbinate, offering biopesticidal properties (Elteraifi and Hassanali, 2011; Alzohairy, 2016; Chaudhary et al., 2017). Lemongrass oil (T6) primarily contains citral (>45%), known for its antibacterial and antifungal activities (Moore-Neibel et al., 2012).

This experimental design allowed for a comprehensive exploration of the effects of these oils across different concentrations, offering valuable insights into the potential impact of varying dosage levels on the inhibition of pathogen growth.

General Laboratory Procedure

Sterilization

The inoculation needle, forceps, and cork borer were sterilized by heating them until they were red-hot, and this process was repeated 2-3 times. The Petri plates were washed with liquid detergent under running tap water, rinsed with distilled water, air-dried, wrapped in aluminum foil, and then placed in a hot air oven at 105°C for up to 24 hours.

Disinfection of the Inoculation Chamber

Laminar airflow was used to conduct all experiments, including isolation, sub-culturing, and other studies, under aseptic conditions. The laminar airflow chamber was sterilized for fifteen minutes using UV light, followed by wiping the inner walls and base with 70% ethanol.

Preparation of Culture Media

Potato dextrose agar (PDA) containing 2% agar, readily available in the market, was used as the growth medium for *S. rolfsii*. According to the label instructions, the required amount of PDA powder was mixed with distilled water to

prepare the medium, with a mixing ratio of 7.8 grams of powder per 1000 ml of distilled water. The prepared medium was then sterilized in an autoclave at 15 psi and 121°C for 20 minutes and allowed to cool. When the temperature of the medium reached approximately 40°C, it was poured into sterile petri plates inside a laminar flow cabinet and left to solidify. Subsequently, the medium was infused with essential oils and used to inoculate the pathogen.

Dilution of Essential Oils

All activities were conducted inside a laminar airflow chamber under sterile conditions. Six different essential oils were assessed against *S. rolfsii* on Potato Dextrose Agar (PDA) medium using the poisoned food technique at concentrations of 500, 1000, and 1500 ppm. To prepare the stock solution, 1 ml of each essential oil was mixed with 19 ml of acetone, resulting in concentrations of 50,000 ppm for each essential oil. The required concentrations of 500 ppm, 1000 ppm, and 1500 ppm for the stock solutions were meticulously prepared using the following formula:

$$C_1V_1=C_2V_2$$

where,

C_1 = concentration of essential oil (ppm)

V_1 = desired volume of essential oils (ml)

C_2 = desired concentration of essential oils (ppm)

V_2 = measured volume of PDA (ml)

Thus-prepared essential oil dilutions were thoroughly mixed by vortexing using a lab vortex mixer before their application to ensure uniform distribution. Streptomycin (0.25 g/l) was added to a sterilized medium to inhibit bacterial growth. For the Potato Dextrose Agar (PDA) medium, a specific quantity of the stock solution was added to achieve final concentrations of 500 ppm, 1000 ppm, and 1500 ppm.

Inoculation and Incubation

On sterile petri plates, 20 ml of culture medium (PDA) mixed with the poisoned medium was poured and allowed to solidify. Mycelium with a 6 mm diameter was cut from the mother culture using a cork borer and placed in the center of a Petri plate with the poisoned medium. All six treatments, including controls, were replicated three times following the same procedure. Finally, parafilm wax was used to seal the Petri plates, which were then incubated at 27°C. The Petri plates were stored inverted in the incubator.

Growth Inhibition Test

The petri plates were positioned within a laminar flow cabinet, and data were collected using a Vernier caliper. The initial data collection was taken 24 hours after inoculation, followed by subsequent measurements at 24-hour intervals for up to 72 hours post-inoculation. The percentage inhibition of mycelial growth was calculated using the formula described by Vincent (1947).

$$\text{PGI} = \frac{C-T}{C} X\ 100$$

where,

PGI = Percent Growth Inhibition

C is the average diameter of the colony in the control treatment.

T is the average diameter of the colony in essential oil treatment.

Statistical Analysis

All data were entered into Microsoft Excel and analyzed using R-Stat (version 4.2.1). The data were subjected to analysis of variance, and treatment means were separated using the least significant difference (LSD) and Duncan's multiple range test (DMRT) at a significance level of 5%.

RESULTS AND DISCUSSION

The in vitro evaluation of various essential oils (thyme, peppermint, cinnamon, lemongrass, neem, and juniper) in inhibiting the growth of *S. rolfsii* was assessed using the Poisoned Food Technique, and the results are presented in Table 2.

Table 2. Efficacy of different essential oils on the growth of S. *rolfsii* in vitro

S.N	Essential Oils	Concentration (ppm)	Percentage Growth Inhibition (%)		
			24 hours	48 hours	72 hours
1	Thyme Oil	500	100^{a}	100^{a}	100^{a}
		1000	100^{a}	100^{a}	100^{a}
		1500	100^{a}	100^{a}	100^{a}
2	Lemon Grass Oil	500	73.49^{c}	73.93^{c}	75.39^{c}
		1000	83.90^{b}	86.31^{b}	86.47^{b}
		1500	100^{a}	100^{a}	100^{a}
3	Cinnamon Oil	500	52.95^{d}	53.76^{d}	59.78^{d}
		1000	71.63^{c}	75.73^{c}	79.12^{c}
		1500	96.55^{a}	98.06^{a}	99.38^{a}
4	Peppermint Oil	500	44.40^{e}	50.23^{d}	51.76^{e}
		1000	75.80^{c}	76.15^{c}	78.36^{c}
		1500	82.69^{b}	82.73^{c}	85.29^{b}
5	Juniper Oil	500	33.58^{g}	34.03^{fg}	36.27^{gh}
		1000	36.62^{fg}	37.19^{ef}	37.84^{fgh}
		1500	38.34^{f}	39.86^{e}	41.79^{f}
6	Neem Oil	500	25.68^{h}	26.77^{h}	27.56^{i}
		1000	29.11^{h}	31.27^{gh}	34.62^{h}
		1500	38.28^{f}	38.41^{ef}	39.26^{fg}
	Grand Mean		65.73	66.91	68.49
	SE_m ()		1.47	1.67	1.43
	CV (%)		3.87	4.33	3.63
	LSD (5%)		4.21	4.79	4.12

LSD: Least Significant Difference, SE_m: Standard error of the mean deviation, CV: Coefficient of Variance, Treatment means separated by DMRT and columns represented with different letter (s) are significant based on DMRT P = 0.05.

The results indicated significant differences in the ability of essential oils to inhibit the growth of *S. rolfsii* under in vitro conditions. Enhanced efficacy was observed as the concentration of the essential oils increased. Thyme oil and lemongrass oil resulted in the most substantial inhibition of growth at various concentrations. Regardless of the concentration used, the range of growth inhibition ranged from 27.56% to 100% after 72 hours of inoculation. Different essential oils displayed varying levels of fungicidal properties against the tested fungus, and a significant difference ($P \leq 0.001$) was observed among different extracts in their inhibitory effects.

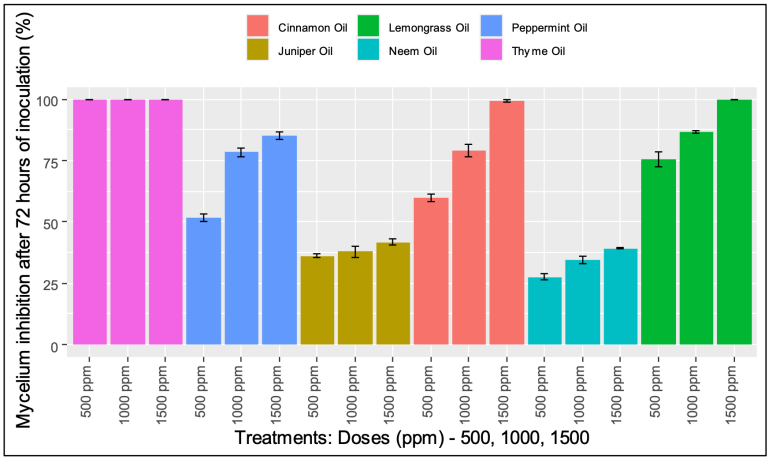

Figure 2. Mycelium inhibition (%) after 72 hours of inoculation across different treatments

As the concentration of the plant extracts increased, a proportional rise in the suppression of *S. rolfsii* mycelial growth was observed. After 24 hours of inoculation, thyme oil achieved 100% inhibition at all concentration levels, followed by 1500 ppm of lemongrass oil. The lowest inhibition was observed with 500 ppm of neem oil (25.68%), which was statistically similar to its 1000 ppm concentration (29.11%). After 36 hours of inoculation, thyme oil at all concentrations, 1500 ppm of lemongrass oil, and cinnamon oil showed the highest percentage of inhibition. Lemongrass oil at 500 ppm (73.93%) showed similar efficacy to cinnamon oil at 1000 ppm (71.63%). At a concentration of 1500 ppm, peppermint oil led to the highest inhibition rate of 82.73%. Cinnamon oil at 1000 ppm (75.73%) showed statistically similar results to peppermint oil at 1000 ppm. Neem oil at 500 ppm was the least effective among the tested essential oils, with the lowest growth inhibition rate at 25.68%.

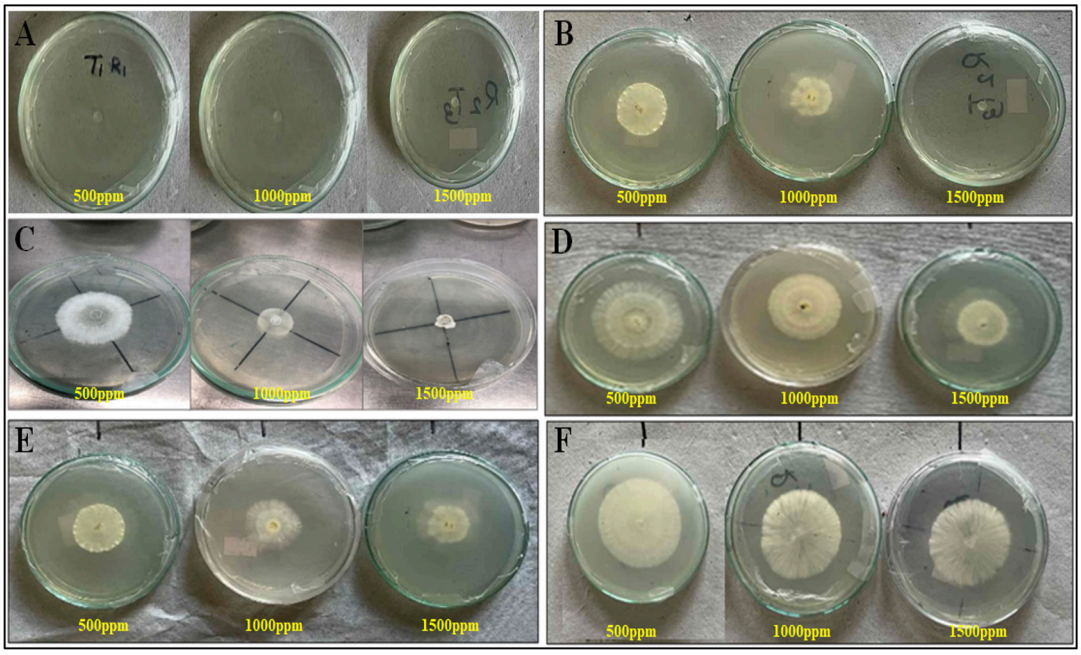

Figure 3. Mycelial growth of *S. rolfsii* in PDA amended media with various essential oils used at different concentrations; A) Thyme oil, B) Lemongrass oil, C) Cinnamon oil, D) Juniper oil, E) Peppermint oil, F) Neem oil

After 72 hours of inoculation, thyme oil at all concentrations achieved 100% inhibition, which was statistically similar to lemongrass oil and cinnamon oil at 1500 ppm (99.38%). Significant results were observed for lemongrass oil at 500 ppm and 1000 ppm (75.39% and 86.47%, respectively) and cinnamon oil at 500 ppm and 1000 ppm (59.78% and 79.12%, respectively). Lemongrass oil at 1000 ppm (86.47%) showed statistically similar efficacy to peppermint oil at 1500 ppm (85.29%). Juniper oil at 1500 ppm (41.79%) was statistically similar to neem oil at 1500 ppm (39.26%). Neem oil at 500 ppm and 1000 ppm resulted in the lowest inhibition rates (24.56% and 34.62%, respectively).

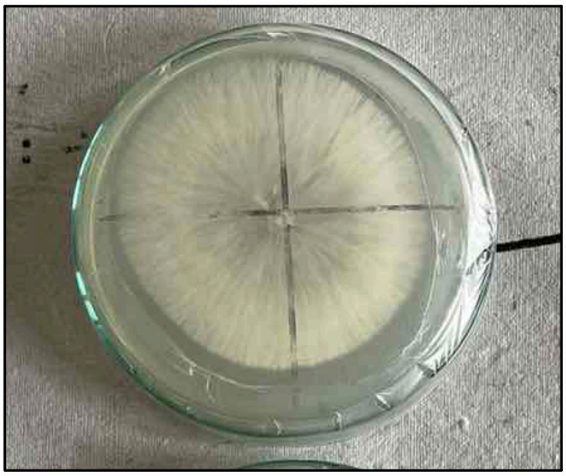

Figure 4. Mycelial growth of *S. rolfsii* in PDA-unamended media as a control

Natural compounds have a profound impact on the internal processes of the fungus. They lead to significant alterations in essential cellular structures, such as the formation of cytoplasmic granules, disruption of cell contents, disturbances in the plasma membrane, and the inhibition of fungal enzymes. These effects, in turn, hinder germination and germ tube elongation, potentially leading to a reduction or complete cessation of mycelial growth (Kim et al., 1995; Schwan-Estrada et al., 2000; Kotzekidou et al., 2008). The current findings are consistent with previous studies by El-Mohamedy et al. (2013) and Abdel-Kader et al. (2011), which also identified thyme oil as the most potent inhibitor of mycelial growth in various *S. rolfsii* strains. This inhibitory effect is attributed to thymol and carvacrol, which constitute a significant portion (20%–54%) of thyme oil's total content (Porte and Godoy, 2008). Thymol's hydrophobic properties allow it to interact with the pathogen's outer cytoplasmic membrane, leading to pathogen death. Thyme oil had exceptional effectiveness, achieving complete mycelial inhibition of *S. rolfsii* at concentrations ranging from 1% to 4% (Abdel-Kader et al., 2011). Additionally, the vapors of lemongrass and thyme oils, each at a concentration of 100 µl/L, resulted in the complete inhibition of linear growth and spore germination. Similar outcomes were observed for the vapors of thyme, oregano, and lemongrass, along with their major components, which manifested complete growth inhibition against Botrytis cinerea and Alternaria arborescens, as documented by Plotto et al. (2003).

Lemongrass demonstrated significant effectiveness in inhibiting the growth of *S. rolfsii* across all concentrations tested. This complete inhibition of pathogen growth can be attributed to the presence of antimicrobial constituents, including citral-a (33.1%), citral-b (30.0%), geranyl acetate (12.0%), and linalool (2.6%) in lemongrass oil, as reported by Nurmansyah et al. (2022). These constituents contribute to its dual antibacterial and antifungal properties, which include reducing spore germination, inhibiting germ tube development, and effectively suppressing fungal sporulation.

Our results at 1500 ppm of cinnamon oil have similar implications and closely align with the findings of Sukatta (2008), who reported 100% antifungal activity of cinnamon oil against various postharvest pathogens, including *Aspergillus niger*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, and *Phomopsis viticola*. Kowalska et al. (2020) also reported an 81.4% inhibition in the mycelium growth of *Botrytis cinerea* using cinnamon water filtrates at a 1% concentration. These consistent findings highlight the potential of cinnamon's active ingredient as an effective antifungal agent in various studies and applications.

Similarly, peppermint oil also showed pronounced mycelium growth inhibition in *Sclerotium rolfsii*, consistent with the findings reported by Souza et al. (2014) and Chandra Sekhar et al. (2020). Furthermore, in an in vitro antifungal assessment conducted by Falasca et al. (2016), they observed that the essential oil extracted from both green and ripe juniper berries effectively inhibited the growth of *S. rolfsii*, which is in accordance with our findings. This antifungal activity appeared to be closely linked to the concentration of sesquiterpenes. Additionally, it might be influenced by potential synergistic or antagonistic interactions among different terpenoid components, as suggested by several researchers (Jing et al., 2014). Several potential mechanisms have been proposed to explain this antifungal effect. One possibility is that terpenes such as α-pinene, p-cymene, and β-pinene could increase the levels of lipid peroxides, leading to cellular death (Filipowicz et al., 2003; Lucini et al., 2006). Alternatively, they may act on the mycelium's hyphae, causing the release of cytoplasmic components and ultimately resulting in the death of the mycelium (Sharma and Tripathi, 2008).

Besides, Salome and Zacharia (2021) observed that neem oil and neem seed cake at a 5% concentration showed the least inhibition of *S. rolfsii* growth, with rates of 37.71% and 47.80%, respectively, when compared to other essential oils, which is in accordance with our findings. This antifungal activity is primarily attributed to bioactive compounds like nimbin, nimbidin, and gedunin, which are found in neem oils and neem leaf extracts. These compounds disrupt fungal cell membranes, hinder fungal enzyme activity, and interfere with fungal metabolic processes (Suleiman, 2011; Raghavendra and Balsaraf, 2014).

CONCLUSION

In conclusion, it is advisable to use thyme oil at lower concentrations to effectively inhibit mycelium growth in vitro while reducing the risks associated with chemical fungicides. Thyme oil, along with other essential oils, presents a promising eco-friendly alternative to chemical fungicides, given their environmental hazards and potential impact on human health. Future research should focus on investigating the mechanisms of action of essential oils to enhance efficacy and reduce the likelihood of resistance development. Comprehensive field trials, covering diverse crop types and application methods, are essential for validating their real-world efficacy. Furthermore, further evaluation is necessary to understand how regional variations in essential oil compounds may affect their effectiveness against fungal pathogens such as *S. rolfsii* and others.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The author order aligns with their respective contributions, with K.R. Pandey contributing more to major aspects of the experiment and paper publishing, followed by A. Pant and N. Gajurel, both making significant contributions to experiments, data analysis, material provision, and paper writing.

All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Energy utilization and greenhouse gas (GHG) emissions in cherry cultivation

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Abstract

This study was performed with the purpose of shedding light on the energy balance (EB) and greenhouse gas (GHG) emissions of cherry cultivation. It was performed in Gönen district of Isparta province of Türkiye during the 2021 production period. Data related to energy inputs (EI) and outputs (EO) were gathered in cherry cultivation. They were then usaged to reveal the EB and GHG in the process. According to the results of the study, EI in cherry cultivation were 8 141.40 MJ/ha (57.04%) chemical fertilizers energy, 3 575.69 (25.05%) diesel fuel energy, 1 186.02 (8.31%) machinery energy, 469.80 (3.29%) electricity energy, 366.25 (2.57%) human labour energy, 290.30 (2.03%) irrigation water energy, 231.30 (1.62%) chemicals energy and 13.20 MJ/ha (0.09%) lime energy, respectively. Total input energy was computed to be 14 273.96 MJ/ha while output energy was found to be 29 593 MJ/ha. Energy utilization efficiency (EUE), specific energy (SE), energy productivity (EP) and net energy (NE) values were found as 2.07, 1.41 MJ/kg, 0.71 kg/MJ and 15 319.04 MJ/ha, respectively. The total energy inputs that were involved in cherry cultivation were categorized as: 32.94% (4 702.04 MJ/ha) direct (IE), 67.06% (9 571.92 MJ/ha) indirect (IDE), 4.60% (656.55 MJ/ha) renewable (RE) and 95.40% (13 617.41 MJ/ha) non-renewable (NRE). Total GHG emission was computed as 550.71 $kgCO_{2eq}$/ha for cherry cultivation with the greatest share for diesel fuel (31.82%). GHG ratio value was computed as 0.05 $kgCO_{2eq}$/kg in cherry cultivation.

Keywords: Cherry, Energy balance, Energy utilization efficiency, GHG emissions, Specific energy

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INTRODUCTION

Cherry tree, called 'Prunus avium' in Latin, is a member of the Rosaceae family (Çelik and Sarıaltın, 2019; İncekara and Selek, 2020). There are around 1 500 cherry varieties in the world and it is a sweet-flavored, juicy and stone fruit type. Cherry contains plenty of calcium, zinc, potassium, carotenoids, fiber, and vitamin C, iron, thiamine, riboflavin, niacin, magnesium, vitamins E and B6 (Anonymous, 2020a; İncekara and Selek, 2020). Cherry, a type of sweet-flavored, juicy and drupe fruit, is rich in calcium, zinc, potassium, fiber, vitamin C, iron, thiamine, riboflavin, niacin, magnesium, vitamins E and B6 (İncekara and Selek, 2020). Türkiye is home to many types of fruit. The climate zone in which Türkiye is located is suitable for the ecological demands of many fruit varieties. For this reason, Türkiye is one of the prominent countries in world fruit production and has a significant share in the world's production of hazelnut, fig, cherry, apricot, quince, pistachio and sour cherry. Compared to others, the importance of cherries in the Turkish economy is increasing due to reasons such as being consumed fresh, being usaged as raw material in the food industry, being subject to export, and contributing to

employment (İşleyen and Erden, 2019).

Cherry production in Türkiye's neighbours remains low compared to Türkiye. In addition, due to its ecological diversity, Türkiye can offer higher quality products to foreign markets at earlier times. For this reason, the Middle East and Arab countries are good markets. Compared to the leading European countries in cherry cultivation, our country has a significant potential in high quality, early varieties with high market value. If the advantage in terms of ecological factors is usaged well, it is possible to become one of the prominent countries in cherry exports and generate high revenues (Sütyemez and Eti, 1999; Çelik and Sarıaltın, 2019).

According to FAO data, Türkiye ranks first in the world cherry production area and production amount with 83 thousand hectares of cherry planting area and approximately 725 thousand tons of production in 2020. Chile follows Türkiye in cherry planting area with 40 thousand hectares. The USA is in third place with 34 thousand hectares, and Syria is in fourth place with 30 thousand hectares. In terms of cherry production, Türkiye is followed by the USA in second place with 295 thousand tons, Chile in third place with 255 thousand tons, and Uzbekistan in fourth place with 185 thousand tons (Anonymous, 2023a).

In order to perform energy balance, it is necessary to carry out economic and technic comprehensive reseaches. However, it is basically done to examine whether the production of the product or service to be offered to the market is possible in terms of EUE. Comparing the total energy value of inputs usaged in agricultural cultivation processes to the energy value of the acquired product is a more realistic approach for the assessment of the productivity (Öztürk, 2011; Bayhan, 2016; Karaağaç et al., 2018). Climate changes are occurring in the world and in our country. Among these, increasing air temperatures attract attention. It is evaluated that this rise in air temperature will cause serious climate change in the world. Climate change due to global warming causes sea level rise, shifting climate zones, severe weather events, floods and floods to occur more frequently and their effects to become stronger. In addition, it is estimated that it will lead to significant consequences by directly or indirectly affecting socio-economic sectors and ecological systems, as well as deterioration of human health along with wildlife species due to drought, erosion, desertification, epidemic diseases, agricultural pests, and disruption of natural balance (Anonymous, 2001, 2002; Korkmaz, 2007).

There has been progress in agriculture in areas such as mechanization, fertilization, spraying and irrigation. As a result of these progress, significant increases have been achieved in the amount of product taken per unit area. However, production, income and productivity have not reached the desired level due to some basic problems such as the use of traditional agricultural techniques in the agricultural sector, the use of incomplete inputs, the small and fragmented agricultural lands and the ineffective use of existing production resources. In order to solve the current problems encountered in agricultural production, it is necessary to determine whether the current structures of agricultural enterprises, production processes and resources are usaged effectively. Studies carried out to determine the amounts and costs of materials, labour and power usaged in the production of agricultural products form the basis of the steps taken in this direction. Studies conducted in this direction reveal the details of the production process, determine the participation amounts and shares of production factors in production, and provide some basic data that can be usaged in agricultural cultivation planning and economic analysis (Anonymous, 1998; İşleyen, 2019).

Non renewable energy sources are usaged to increase input density. These include chemical fertilizers, chemical pesticides, diesel fuel and the like. Non renewable energy resources containing fossil fuels decompose due to their structure and spread into the environment. As a result, soil, water and air are polluted and GHG are released into the environment. As a direct result of this, GHG have negative effects on the environment and human health (such as climate change, the emergence of diseases and pests, and the extinction of species). In other words, with the increase in the use of input energy per unit area, the environment and nature are polluted and resources such as soil and water, which are essential for nutrition, are damaged (Gökırmaklı and Bayram, 2018; Anonymous, 2020b; Şahin and Külekçi, 2022). A number of studies were performed on EB and GHG of agricultural production. A number of various studies were conducted on cherry (Demircan et al., 2006; Kizilaslan, 2009; Vahid-Berimanlou and Nadi, 2021), apricot (Gezer et al., 2003), pomegranate (Ozalp et al., 2018), apple (Çelen et al., 2017), sunflower (Akdemir et al., 2017), lavender (Demir et al., 2022), pepper (Baran et al., 2022), tea (Yıldız, 2023), watermelon (Demir, 2023), garlic (Baran et al., 2023), among others. A research on the literature has revelaed that no studies were conducted on the energy balance and GHG emission of cherry emission in the area and therefore the significance of this current study is quite high.

MATERIALS AND METHODS

Isparta province is located in the lakes region in the north of the Mediterranean Region. The city has a surface area of 8 933 km^2 and an average altitude of 1 050 meters. 68.4% of the province includeds of mountains, 16.8% plains and 14.8% plateaus. Gönen district generally reflect the steppe climate, which is a characteristic feature of Central Anatolia (Anonymous, 2023b). Gönen district is in the north of Isparta and is surrounded by Atabey in the east, Uluborlu in the

north, Burdur province in the southwest, and Keçiborlu district in the west. The district is 5 km away from the Isparta-Burdur highway. The district's surface area is 356 km^2. The district's altitude above sea level is 1 820 meters. It is 23 km away from Isparta city center. Agriculture and animal husbandry are important sources of income in the district (Anonymous, 2023c). The soils in Isparta generally have a calcareous main structure. Tectonic depression grooves in Isparta were filled with I. period alluviums. In the topsoil of agriculture, soils that constitute the basic source of 8-40 cm have emerged. According to temperature observations of Isparta over 30 years, the annual average temperature of the province is (12 °C). The highest temperature detected in the province is (38.7 °C) and the lowest temperature is (-21 °C). The average annual total rainfall in the city center is 508.3 mm (Anonymous, 2023d).

This current study was conducted in Gönen district of Isparta of Türkiye during the 2021 production period. The area that was studied spanned over a 2 ha cherry cultivation area. Randomized complete-block design with three replications was usaged. The amount of fuel consumption was computed and full-tank method was usaged to achieve this. The amount of fuel usaged per unit area was determined to measure the trial area and the amount of fuel that was placed in the tank (Göktürk, 1999; El Saleh, 2000; Sonmete and Demir, 2007). The work productivity for the area was computed and it was deemed to be an effective productivity. Work productivity in (ha/h) was achieved by calculating the effective working time (t_{ef}) (Güzel, 1986; Özcan, 1986; Sonmete, 2006). Time durations were measured in the study with the help of a chronometer (Sonmete, 2006). The energy equivalents and GHG equivalents of inputs in cherry cultivation are shown in Table 1 and Table 2, respectively. According to Mohammadi et al. (2010); EUE, SE, EP and NE were computed by using the formulates (Mandal et al., 2002; Mohammadi et al., 2008).

$$\text{Energy utilization efficiency} = \frac{\text{Energy output } (\frac{MJ}{ha})}{\text{Energy input } (\frac{MJ}{ha})} \quad (1)$$

$$\text{Specific energy} = \frac{\text{Energy input } (\frac{MJ}{ha})}{\text{Product output } (\frac{kg}{ha})} \quad (2)$$

$$\text{Energy productivity} = \frac{\text{Product output } (\frac{kg}{ha})}{\text{Energy input } (\frac{MJ}{ha})} \quad (3)$$

$$\text{Net energy} = \text{Energy output (MJ/ha)} - \text{Energy input (MJ/ha)} \quad (4)$$

Table 1. Energy Equivalents in Cherry Production.

Inputs	Unit	Energy Equivalent (MJ/unit)	References
Human labour	h	1.96	Mani et al. 2007; Karaağaç et al. 2011
Tractor	h	25.40	Singh, 2002; Akbolat et al., 2014
Rotary tiller	h	23.60	Singh, 2002; Akbolat et al., 2014
Disc harrow	h	19.60	Singh, 2002; Akbolat et al., 2014
Spraying	h	21.40	Singh, 2002; Akbolat et al., 2014
Chemical fertilizers			
N	kg	60.60	Singh, 2002; Ekinci et al., 2020
P	kg	11.10	Singh, 2002; Ekinci et al., 2020
S	kg	1.12	Nagy, 1999; Mohammadi et al., 2010
Chemicals			
Fungicide	kg	99	Fluck, 1992; Ekinci et al., 2020
Insecticide	kg	363.60	Pimentel 1980; Mrini et al., 2002
Diesel fuel	L	56.31	Singh 2002; Demircan et al., 2006
Lime	kg	1.32	Pimentel, 1980; Bilgili, 2012
Irrigation water	m^3	0.63	Yaldız et al., 1993; Ozkan et al., 2011
Electricity	kWh	3.60	Ozkan et al., 2004
Cherry fruit (Output)	kg	2.93	Proebsting (1980); Vahid-Berimanlou and Nadi (2021)

Table 2. GHG Emissions Coefficients in Cherry Cultivation.

Inputs	Unit	GHG Equivalent ($kgCO_{2-eq}$/unit)	References
Machinery	MJ	0.071	Dyer, J.A. and Desjardins, 2006; Ekinci et al., 2020
N	kg	1.300	Lal, 2004; Ozalp et al., 2018
P	kg	0.200	Lal, 2004; Ozalp et al., 2018
S	kg	0.370	Maraseni et al., 2010; Eren et al., 2019
Fungicide	kg	3.900	Graefe et al., 2013; Ozalp et al., 2018
Insecticide	kg	5.100	Lal, 2004; Ozalp et al., 2018
Diesel fuel	L	2.760	Clark et al., 2016; Eren et al., 2019
Electricity	kWh	0.608	Khoshnevisan et al., 2013; Ozalp et al., 2018

Eren et al. (2019) concluded that the GHG emissions ($kgCO_{2eq}$/ha) that take place through the inputs usaged to grow 1 ha of fruit were computed as follows, as adapted by Hughes et al. (2011).

$$GHG_{ha} = \sum_{i=1}^{n} R(i)\, x\, EF(i) \quad (5)$$

Eren et al. (2019) stated as follows ∑ where *R(i)* is the application rate of input *i* ($unit_{input}$/ha) and EF(*i*) is the GHG emission coefficient of input *i* ($kgCO_{2-eq}$/$unit_{input}$). However, an index is defined to evaluate the amount of emitted $kgCO_{2-eq}$ per kg yield. This is indicated in the following formula adapted Houshyar et al. (2015) and Khoshnevisan et al. (2014), where I_{GHG} is GHG ratio and Y is the yield as kg per ha.

$$I_{GHG} = \frac{GHG_{ha}}{Y} \quad (6)$$

The input energy can be categorized into into D, IDE, RE and NRE forms (Mandal et al., 2002; Singh et al., 2003; Koctürk and Engindeniz, 2009). Energy balance, energy utilization efficiency computations, energy inputs types, GHG emissions of inputs related to cherry cultivation are presented in Tables 3 to 6, respectively.

RESULTS AND DISCUSSION

As a result of the current study conducted in a cherry orchard, the average amount of cherry cultivated per hectare was computed as 10 100 kg for the 2021 production season. As Table 3 indicates, EI in cherry cultivation were, respectively: 8 141.40 (57.04%) chemical fertilizers energy, 3 575.69 (25.05%) diesel fuel energy, 1 186.02 (8.31%) machinery energy, 469.80 (3.29%) electricity energy, 366.25 (2.57%) human labour energy, 290.30 (2.03%) irrigation water energy, 231.30 (1.62%) chemicals energy and 13.20 MJ/ha (0.09%) lime energy. Total inputs energy was computed as 14 273.96 MJ/ha. Output energy (cherry fruit) was computed as 29 593 MJ/ha. In previous studies on the subject, Demircan et al. (2006) reported that fertilizer utilization energy had the biggest share by 40.82% in sweet cherry cultivation, while Ekinci et al. (2020) reported that diesel fuel energy had the biggest share by 24.69% in apple cultivation, etc. Cherry fruit, EI, EO, EUE, SE, EP and NE in cherry cultivation were computed as 10 100 kg/ha, 14 273.96 MJ/ha, 29 593 MJ/ha, 2.07, 1.41 MJ/kg, 0.71 kg/MJ and 15 319.04 MJ/ha, respectively (Table 4). In previous studies on the subject, Demircan et al. (2006) computed (cherry) EUE as 1.23, Vahid-Berimanlou and Nadi (2021) computed (cherry) EUE as 0.43, Oğuz et al. (2019) computed (nectarine) EUE as 1.86.

As indicated in Table 5, the total EI usaged in cherry cultivation can be classified as 32.94% (4 702.04 MJ/ha) DE, 67.06% (9 571.92 MJ/ha) IDE, 4.60% (656.55 MJ/ha) RE and 95.40% (13 617.41 MJ/ha) NRE. NRE was higher than the ratio of RE in EI of cherry cultivation. Similarly, in previous studies on sweet cherry (Demircan et al., 2006), on cherry (Vahid-Berimanlou and Nadi, 2021), on nectarine (Oğuz et al., 2019), among others, yielded results where the ratio of NRE was higher than the ratio of RE.

The results of GHG emissions of cherry cultivation are presented in Table 6. The total GHG emission was computed as 550.71 $kgCO_{2eq}$/ha (0.55 $tonCO_{2eq}$/ha). The results of the research pointed to the fact that the share of diesel in total GHG emissions had the highest value 31.82%, N (nitrogen) 21.25% and machinery 15.29% held the second and third place. GHG ratio (per kg) was computed as 0.05. In previous studies on the subject, Ekinci et al. (2020) computed the total GHG emission of apple cultivation as 1.46 $tonCO_{2eq}$/ha, Baran et al. (2023) computed the total GHG emission of garlic cultivation as 8.63 $tonCO_{2eq}$/ha, Demir (2023) computed the total GHG emission of watermelon cultivation as 0.43 $tonCO_{2eq}$/ha.

Table 3. Energy Balance in Cherry Production.

Inputs	Unit	Energy Equivalent (MJ/unit)	Input Per Hectare (Unit/ha)	Energy Value (MJ/ha)	Ratio (%)
Human labour	h	1.96	186.86	366.25	2.57
Tractor	h	25.40	24.75	628.65	4.40
Rotary tiller	h	23.60	15.84	373.82	2.62
Disc harrow	h	19.60	3.96	77.62	0.54
Spraying	h	21.40	4.95	105.93	0.74
Chemical fertilizers					
N	kg	60.60	90	5 454	38.21
P	kg	11.10	230	2 553	17.89
S	kg	1.12	120	134.40	0.94
Chemicals					
Fungicide	kg	99	0.50	49.50	0.35
Insecticide	kg	363.60	0.50	181.80	1.27
Diesel fuel	L	56.31	63.50	3 575.69	25.05
Lime	kg	1.32	10	13.20	0.09
Irrigation water	m^3	0.63	460.80	290.30	2.03
Electricity	kWh	3.60	130.50	469.80	3.29
Total inputs	-	-		14 273.96	100
Output	Unit	Energy equivalent (MJ/unit)	Output per hectare (unit/ha)	Energy value (MJ/ha)	Ratio (%)
Cherry fruit	kg	2.93	10 100	29 593	100
Total output	-	-	-	29 593	100

Table 4. EUE Computations in Cherry Cultivation.

Computations	Unit	Values
Cherry fruit	kg/ha	10 100
EI	MJ/ha	14 273.96
EO	MJ/ha	29 593
EUE	-	2.07
SE	MJ/kg	1.41
EP	kg/MJ	0.71
NE	MJ/ha	15 319.04

Table 5. EI in the Forms of Energy for Cherry Cultivation.

Energy Types	EI (MJ/Ha)	Ratio (%)
DE[a]	4 702.04	32.94
IDE[b]	9 571.92	67.06
Total	14 273.96	100
RE[c]	656.55	4.60
NRE[d]	13 617.41	95.40
Total	14 273.96	100

[a]Human labour, diesel fuel, electricity and irrigation water

[b]Chemical fertilizers, chemicals, lime and machinery

[c]Human labour and irrigation water

[d]Diesel fuel, chemicals, chemical fertilizers, machinery, lime and electricity

Table 6. GHG Emissions in Cherry Cultivation.

Inputs	Unit	GHG Coefficient ($kgCO_{2eq}$/unit)	Input usaged per area (unit/ha)	GHG Emissions ($kgCO_{2eq}$/ha)	Ratio (%)
Machinery	MJ	0.071	1 186.02	84.21	15.29
N	kg	1.300	90	117	21.25
P	kg	0.200	230	46	8.35
S	kg	0.370	120	44.40	8.06
Fungicide	kg	3.900	0.50	1.95	0.35
Insecticide	kg	5.100	0.50	2.55	0.46
Diesel fuel	L	2.760	63.50	175.26	31.82
Electricity	kWh	0.608	130.50	79.34	14.41
Total	-	-	-	550.71	100.00
GHG ration (per kg)	-	-	-	0.05	-

CONCLUSION

This current study aimed to reveal the energy balance and GHG emissions in cherry cultivation. EUE, SE, EP and NE in cherry cultivation were computed as 2.07, 1.41 MJ/kg, 0.71 kg/MJ and 15 319.04 MJ/ha, respectively. The highest energy input in cherry production was deemed to be chemical fertilizers energy by 57.04%. The total energy inputs usaged in cherry cultivation can be classified as 4.60% RE and 95.40% NRE. Use of chemical fertilizers usage should be decreased and use of farm fertilizers should be increased in order to rise EUE.

The total GHG emissions were computed as 550.71 $kgCO_{2eq}$/ha (0.55 $tonCO_{2eq}$/ha) and GHG rate (per kg) as 0.05. The findings of the research indicate that the rate of diesel fuel in total GHG emissions had the highest value by 31.82%. Eren et al. (2019) performed that it is recommended to make soil analysis to determine the type of soil fertilizer needed (to reduce high chemical fertilizers causing GHG emissions), and diesel fuel efficiency (to reduce the diesel fuel consumption).

According to the findings of this current study, cherry cultivation is a profitable production activity in terms of EUE (2.07). Machinery-use related fuel expenses can be decreased by using RE terms (Akbolat et al., 2014; Yıldız, 2023). The energy saving potential is huge. Observance of optimum requirement levels rises energy efficiency and decreases GHG (Imran and Ozcatalbas, 2021; Yıldız, 2023). Balanced fertilization programs based on soil and plant assessments can be important in reducing GHG (Seydoşoğlu et al., 2023). Energy utilization efficiency can be enhanced by taking the given recommendations into consideration.

The results of the energy balance given that cherry cultivation is a profitable production. Yılmaz and Bayav (2023) reported that; applications that improve profits should be encouraged; moreover, energy efficiency should be provided. Otherwise, it has not possible to talk about sustainability in agriculture production. It has important to support organic agriculture and good agricultural appliations, which some researches have defined to be highly energy efficient.

Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no competing interests in this study.

Author contribution

The contribution of the authors to this study is equal. The authors read and approved the last manuscript. The authors approve that the manuscript are original and not been published before.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Determining the genetic diversity of some black cumin genotypes collected in different regions of Türkiye using RAPD markers

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Abstract

Black cumin is one of the important medicinal aromatic plants belonging to the Ranunculaceae family. It is mostly used in the Middle East and for some diseases, especially in the Iranian region. It is important to know the genetic resources of such important medicinal and aromatic plants. Characterization of genetic resources sheds light on both the conservation of genetic resources and the future breeding studies. In this study, a total of 8 black cumin plants were characterized with 17 RAPD primers. Presence (1) and absence (0) scoring of gel images was performed using the Agarose Gel Electrophoresis (AGE) method. In genetic characterization, phylogenetic dendrogram with Bayesian statistics and Principal Coordinate Analysis (PCoA) with Jaccard similarity index were performed. As a result of the findings, the *Nigella damascena*, one of the black cumin plant species, was 100% separated from the *Nigella sativa* species. Additionally, Nigella sativa species differed among Konya, Eskişehir and Çameli genotypes. It gave similar results to Bayesian statistics in PCoA. The analysis indicated that Konya, Eskişehir and Çameli genotypes of *Nigella sativa* species have a higher potential to be used in breeding studies compared to other genotypes.

Keywords: *Nigella sativa*, *Nigella damascena*, RAPD, Plant Breeding, Diversity

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INTRODUCTION

Medicinal and aromatic plants have been used to treat diseases since time immemorial and have a wide range of traditional uses. Even in the Ranunculaceae family, which includes some important medicinal and aromatic plants, more than 2000 species are reported (Luan Nguyen et al. 2023). *Nigella segetalis*, *Nigella sativa*, *Nigella stellaris*, *Nigella koyuncui*, *Nigella gallica*, *Nigella fumariifolia*, and *Nigella damascene* are some of the species belonging to this family. The most studied species among these is *N. sativa*. This plant has different pharmacological effects (for example, it is used in dermatological complications, cancer, and type 2 diabetes) and is also used in traditional treatments (Srinivasan, 2018). In particular, thymoquinone, which is the main component of *N. sativa* oil, has been reported to have antibacterial, anti-cancer, immunostimulant, and antioxidant properties (Havlik et al. 2006). This plant is cultivated in different countries of the world and attracts attention due to its therapeutic properties (Luan Nguyen et al. 2023). In addition, black cumin seeds are used as flavoring in the edible cheese industry (Bourgou et al. 2010). The regions where the plant is most cultivated are Southern Europe, Middle East, and North and East Africa. It is of great importance to know the genetic resources of such an important plant.

Today, with the advancement of technology and molecular biology, the breeding

process is moving even faster. Since morphological markers require expertise and are affected by environmental conditions, researchers have started to use molecular marker methods more frequently (Aydin, 2023). Since molecular markers utilize sequences directly on the genome without being affected by time and environmental conditions, the reliability of the data is higher (Grover and Sharma, 2016). Molecular markers are used in a wide range of fields such as sex determination, species identification, genetic relationships, and determination of parents (Han et al. 2020; Soller, 2020; Song et al. 2023). In addition, it shortens the breeding process by making selection with the help of markers in plant breeding (Hasan et al. 2021). With the help of molecular markers, both population structure and genome structure can be revealed (Song et al. 2023; Yanez et al. 2023). Molecular markers commonly used in such studies are AFLP (Mei-Chao et al. 2020), RFLP (Manjunathagowda, 2021), RAPD (Türkoğlu et al. 2023), SCAR (Xu et al. 2020), ISSR (Venkatesan et al. 2021), SSR (Karaca et al. 2013), and SNP (Meng et al. 2022). Among these markers, RAPD markers were the first PCR-based marker technique and are still very actively used (Bi et al. 2021). RAPD markers are unidirectional universal markers consisting of 10 base pairs developed according to operon technology. The main advantages of this marker technique are that it does not require genomic information, is PCR-based and inexpensive (Amiteye, 2021). Primers form amplicons in PCR and bands in Agarose Gel Electrophoresis (AGE) can be seen when the same primer has reverse binding points on both strands. These markers can produce a large number of amplicons and can be used in genetic studies (Al-Hadeithi and Jasim, 2021). Their major disadvantage is that their primers bind at low temperatures and therefore, if not sensitive, nonspecific bands may appear (Al-Khayri, 2022). Different results may occur in various laboratories. Therefore, conversion of polymorphism bands into SCAR markers shows more permanent results (El-Haggar et al. 2023).

When the web of science database is sought, there are very few molecular marker studies on *Nigella* species. More molecular studies are needed to better understand the genetic structure of such an important plant in the medicinal and aromatic plant group. In this study, 17 RAPD markers were used to reveal the genetic relationships of some *Nigella* genotypes and it was aimed to reveal the genetic relationship in the existing genotypes by analyzing the data in the gel images obtained by agarose gel electrophoresis method.

MATERIALS AND METHODS

Plant Material

A total of 8 *Nigella* sp. genotypes were used in the study. Seven of them belonged to *Nigella sativa* and one genotype belonged to *Nigella damascene*. Six of the genotypes belonging to *Nigella sativa* (Çameli, Eskişehir, Konya, Şanlıurfa, Samsun, and Tokat) were obtained from different regions of Türkiye and one from Syria. The seeds of each plant were planted in small vials and grown under suitable climatic conditions. Leaf samples were collected under sterile conditions after the fourth true leaf was removed and stored at -20 °C for molecular studies.

DNA isolation and Quality-Quantity Determination

After the leaf samples were collected, they were pulverized with the help of liquid nitrogen. DNA isolation was performed according to Karaca et al., (2005) with some modifications. The quantity and quality of the obtained genomic DNAs (gDNA) were determined by Nano-Drop and agarose gel electrophoresis methods.

Primers and Polymerase Chain Reaction (PCR)

Ten nucleotide long unidirectional universal primers developed from operon technology were used in the study (Table 1). In PCR studies, amplicons were generated using Thermo Fisher Scientific (Cat:EP0402) thermal cycling device and Touch-Down PCR method (Karaca et al. 2019). In PCR, the temperature was reduced by 0.5 °C for each cycle from 42 °C to 37 °C and continued with 30 cycles after the first ten cycles. The pre-denaturation phase was continued at 94 °C for 5 min, the denaturation phase at 94 °C for 1 min, the binding temperature at 37 °C for 1 min and the renaturation phase at 72 °C for 2 min. In addition, PCR processes were completed after 10 min at the final renaturation temperature. PCR components and concentrations used were 50 ng gDNA, 2.4 µM of each primer, dNTP 0.28 mM, MgCl2 2.5 mM, 10X buffer 2.5 µL, and 1 Unit of Taq DNA polymerase 25 µL final volume.

Molecular Analysis

Amplicons obtained after PCR were generated by presence (1) and absence (0) scoring. Phylogenetic dendrogram tree was constructed using polymorphic information content (PIC) of primers, principal coordinate analysis (PCoA) and Bayesian statistics. Primer PIC values were calculated according to Smith et al., (1997). Principal coordinate analyses were performed using the Multivariate Statistical Package (MVSP) and Jaccard similarity index. MrBayes program and FigTree version 1.4.4 were used to construct the phylogenetic dendrogram tree.

Table 1. Information on RAPD primers used in the study.

NO	Primer ID	Primer sequence 5'→ 3'
1	OPA-05	AGGGGTCTTG
2	OPA-06	GGTCCCTGAC
3	OPB-12	CCTTGACGCA
4	OPB-13	TTCCCCCGCT
5	OPC-08	TGGACCGGTG
6	OPC-09	CTCACCGTCC
7	OPD-01	ACCGCGAAGG
8	OPD-02	GGACCCAACC
9	OPF-16	GGAGTACTGG
10	OPF-17	AACCCGGGAA
11	OPG-03	GAGCCCTCCA
12	OPG-04	AGCGTGTCTG
13	OPH-07	CTGCATCGTG
14	OPH-10	CCTACGTCAG
15	OPI-11	ACATGCCGTG
16	OPI-14	TGACGGCGGT
17	OPK-15	CTCCTGCCAA
18	OPK-18	CCTAGTCGAG
19	OPN-19	GTCCGTACTG
20	OPN-20	GGTGCTCCGT

RESULTS AND DISCUSSION

Determination of genetic relationships of *Nigella* sp. genotypes in the medicinal and aromatic plant group at molecular level is important for researchers working in this plant group. In this study, genetic characterization of different black cumin genotypes was carried out by using molecular markers. Black cumin seeds available in our unit were germinated and DNA isolation was performed. The quality and quantity of the obtained gDNAs were measured by Nano-Drop (Maestrogen, Hsinchu City, Taiwan, MN-013) and agarose gel electrophoresis methods. The A_{260}/A_{280} reads of around 1.8 should be a quality gDNA, free of proteins (Karaca et al., 2005). Since the ratios of the obtained gDNAs in the specified readings ranged between 1.7-1.9, a quality DNA free of proteins was obtained. In addition, it is not possible to determine whether the gDNA obtained in spectrophotometric readings is broken or not. Therefore, this result can be seen visually by agarose gel electrophoresis method. When agarose gel electrophoresis images were analyzed, it was determined that there was no broken DNA with high molecular weight that would negatively affect the studies.

After PCR, agarose gel electrophoresis method was used to visualize the amplicons. 2% gels were prepared and run for 2, 4 or 6 hours. The images were recorded on computer. Gel images were analyzed with GelAnalyzer (19.1v) and presence (1) and absence (0) bands were recorded. Only 17 of the 20 primers screened produced polymorphic and clearly readable amplicons. The PIC values of these primers varied between 0.218-0.857 for these genotypes. While he lowest PIC value was shown by primer OPD-02, the highest PIC value was shown by primer OPK-15 (Table 2). Only 3 of the primers were below 0.5. The remaining primers were found to be highly polymorphic. In addition, the number of alleles formed by the primers was calculated as 200 in total and the number of alleles per primer was determined as 11.764. The number of alleles for the primers varied between 6-18. The lowest number of alleles was observed in primer OPA-06, while the highest number of alleles was observed in primer OPF-16. The number of patterns formed by the primers in the population was calculated, as well. It was determined that the total number of patterns was 75 and the number of patterns per perimer was 4.411. The lowest number of patterns was 2 in primer OPD-02 and the highest number of patterns was 7 in primer OPK-15. PIC values of the primers are calculated to reveal the efficiency and discrimination power of the molecular markers used in the population and the importance of the primer used (Serrote et al. 2020). It was also determined that the primers used for the black cumin population in this study were effective primers that can be used to distinguish and characterize this population.

Table 2. Polymorphic Information Content of Primers.

NO	Primer ID	Primer sequence 5'→ 3'	Total Alel	Patern Number	PIC
1	OPA-06	GGTCCCTGAC	6	5	0,703
2	OPB-12	CCTTGACGCA	15	6	0,812
3	OPB-13	TTCCCCCGCT	16	5	0,750
4	OPC-08	TGGACCGGTG	12	3	0,593
5	OPC-09	CTCACCGTCC	12	5	0,687
6	OPD-01	ACCGCGAAGG	18	4	0,656
7	OPD-02	GGACCCAACC	7	2	0,218
8	OPF-16	GGAGTACTGG	18	4	0,656
9	OPF-17	AACCCGGGAA	8	4	0,687
10	OPG-03	GAGCCCTCCA	16	6	0,812
11	OPG-04	AGCGTGTCTG	13	3	0,406
12	OPH-10	CCTACGTCAG	8	4	0,562
13	OPI-11	ACATGCCGTG	9	5	0,775
14	OPI-14	TGACGGCGGT	9	5	0,750
15	OPK-15	CTCCTGCCAA	12	7	0,857
16	OPK-18	CCTAGTCGAG	10	3	0,406
17	OPN-19	GTCCGTACTG	11	4	0,612

One of the analyses used in this study is PCoA analysis. In PCoA analysis, principal components are processed and a graph is created with a proximity or distance matrix and reveals the distance between samples (Gower, 2014). In the current study, we revealed the genetic similarity of the existing alleles by using the Jaccard similarity index (Figure 1). As a result of the analysis, *Nigella damascena*, which showed different species characteristics, was completely differentiated from the existing *Nigella sativa* species. Genotypes belonging to *Nigella sativa* species also formed 3 clusters among themselves. One of them was Şanlıurfa, Tokat and Syrian genotypes, another one was Samsun, Konya and Eskişehir and the third one was Çameli variety which was developed as a variety. Here, although the Syrian genotype was obtained from a different country, it was grouped in the same group with Şanlıurfa and Tokat varieties in Türkiye and showed a high rate of similarity. In the PCoA analysis, our population was divided into 4 different clusters and it was determined that it was suitable for use in genetic studies.

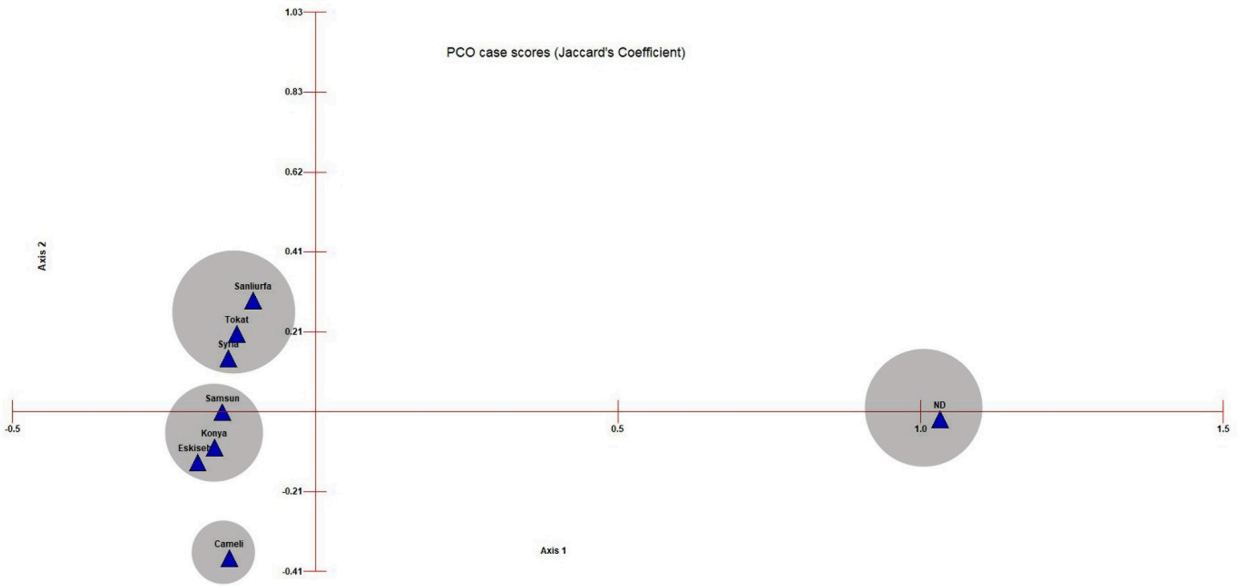

Figure 1. Principal Coordinate Analysis (PCoA).

In addition to these analyses, genetic relationships were analyzed with Bayesian statistics. MrBayes reveals the best relationship between samples or population by including "post probability" estimates in Bayesian statistics in a wide range of phylogenetic and evolutionary models. In this method, the analysis starts with a topology with the highest probability (a prior) and the trees are simulated using the Markov Chain Monte Carlo (MCMC) method and the tree/trees with high post probabilities is/are selected by capturing the best topologies (Karaca et al., 2015). The posterior probability of phylogenetic trees cannot be determined analytically. Instead, MCMC calculates the posterior probability by using the data with the tree created by drawing samples from the "posterior" distribution. As a result of the analyses conducted using MrBayes program, the best tree for revealing the genetic relationship was created with FigTree program. In this study, the analysis was performed with 10 million replications and two main clusters were formed (Figure 2). In one of these clusters, there were genotypes belonging to *Nigella damascena* species and the other cluster included genotypes belonging to *Nigella sativa* species. Among the genotypes of *Nigella sativa* species, Konya, Eskişehir and Çameli genotypes were grouped within themselves.

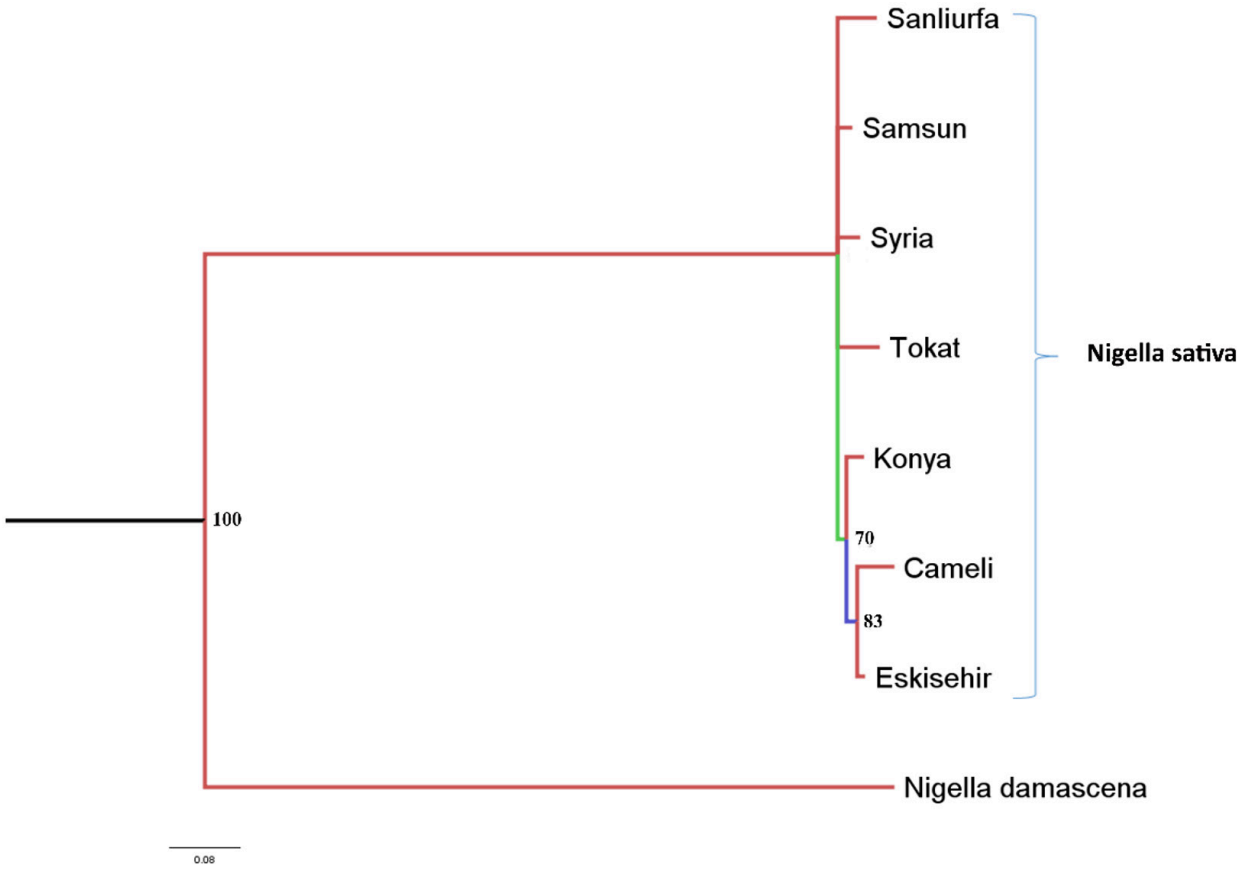

Figure 2. Phylogenetic Dendrogram of Bayesian Statistics.

CONCLUSION

The main objective of the study was to characterize the black cumin plant using molecular markers. In this context, molecular characterization of 7 *Nigella sativa* genotypes and 1 *Nigella damascena* genotype was carried out using 17 RAPD machines. Agarose gel electrophoresis method was used to separate the amplicons generated after PCR. By analyzing the gel images, 17 RAPD primers generated 200 alleles in total and 11.764 alleles were detected per primer. It was determined that 13 of the primers used had high PIC values. Two analysis methods were used for characterization of the genotypes. One of these methods was PCoA analysis with the Jaccard similarity index, and the other was dendrograms using Bayesian statistics. Although there were very few differences between the analyses, they supported each other. While 4 clusters were formed with PCoA analysis, 2 main clusters and one of the clusters formed two clusters in Bayesian statistics.

Consequently, the RAPD primers used in the outputs of this study were found to be effective for this population and can be used in other black cumin populations. Another output was detection of the genetic relationships between

these genotypes. Although the population density was low, the analyses revealed that the variation in the population was high. Especially in PCoA analysis, 4 different clusters were observed. High level of differences was observed in the genotypes of *Nigella sativa* species. These results indicate that *Nigella sativa* genotypes, which is an important medicinal and aromatic plant group, would be used in breeding studies.

Compliance with Ethical Standards

Conflict of interest

There is no conflict of interest regarding the article.

Author contribution

AA: desing, writing and laboratory studies.

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Data availability

Not applicable

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RESEARCH ARTICLE

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Determining the relationship between physical activity and intuitive eating and mindful eating in university students

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Abstract

In this study, it was aimed to determine the relationship between physical activity and intuitive eating (IE) and mindful eating (ME) in university students. The "International Physical Activity Short Form", "Mindful Eating Test (MET)" and "Intuitive Eating Scale (IES)" were applied by questioning the demographic characteristics, anthropometric measurements, health information and nutritional habits of 255 university students studying in Health and Sports sciences. Among the students whose mean age is 20.34±2.06, 86.7% of the students who are in health science are female, and 67.7% of them, who are in sports sciences are male ($p<0.05$). Students (51.4%) with normal Body Mass Index (BMI) consume three main meals and go on a diet for aesthetic reasons. Eating discipline (ED), emotional eating (EE), and intuitive eating total (IET) scores are higher in females, while control of eating (EC) scores are higher in males ($p<0.05$). Intuitive eating total score, reliance on hunger and satiety cues (RHSC), unconditional permission to eat (UPE) and EC cores are positively related to BMI. Students who are physically inactive have higher IET score, RHSC, body-food choice congruence, focusing and ED, and those who do adequate physical activity have higher eating control scores ($p<0.05$). Mindful eating total score and sub-dimensions are positively correlated with the score of all sub-dimensions except UPE, which is one of the sub-dimensions of IE ($p<0.05$). In addition, as the UPE score increases, the total scores of disinhibitions, ED, focusing, interference, EE, and ME decrease ($p<0.05$). In conclusion, ME and IE are positively related to each other. It is understood that adequate and balanced nutrition along with being physically active at the same time is quite important for younger individuals to be healthier.

Keywords: Intuitive eating, Mindful eating, Physical activity

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INTRODUCTION

Eating behavior, which takes place in every period of life, continues by developing from infancy to school age (Canetti et al., 2002). Eating behavior is affected by many factors. These factors can be individual such as genes, hormones, mood and body image; it can also be environmental, such as experiences, cultural background, religious beliefs, media (Özkan & Bilici, 2021). The relationship between eating behavior and mood in these processes is one of the important areas of study (Özkan & Bilici, 2018). Combined treatment of physical activity and calorie restriction has been recommended for weight control for many years (Kayar & Utku, 2013). On the other hand, cognitive restriction of food intake can create negative effects on the eating behavior. Deficiencies in stress management and long-term dietary practices may cause malnutrition habits. For that reason, the concepts of intuitive nutrition and mindful eating (ME) have come to the fore in gaining healthy eating attitudes and behaviors (Özkan & Bilici, 2018).

Intuitive nutrition is a self-care eating framework, which integrates instinct, emotion, and rational thought and was created by two dietitians, Evelyn Tribole and Elyse Resch in 1995 (Tribole & Resch, 2003). Intuitive eating (IE) aims to break the cycle of constant dieting by reconnecting with the body's natural signals of hunger, satiety and satisfaction (Tribole & Resch, 2003; Tylka, 2006; Camilleri et al., 2015). Intuitive eating aims to establish a healthy relationship between food, mind, and body. In addition, it supports awareness of emotions and experiencing the pleasure of eating. In the IE approach, main is to allow the body to recognize the internal hunger – fullness signals and amount and type of food consumed to feel more satisfied with meal (Van Dyke & Drinkwater, 2014). The concept of IE is explained by four sub-dimensions:

1) Eating for physical rather than emotional reasons (FRE)

2) Unconditional permission to eat (UPE) (desired food consumption in accordance with physical hunger signals),

3) Reliance on hunger and satiety cues (RHSC) (determining when and how much to eat)

4) Body-food choice congruence (BFCC) (Özkan & Bilici, 2018; Tylka, 2006)

Mindful eating is defined as acting consciously in food selection, developing an mindfulness in evaluating physical and psychological hunger and satiety cues, and making healthy food choices in response to these cues (Miller et al., 2014; Dalen et al., 2010).

In mindful eating, it is aimed to develop an mindfulness without prejudice to the physical and emotional feelings of the individual about eating (Jordan et al., 2014). The individual is aware of the moment during the meal and pays attention to the effect of food on the senses and thus realizes the physical and emotional sensations that occur in response to eating (Warren et al., 2017). Mindful eating consists of 7 sub-dimensions: disinhibition, emotional eating (EE), control of eating (EC), focusing, eating discipline (ED), mindfulness, external cues (Özkan & Bilici, 2018). Studies have linked the ability to eat mindfulness to less impulsive eating behavior, thereby reducing energy consumption and healthier snack choices (Bor & Saka, 2021).

Physical activity is also one of the most important parameters of a healthy life. Studies report the positive effect of physical activity on health in case of chronic disease. Regular physical activity: it contributes to the reduction of many diseases, to increase the individual's work efficiency, to the regulation of cognitive functions and school success, and to psychological well-being (WHO, 2023).

The aim of the study; to examine the relationship between IE, ME and physical activity status in university students who are a sensitive population in terms of irregular diet and eating disorders.

MATERIALS AND METHODS

This descriptive and cross-sectional study was carried out between May 2022 and July 2022 at İstanbul Rumeli University via Google Survey. Permission for the study was obtained from the Ethics Committee of İstanbul Rumeli University with the desicion no 02 dated 20.05.2022. Participants were asked to read and approve the participation before the survey. The population of the research consists of 374 individuals from the Faculty of Health and Sports Sciences. The sample includes a total of 255 students, 128 from the Faculty of Health Sciences and 127 from the Faculty of Sport Sciences, who agreed to participate in the study. Questionnaires applied to the participants consists of 4 parts which are given below;

1. Demographic Information

2. Intuitive Eating Scale (IES)

3. Mindful Eating Test (MET)

4. Physical Activity Status

In the first part of the questionnaire, the demographic information of the participants (general information, anthropometric measurements, health information, eating habits) was questioned. The Intuitive Eating Scale was used to determine IE behaviors. The Turkish validity and reliability of the scale developed by Tylka et al. was performed by Baş et al (Bas et al., 2017; Tylka & Kroon Van Diest, 2013). The scale consists of the sub-headings of unconditional consent to eat, eating for physical reasons rather than emotional reasons, RHSC, and BFCC. The scale is scored on a five-point Likert type (1=Strongly Disagree, 2=Disagree, 3=Neither Agree Neither Disagree, 4=Agree, 5=Strongly Agree). A minimum of 21 points and a maximum of 105 points are obtained on the scale. The Mindful Eating Test was used to detect eating awareness. The scale, which was validated and reliable by Kose et al., consists of 30 questions (Kose et al., 2017). The scale includes sub-dimensions of mindless eating, EE, eating control, awareness, ED, conscious eating, and interference. The scale is scored on a five-point Likert type (1=Never, 2=Rarely, 3=Sometimes, 4=Often, 5=

Always). A minimum of 30 and a maximum of 150 points are obtained on the scale. The physical activity status of the participants was evaluated with the International Short Form of Physical Activity (Öztürk, 2005).

Statistical Evaluation

SPSS 26 (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.) statistical package program was used to evaluate the data obtained from the study. Quantitative data are expressed as number (n), percantage (%), descriptive values as frequency, arithmetic mean (x), standard deviation (ss). The conformity of the variables to the normal distribution was examined by histogram, probability graphs and Shapiro-Wilk test. When using independent sample t-test to compare 2 groups with normal distribution; Mann-Whitney U test was used to compare the data that did not have normal distribution. When comparing 3 or more groups, normally distributed groups were evaluated with ANOVA, and non-normally distributed groups were evaluated with Kruskal Wallis tests. In evaluating the relationship between continuous variables, Pearson Correlation Analysis has been used for the data which has a normal distribution and Spearman Correlation Analysis has been used for the data which were not normally distributed. Statistically significance level was determined as $p<0.05$.

RESULTS AND DISCUSSION

Results

Demographic information of the participants is given in Table 1. While the avarege age of participants who are studying in Health Sciences is 20.65±2.20, it is 20.03±1.87 in Sports Sciences ($p<0.05$). Female participants mostly (86.7%) are studying Health Sciences, male participants (67.7%) are studying Sports Sciences. Cigarette and alcohol consumption were higher in Sport Sciences' participants ($p<0.05$, $p>0.05$ respectively). While 91% of the participants do not have a chronic disease, in others asthma, diabetes mellitus and allergies are most common. The mean Body Mass Index (BMI) of those studying in Health and Sports Sciences is 22.26±3.28 and there is no statistical difference between the groups. In addition, although the level of physical activity is not desired level in both groups, it is related to the department of education ($p<0.05$).

According to nutritional characteristics (Table 2), 51.4% of the participants consume 3 main meals, while 36.5% do not consume snacks. It was determined that especially the number of main meals consumed was related to the department ($p<0.05$). While skipping meals is seen in 62.4% of the participants, the most skipped meal is breakfast for students who were studying health sciences and lunch for students who were studying sport sciences. The main reason of this was determined that the lack of time in both groups. While 60.4% of the participants reported that they have never had diet before, it was determined that the others had dieted mostly due to aesthetic reasons for both groups. In addition, it has been shown that those who diet for health are more likely than those who study health sciences. Accordingly, it was found that the reasons for dieting were related to the department of education ($p<0.05$).

Table 3 shows the BMI classification according to gender, department of education and physical activity level 69.7% of women and 73.8% of men are in the normal range. Below 70% of those studying in both departments are in the normal BMI range. In the majority of participants in each BMI group, the level of physical activity was lower than expected.

Table 4 shows the relationship between ME and its sub-dimensions and gender, department of education, BMI classification and physical activity level. The mean of the total score of ME was calculated as 2.55. Students above this score (45.88%) were determined as "Those with more awareness of eating", and those below (54.12%) as "Those with less awareness of eating". According to this evaluation, there was no statistical difference in terms of gender, department of education, BMI and physical activity level ($p>0.05$). Eating discipline and EE scores were higher in females ($p<0.001$), while eating control was higher in males ($p<0.001$). On the other hand, according to the department of education, ED is higher in health sciences and eating control is higher in sports sciences ($p<0.05$). There was no difference between underweight, normal, overweight and obese individuals in the total score and subdimensions of mindful eating ($p>0.05$). Mindful eating total score, interference, EE and mindfulness scores ($p>0.05$) and ED score ($p<0.001$) were higher in inactive individuals, while focusing score was lower in those with low physical activity level ($p<0.05$). Eating control score is higher in those with sufficient physical activity level ($p<0.05$).

Table 5 shows the relationship between IE and its sub-dimensions and gender, department of education, BMI classification and physical activity level. The mean of IET score was determined as 2.52. Students above this score (45.49%) were determined as "Those with more IE behavior", and students below (54.51%) as "Those with less IE behavior". According to this evaluation, women eat more intuitively than men ($p<0.05$). In addition, individuals who eat intuitively are statistically higher in BMI. There was no statistically significant difference between the sub-dimensions of IE between men and women ($p>0.05$).

However, IET score was much higher in women ($p<0.05$). When evaluated according to the department of education,

the UPE score is higher in those studying in sports sciences (p<0.05). When IET score was evaluated, it was lowest in normal individuals and highest in overweight participants (p<0.05). Unconditional permission to eat score, which is one of the sub-dimensions of IE, was highest in obese subjects and lowest in normal individuals (p<0.05). In inactive individuals, IET score and BFCC score were higher than the others (p<0.05), while the eating score due to hunger and satiety cues was lower in those with low physical activity level (p<0.05).

The relationship between ME and IE is shown in Table 6. The total score and sub-dimensions of ME are positively related to the score of all dimensions except UPE, which is one of the sub-dimensions of IE. Mindful eating increases stastistically as the UPE score which covers when people are hungry and what food they desire, decreases (p<0.05). Although not statistically significant, a negative relationship was observed in the sub-dimensions of eating control and mindfullnes.

Table 1. Demographic Information of Participants

Demographic Informations	Total (n_T=255)	Department of education		*p*
		Health Sciences (n_T=128)	Sports Sciences (n_T=127)	
	n (%)	n (%)	n (%)	
Age (years) (minimum-maximum)	20.34±2.06 (18-36)	20.65±2.20 (18-34)	20.03±1.87 (18-36)	**0.010***
Sex				**<0.001****
Female	152 (59.6)	111 (86.7)	41 (32.3)	
Male	103 (40.4)	17 (13.3)	86 (67.7)	
Tobacco Use				**0.022****
Yes	57 (22.4)	21 (16.4)	36 (28.3)	
No	198 (77.6)	107 (83.6)	91 (71.7)	
Alcohol Use				0.416
Yes	51 (20.0)	23 (18.0)	28 (22.0)	
No	204 (80.0)	105 (82.0)	99 (78.0)	
Disease status				0.051
Yes	23 (9.0)	16 (12.5)	7 (5.5)	
No	232 (91.0)	112 (87.5)	120 94.5)	
Disease type	**(n=23)**	**(n=16)**	**(n=7)**	0.243
Asthma	4 (17.4)	2 (12.5)	2 (28.6)	
Diabetes mellitus	4 (17.4)	1 (6.3)	3 (42.9)	
Allergies	3 (13.0)	3 (18.8)	0 (0.0)	
Familial Mediterranean Fever	1 (4.4)	1 (6.3)	0 (0.0)	
Hernia	2 (8.7)	2 (12.5)	0 (0.0)	
Psoriasis	1 (4.4)	0 (0.0)	1 (14.3)	
Thyroid disease	1 (4.4)	1 (6.3)	0 (0.0)	
Iron deficiency	1 (4.4)	0 (0.0)	1 (14.3)	
Peptic ulcer	1 (4.4)	1 (6.3)	0 (0.0)	
Rheumatic diseases	1 (4.4)	1 (6.3)	0 (0.0)	
Familial Mediterranean Fever and Psoriasis	1 (4.4)	1 (6.3)	0 (0.0)	
Allergies and Asthma	1 (4.4)	1 (6.3)	0 (0.0)	
Diabetes mellitus and Asthma	2 (8.7)	2 (12.5)	2 (28.6)	
Medication use				0.099
Yes	19 (7.5)	13 (10.2)	6 (4.7)	
No	236 (92.5)	115 (89.8)	121 95.3)	
BMI (kg/m²)	22.26±3.28	21.87±3.40	22.66±3.13	0.056
BMI classification				0.612
<18,5 kg/m²	25 (9.8)	14 (10.9)	11 (8.7)	
18,5-24,9 kg/m²	182 (71.4)	93 (72.7)	89 (70.1)	
25-29,9 kg/m²	39 (15.3)	16 (12.5)	23 (18.1)	
>30 kg/m²	9 (3.5)	5 (3.9)	4 (3.1)	
Level of physical activity				**<0.001****
Inactive	46 (18.0)	30 (23.4)	16 (12.6)	
Low level of physical activity	129 (50.6)	72 (56.3)	57 (44.9)	
Adequate physical activity level	80 (31.4)	26 (20.3)	54 (42.5)	

*Mann-Whitney U Test **Chi-square Test BMI: Body Mass Index n: number of participants nT: total number of participants

Table 2. Nutritional characteristics of the participants

Nutrition Status	Total (n_T=255)	Department of education		p
		Health Sciences (n_T=128)	Sports Sciences (n_T=127)	
	n (%)	n (%)	n (%)	
Main Meals				**0.008***
1	4 (1.6)	4 (3.1)	0 (0.0)	
2	103 (40.4)	62 (48.4)	41 (32.3)	
3	131 (51.4)	54 (42.2)	77 (60.6)	
4 and more	17 (6.7)	8 (6.3)	9 (7.1)	
Snacks				0.245
None	93 (36.5)	40 (31.3)	53 (41.7)	
1	65 (25.5)	32 (25.0)	33 (26.0)	
2	71 (27.8)	44 (34.4)	27 (21.3)	
3	19 (7.5)	8 (6.3)	11 (8.7)	
4 and more	7 (2.8)	4 (3.1)	3 (2.4)	
Meal skipping status				0.279
Yes	159 (62.4)	84 (65.6)	75 (59.1)	
No	96 (37.6)	44 (34.4)	52 (40.9)	
Skipped meals	**(n=159)**	**(n=84)**	**(n=75)**	0.279
Breakfast	63 (39.9)	35 (41.7)	28 (37.3)	
Lunch	69 (43.7)	34 (40.5)	35 (46.7)	
Dinner	10 (6.3)	6 (7.1)	4 (5.3)	
Snack	11 (6.9)	4 (4.8)	7 (9.3)	
Breakfast and lunch	2 (1.3)	1 (1.2)	1 (1.3)	
Breakfast and dinner	2 (1.3)	2 (2.4)	0 (0.0)	
Lunch and snack	2 (1.3)	2 (2.4)	0 (0.0)	
Reasons for skipping meals	**(n=141)**	**(n=70)**	**(n=71)**	0.273
Lack of hunger/loss of appetite	24 (9.4)	8 (11.4)	16 (22.5)	
Late awakening/sleep patterns	18 (7.1)	6 (8.6)	12 (16.9)	
Lack of time	59 (23.1)	31 (44.3)	28 (39.4)	
Lack of appetite for snack foods	4 (1.6)	3 (4.3)	1 (1.4)	
Bother to eat	8 (3.1)	4 (5.7)	4 (5.6)	
Habit	2 (0.8)	2 (2.9)	0 (0.0)	
Diet-dependent	1 (0.4)	0 (0.0)	1 (1.4)	
Lack of opportunity	6 (2.4)	5 (7.1)	1 (1.4)	
Forgetting to eat	4 (1.6)	2 (2.9)	2 (2.8)	
Overeating at the previous meal/late	10 (3.9)	6 (8.6)	4 (5.6)	
hour	5 (2.0)	3 (4.3)	2 (2.8)	
Other				
Diet following status				0.107
Yes	101 (39.6)	57 (44.5)	44 (34.6)	
No	154 (60.4)	71 (55.5)	83 (65.4)	
Reason for following a diet	**(n=176)**	**(n=89)**	**(n=87)**	**0.002***
Health	24 (9.4)	18 (20.2)	6 (6.9)	
Aesthetics	105 (41.2)	56 (62.9)	49 (56.3)	
Other	47 (18.4)	15 (16.9)	32 (36.8)	
Duration of maintenance of weight loss	**(n=90)**	**(n=48)**	**(n=42)**	0.289
None				
0-6 m	4 (1.6)	3 (6.3)	1 (2.4)	
6-12 m	47 (18.4)	22 (45.8)	25 (59.5)	
12-18 m	19 (7.5)	14 (29.2)	5 (11.9)	
18-24 m	1 (0.4)	0 (0.0)	1 (2.4)	
>24 m	8 (3.1)	4 (8.3)	4 (9.5)	
	11 (4.3)	5 (10.4)	6 (14.3)	

*Chi-square Test n: number of participants nT: total number of participants

Table 3. Classification of BMI according to gender, department of education and physical activity

	Underweight (<18.5 kg/m²) n (%)	Normal weight (18.5-24.9 kg/m²) n (%)	Overweight (25.0-29.9 kg/m²) n (%)	Obese (>30.0 kg/m²) n (%)	Total n (%)
Sex					
-Female	23 (15.1)	106 (69.7)	17 (11.2)	6 (4.0)	152 (59.6)
-Male	2 (1.9)	76 (73.8)	22 (21.4)	3 (2.9)	103 (40.4)
Department of education					
-Health Sciences	14 (10.9)	93 (72.7)	16 (12.5)	5 (3.9)	128 (50.2)
-Sport Sciences	11 (8.7)	89 (70.1)	23 (18.1)	4 (3.2)	127 (49.8)
Level of physical activity					46 (18.0)
					129 (50.6)
-Inactive	4 (16.0)	31 (17.0)	9 (23.1)	2 (22.2)	
- Low level of physical activity	16 (64.0)	91 (50.0)	18 (46.2)	4 (44.4)	80 (31.4)
- Adequate physical activity level	5 (20.0)	60 (33.0)	12 (30.8)	3 (33.3)	
Total	25 (9.8)	182 (71.4)	39 (15.3)	9 (3.5)	***255***

n: number of participants *Chi-square Test

Table 4. The relationship between eating awareness and its sub-dimensions and gender, department of education, BMI classification and physical activity level

Mindful Eating Score		Sex		Department of education		Level of physical activity		
		Female	Male	Health Sciences	Sport Sciences	Inactive	Low level of physical activity	Adequate physical activity level
Mindful Eating total		2.55±0.49	2.55±0.53	2.55±0.51	2.55±0.50	2.66±0.44	2.49±.49	2.59±.55
p		0.946		0.943			0.086	
Disinhibition		2.41±0.85	2.54±1.04	2.43±0.88	2.50±0.99	2.41±.87	2.37±.84	2.65±1.09
p		0.282		0.563			0.184	
Eating discipline		3.07±0.74	2.60±0.98	3.02±0.82	2.74±0.90	3.26±.80[ab]	2.90±.80[a]	2.63±.93[b]
p		**<0.001***		**0.011***			**<0.001*****	
Focusing		2.63±0.51	2.74±0.51	2.61±0.50	2.74±0.52	2.85±.42[c]	2.60±.51[c]	2.69±.54
p		0.080		0.055			**0.006********	
Interference		2.29±0.86	2.38±1.04	2.25±0.92	2.41±0.95	2.51±.93	2.26±.82	2.33±1.10
p		0.417		0.175			0.278	
Emotional eating		2.52±1.00	2.13±1.11	2.47±1.03	2.26±1.09	2.46±1.03	2.29±.96	2.44±1.22
p		**<0.001****		0.114			0.592	
Control of eating		1.98±0.84	2.50±0.95	2.02±0.88	2.36±0.94	2.11±.78	2.07±.90[d]	2.43±1.00[d]
p		**<0.001****		**0.001****			**0.027********	
Mindfulness		2.81±0.40	2.84±0.43	2.86±0.36	2.79±0.45	2.94±.32	2.81±.40	2.78±.46
p		0.607		0.336			0.096	
ME Status (ME score)	**Higher** n (%)	68 (58.1)	49 (41.9)	55 (47.0)	62 (53.0)	22 (18.8)	53 (45.3)	42 (35.9)
	Lower n (%)	84 (60.9)	54 (39.1)	73 (52.9)	65 (47.1)	24 (17.4)	76 (55.1)	38 (27.5)
	p	0.656		0.349			0.262	

Table 4. The relationship between eating awareness and its sub-dimensions and gender, department of education, BMI classification and physical activity level (continue)

Mindful Eating Score		BMI classification				BMI (kg/m²)
		Underweight (<18.5 kg/m²)	Normal (18.5-24.9 kg/m²)	Overweight (25.0-29.9 kg/m²)	Obese (>30.0 kg/m²)	r p
Mindful Eating total		2.68±0.52	2.54±0.51	2.59±0.48	2.38±0.33	0.040
p			0.358			0.520
Disinhibition		2.42±0.98	2.50±0.92	2.41±1.05	2.00±0.48	-0.010
p			0.370			0.869
Eating discipline		3.31±0.56	2.84±0.85	2.83±1.07	2.81±0.86	-0.108
p			0.082			0.086
Focusing		2.86±0.52	2.64±0.52	2.71±0.46	2.64±0.33	0.003
p			0.310			0.967
Interference		2.48±0.70	2.35±0.96	2.23±1.01	1.83±0.56	-0.126
p			0.165			**0.044**[£]
Emotional eating		2.55±1.07	2.33±1.06	2.51±1.18	1.98±0.48	-0.048
p			0.523			0.447
Control of eating		2.23±0.96	2.13±0.92	2.41±0.92	2.28±0.72	0.221
p			0.283			**<0.001**[£]
Mindfulness		2.85±0.35	2.82±0.41	2.80±0.47	2.87±0.33	0.027
p			0.995			0.663
ME Status (ME score)	**Higher** n (%)	15 (12.8)	79 (67.5)	20 (17.1)	3 (2.6)	22.41±3.29
	Lower n (%)	10 (7.2)	103 (74.6)	19 (13.8)	6 (4.3)	22.14±3.29
	p		0.323			0.393

*Independent Samples t Test **Mann-Whitney U Test ***One-way ANOVA ****Kruskal Wallis Test [£]Spearman Test ME: Mindful Eating BMI: Body Mass Index n: number of participants

[a-d] In the same column, there is a statistically significant difference between those with the same exponential letter.

Table 5. The relationship between IE and its sub-dimensions and gender, major, BMI classification and physical activity level

Intuitive Eating Scale		Sex		Department of education		Level of physical activity		
		Female	Male	Health Sciences	Sport Sciences	Inactive	Low level of physical activity	Adequate physical activity level
Intuitive Eating total score		2.58±0.58	2.44±0.63	2.58±0.62	2.46±0.59	2.72±0.62[ab]	2.51±0.60[b]	2.43±0.59[a]
p		**0.038****		0.113		**0.036*****		
Unconditional Permission to Eat		2.96±0.59	2.93±0.76	2.87±0.64	3.03±0.68	2.86±0.58	2.96±0.65	2.98±0.72
p		0.791		**0.044***		0.197		
Eating for Physical Rather than Emotional Reasons		2.50±0.87	2.31±0.87	2.52±0.88	2.33±0.86	2.59±0.77	2.40±0.89	2.37±0.89
p		0.090		0.076		0.325		
Reliance on Hunger and Satiety Cues		2.40±1.00	2.25±1.26	2.47±1.07	2.22±1.15	2.71±1.21[c]	2.30±1.00	2.19±1.19[c]
p		0.311		0.073		**0.027*****		
Body-food choice congruence		2.37±1.02	2.20±1.24	2.42±1.05	2.18±1.17	2.81±1.16[df]	2.30±1.05[ef]	2.00±1.11[de]
p		0.240		0.088		**<0.001*****		
IES Status (ME score)	**Higher** n (%)	79 (68.1)	37 (31.9)	66 (56.9)	50 (43.1)	27 (23.3)	57 (49.1)	72 (51.8)
	Lower n (%)	73 (52.5)	66 (47.5)	62 (44.6)	77 (55.4)	19 (13.7)	32 (27.6)	48 (34.5)
	p	**0.012¥**		0.051		0.117		

Table 5. The relationship between IE and its sub-dimensions and gender, major, BMI classification and physical activity level (continue)

Intuitive Eating Scale		BMI classification				BMI (kg/m²)
		Underweight (<18.5 kg/m²)	Normal (18.5-24.9 kg/m²)	Overweight (25.0-29.9 kg/m²)	Obese (>30.0 kg/m²)	r p
Intuitive Eating total score		2.51±0.57	2.47±0.62[a]	2.78±0.55[a]	2.55±0.20	0.172 **0.006**[£]
p			**0.034*** **			
Unconditional Permission to Eat		2.89±0.71	2.89±0.69[bc]	3.14±0.43[c]	3.37±0.59[b]	0.139 **0.027**[£]
p			**0.040*** **			
Eating for Physical Rather than Emotional Reasons		2.54±0.92	2.37±0.90	2.61±0.72	2.28±0.68	0.034 0.584
p			0.344			
Reliance on Hunger and Satiety Cues		2.17±1.03	2.28±1.11	2.75±1.18	2.31±0.77	0.144 **0.022**[£]
p			0.062			
Body-food choice congruence		2.39±1.11	2.24±1.12	2.56±1.16	2.07±0.66	0.064 0.310
p			0.257			
IES Status (ME score)	**Higher** n (%)	12 (10.3)	71 (61.2)	28 (24.1)	5 (4.3)	22.85±3.51
	Lower n (%)	13 (9.4)	111 (79.9)	11 (7.9)	4 (2.9)	21.77±3.01
	p		**0.002**[¥]			**0.011****

*Independent Samples t Test **Mann-Whitney U Test ***One-way ANOVA ****Kruskal Wallis Test [£]Spearman Test [¥]Chi-square Test

IES: Intuitive Sating Scale BMI: Body Mass Index n: number of participants

[a-f]In the same column, there is a statistically significant difference between those with the same exponential letter.

Table 6. Correlation of Eating Awareness Scale and its Subscales and IES and its Subscales

	Intuitive Eating Total score	Unconditional Permission to Eat	Eating for Physical Rather than Emotional Reasons	Reliance on Hunger and Satiety Cues	Body-food choice congruence
Disinhibition	**r: 0.143 p=0.022**	**r: -0.268 p <0.001**	**r: 0.372 p <0.001**	**r: 0.012 p <0.001**	**r: 0.006 p <0.001**
Eating discipline	**r: 0.364 p <0.001**	**r: -0.334 p <0.001**	**r: 0.316 p <0.001**	**r: 0.392 p <0.001**	**r: 0.531 p <0.001**
Focusing	**r: 0.131 p=0.037**	**r: -0.239 p <0.001**	**r: 0.199 p=0.001**	r: 0.091 p=0.149	**r: 0.175 p=0.005**
Interference	r: 0.105 p=0.095	**r: -0.343 p <0.001**	**r: 0.310 p <0.001**	r: 0.030 p=0.633	r: 0.094 p=0.136
Emotional eating	**r: 0.331 p <0.001**	**r: -0.332 p <0.001**	**r: 0.565 p <0.001**	**r: 0.178 p=0.004**	**r: 0.155 p=0.013**
Control of eating	**r: 0.272 p <0.001**	r: -0.024 p=0.700	**r: 0.321 p <0.001**	r: 0.109 p=0.082	r: 0.095 p=0.131
Mindfulness	**r: 0.267 p <0.001**	r: -0.091 p=0.149	**r: 0.211 p=0.001**	**r: 0.244 p <0.001**	r: 0.122 p=0.051
MET score	**r: 0.382 p <0.001**	**r: -0.364 p <0.001**	**r: 0.579 p <0.001**	**r: 0.208 p=0.001**	**r: 0.230 p <0.001**

p: Spearman Test MET: Mindful Eating Total

DISCUSSION

In this study, it was aimed to determine the relationship between physical activity and IE and ME in university students studying in health and sports sciences. A statistically significant difference was found between the students studying in both departments in terms of age, gender, smoking consumption and physical activity level. The fact that 67.7% of the students studying in sports sciences are male and accordingly more cigarette consumption is appropriate for the literature (Çakaroğlu et al., 2020; Kuseyri, 2020; Özkan, 2018). Yılmaz et al. they showed that male students studying in sports sciences were more likely to consume cigarettes and alcohol, but there was no statistical difference (Yılmaz et al., 2007). In another study conducted with university students, it was determined that male students consumed more cigarettes ($p<0.05$) due to a greater sense of freedom and less family pressure (Kılıç et al., 2018). When the physical activity levels were examined in our study, it was observed that the students who were inactive or did insufficient physical activity were more in health sciences, those who had sufficient level in sports sciences ($p<0.05$). Similarly, studies indicating that sport sciences students have higher physical activity levels support our results (Çakaroğlu et al., 2020; Şahin et al., 2017).

When the main meal consumption was evaluated, 48.4% of the students in health sciences reported that they consumed two meals, and 60.6% of those in sports sciences consumed three meals. Although there is no statistically significant difference, the fact that breakfast and lunch are skipped more frequently in both departments shows that university students can skip these meals due to reasons such as being away from their families, being late for classes, economic reasons and spending more time outside. When the reasons for dieting of the students in both departments were questioned, it was determined that they dieted more for aesthetic appearance rather than being healthy ($p<0.05$). It is known that women experience more aesthetic anxiety than men and therefore they diet (Özkan, 2018). It is thought that this situation will put more pressure on women day by day, especially with the effect of social media.

It is possible to choose healthier foods depending on the increased sensitivity to the foods consumed because of the ME behavior. Thus, it is known that ME has an effective role in providing weight control (Kose et al., 2017; Özkan, 2018). When the participants' ME scores were evaluated according to gender, it was determined that there was no difference in the MET score, and the studies carried out support our results (Karataş & Müftüoğlu, 2021; Köse, 2017; Kuseyri, 2020; Özkan & Bilici, 2021; Serban et al., 2022). In addition, ED and EE scores were found to be significantly higher in women, and EC scores were significantly higher in men (Table 4). In a study conducted in Romania, it was determined that the EE score was significantly higher in women which is supporting our results. However, since the sub-dimensions of ME were different in the Romanian version of the ME scale, all of our sub-dimensions could not be compared (Serban et al., 2022). Since each society has different characteristics, different sub-dimensions and accordingly different results

may emerge as a result of the validity and reliability studies of the scales. Similar to our study, in a study conducted with university students, which showed that EE score was significantly higher in women, unlike us, the disinhibition score was higher in women and the focusing score was higher in men ($p<0.05$) (Çakaroğlu et al., 2020). However, in another study conducted in adults (19-45 years old) in Turkey, the ED score was higher in women and the EE score was higher in men, which is different from the results of our study (Özkan & Bilici, 2021). Since the age range in this study is different from ours, different results are expected. Therefore, in order to better observe these differences, cross-sectional or intervention studies should be conducted in different populations.

It was determined that the ED score in health science students and the EC score in sports science students were significantly higher. In a study involving a population similar to our study, EE and interference scores were higher in health science students, and mindfullness, ED and focusing scores were higher in sports science students ($p<0.05$) (Çakaroğlu et al., 2020).

Although there was no significant difference between MET and sub-dimension scores according to BMI classification in our study, it was observed that MET, disinhibition, ED, focusing, interference, and EE scores were lowest in obese individuals and highest in those with a underweight or normal BMI. In a study conducted with university students, it was determined that MET, disinhibition, and EC scores were highest in thin individuals and lowest in obese individuals ($p<0.05$), that all are supporting our results (Kuseyri, 2020). In addition, it was determined that BMI and interference score were negatively correlated, while EC score was positively correlated. However, it was shown in a study (Özkan & Bilici, 2021) that MET, disinhibition, EE and EC scores in women and EC scores in men were negatively correlated with BMI ($p<0.05$). In studies conducted with university students, it was determined that BMI decreased as EC and MET scores increased ($p<0.05$, $p>0.05$, respectively) (Karataş & Müftüoğlu, 2021; Köse, 2017; Kuseyri, 2020). Thus, depending on the increase in mindful eating, it is expected that BMI will decrease as a result of preferring to eat healthier foods more accurately.

When the relationship between physical activity levels and ME was examined in our study, it was determined that MET score ($p>0.05$) and ED and focusing scores ($p<0.05$) were highest in inactive individuals, and EC score was at the highest level in those with sufficient physical activity level. In Özmumcu's (2019) study with university staff, it was shown that as the MET score increases, the level of physical activity also increases (Özmumcu, 2019). Although this result that we obtained in university students does not comply with the literature, it is in question that it can not be adapted to the society in general, since the majority of those who do enough physical activity are educated in sports sciences.

Intuitive eating, which includes consuming food according to the internal stimuli of hunger and satiety, focuses on the physical hunger of the body in general, and overeating due to emotional reasons is prevented. It is negatively associated with BMI, especially in early adolescents, young adults, and college students (Ruzanska & Warschburger, 2019). Accordingly, as the IE tendency increases, it is seen that BMI decreases due to decreasing obsessive thoughts and social physical anxiety (Akırmak et al., 2021; Altay et al., 2022; Atalay, 2017; Ateş, 2021; Braun et al., 2022; Horwath et al., 2019; Kuseyri, 2020; Özkan, 2018; Özkan & Bilici, 2018, 2021; Ruzanska & Warschburger, 2019). However, in our study, IET, UPE, and RHSC scores showed a statistically significant positive correlation, although weakly, with BMI (Table 5). It was determined that the lowest IET score was in the normal BMI range, and the highest UPE score was in the obese individuals ($p<0.05$). In addition, it was observed that BMI was statistically higher in those who showed IE behavior. Studies have shown that there is a significant positive relationship (Özkan, 2018) between UPE score and BMI, and a negative relationship between UPE and diet quality (Horwath et al., 2019). However, what is observed in the literature contradicts the behavior of avoiding overeating, depending on the idea that the person can eat the food they want as soon as they feel physical hunger (Ateş, 2021). This situation shows that university students can not distinguish physical hunger from emotional hunger, or they prefer high energy foods more. In addition, since our study was limited to university students with a mean age of 20.34±2.06 years, significant results were revealed for this population. Only the UPE score was significantly higher for students studying sports sciences. However, although IET, FRE rather than emotional reasons, RHSC and BFCC scores were higher in health sciences students, no difference was found between departments. Sports science students are expected to be more physically active and accordingly to pay more attention to their appearance. For this, it is seen that overeating is avoided and can better focus on hunger signals.

In our study, the scores of the IET and its sub-dimensions were higher in women than in men ($p<0.05$ and $p>0.05$, respectively). This result shows that women have more IE behavior. However, it was determined that the scores of men were higher in studies (Horwath et al., 2019; Özkan, 2018; Özkan & Bilici, 2021; Ruzanska & Warschburger, 2017). Further studies with equal gender distribution need to be planned to clarify whether this differential result represents true gender differences.

People who can not accurately assess their hunger and satiety signals have difficulty in limiting their food consumption.

Therefore, EE behavior is seen in these people with more weight gain and accordingly the desire to diet (Atalay, 2017). In addition, it has been shown that people with a high BFCC score, which is associated with consuming delicious and healthy foods, have an increased level of physical activity and are healthier (Horwath et al., 2019; Ruzanska & Warschburger, 2019). However, when the physical activity levels of the participants were examined, it was determined that the IET, RHSC and BFCC scores of those who reported being inactive were significantly higher. This result contradicts the knowledge that people with IE tendencies increase their physical activity levels due to their desire to be healthier (Ateş, 2021). Therefore, these results should be re-evaluated by planning new studies with a wider age range and suitable for the general population.

In our study, it was shown that there is a significant positive relationship between mindful eating and IET scores, and other studies support this result (Kuseyri, 2020; Özkan & Bilici, 2021). When the relationship between the scores of the sub-dimensions of both scales was examined, a significant negative relationship (except for the EC and mindfulness scores; $p>0.05$) was observed only between the scores of the UPE score of ME and the sub-dimensions. In the study conducted by Kuseyri (2020), it was found that there was a negative correlation between UPE and all scores of mindful eating, which was in line with our results (Kuseyri, 2020).

Our study has some limitations. Since there is a difference between the numbers of female and male participants, this situation complicates the evaluations between both genders. Also, since only university students participated in the study, it is difficult to apply our results to the general population.

While there are studies in the literature showing the relationship between ME and IET scores, there are not many studies showing the relationship between all sub-dimensions of both scales. This is the strongest part of our study.

CONCLUSION

In conclusion, ME and IE appear to be positively related to each other. For young individuals to be healthier, the importance of adequate and balanced nutrition and being physically active is understood. However, there are inconsistencies in some results since the study was conducted with a limited group. Therefore, it is necessary to carry out more comprehensive studies that can reflect the general population.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

Conceptualization, ZGY, FMB and SG; methodology, ZGY and SG; data collection and analysis, FMB and SG; writing-original draft preparation, FMB and SG; writing-review and editing, ZGY, FMB and SG. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval

Ethics committee approval was obtained from the Ethics Committee of İstanbul Rumeli University with the desicion no 02 dated 20.05.2022.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Environmentally friendly rapid green synthesis of SeNPs using grapefruit (*Citrus paradisi*) leaves extract, and their antimicrobial potential

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Abstract

The utilisation of plant biomass in the production of nanoparticles is gaining popularity because of its associated benefits. Selenium nanoparticles (SeNPs) are highly valuable due to their involvement in numerous biological functions. In this study, SeNPs were rapidly synthesized using the environmentally friendly and low-cost green synthesis approach using *Citrus paradisi* (Grapefruit) leaves extract. The synthesized SeNPs were characterized using TEM, AFM, DLS, UV-vis, XRD, and EDX data. The data revealed that SeNPs had a spherical and uniform shape, with an average size of 45 nm, a surface charge of -20.54 mV, and a peak absorbance wavelength of 326 nm. The inhibitory impact of SeNPs on harmful strains and cancer cells was investigated using the microdilution method. The development of bacteria was effectively inhibited at concentrations ranging from 4 to 16 µg ml^{-1}.

Keywords: Antimicrobial, Anticancer, Extract, *Citrus paradisi*, Green synthesis, SeNPs

Type: Research Article
Subject: Plant Biotechnology

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INTRODUCTION

Nanotechnology is a crucial interdisciplinary field that encompasses various scientific and technological fields including biomedicine, agriculture, pharmaceuticals, environmental science, chemical engineering, textile technology, electronic physics, and information technology. In this sector, especially the vast diversity of synthesis methods of nanoparticles and some advantages such as environmental sensitivity and inexpensive cost are among the most attractive features of this field (Kumar et al., 2017; Alagesan & Venugopal, 2019; Hashem & Salem, 2022)this study aimed to biosynthesize SeNPs using aqueous extract of Urtica dioica leaf through green and ecofriendly method. Moreover to fully characterize SeNPs using different techniques, and to evaluate it for antimicrobial activity as well as anticancer activity. Main Methods and Major Results: SeNPs were biosynthesis using aqueous leaf extract of U. dioica (stinging nettle. The benefits of synthesizing nanoparticles using plant biomass include ease of method, cost-effectiveness, environmental friendliness, biocompatibility, high product yield, easy resource accessibility, and increased appeal for research in this area (Anu et al., 2017; Chellapandian et al., 2019; Gunti et al., 2019; Alipour et al., 2021; Hashem & Salem, 2022; Mariadoss et al., 2022)phytofabricated selenium nanoparticles (PF-SeNPs. Extracts from plant sources contain bioactive components like alcohol, phenolic compounds, and flavonoids, which have active groups that contribute to reduction, stability, and coating properties (Alipour et al., 2021; Perumal et al., 2021; Vu et al., 2022). Nanoparticles produced by plants are valuable products used in several fields, particularly in biomedical applications, due to their biocompatible architectures (Ndwandwe et al., 2021; Perumal et al., 2021; Adibian et al., 2022).

Selenium (Se) is a vital element involved in antioxidant systems and several biological activities including catalase and superoxide dismutase. SeNPs have a competitive edge over selenium salts as a selenium source due to their reduced toxicity compared to the numerous types of selenium salts present in nature. Selenium (Se) is a vital element involved in antioxidant systems and several biological activities including catalase and superoxide dismutase. SeNPs have a competitive edge over selenium salts as a selenium source due to their reduced toxicity compared to the numerous types of selenium salts present in natüre (Ranjitha & Rai, 2021; Chen et al., 2022)synthesis of selenium at the nanoscale level is important. Selenium nanoparticles (SeNPs. Alcohol/phenol amine groups of phytochemicals in plant extracts are bioactive organic molecules that reduce SeO_3^{-2} to form, stabilize, and coat SeNPs. (Anu et al., 2017; Ezhuthupurakkal et al., 2017; Alagesan & Venugopal, 2019; Cittrarasu et al., 2021; Pon Matheswari et al., 2022).

Citrus paradisi, a member of the *Rutaceae* family, is a perennial species. This plant contains narirutin and naringin, flavanones, ferulic and p-coumaric acids, vanillic, gallic acids phenolic acids, carotenoids, flavonoids, terpenoids, essential oils, and ascorbic acid, which have numerous health benefits due to their pharmacologically active properties (Gupta et al., 2010; Anupama Prasad et al., 2023; Kumar et al., 2020)

The work aims to synthesize selenium nanoparticles using an extract from *Citrus paradisi* plant leaves in an environmentally friendly, cost-effective, and simple approach. The properties of the nanoparticles were characterised, and their antibacterial activities were investigated.

MATERIAL AND METHOD

Plant extract and sodium selenite solution preparation

Citrus paradisi plant leaves were collected at the end of the season in Köyceğiz, Muğla, Turkey. After rinsing with tap water and then distilled water, it was dried on blotting paper at room temperature. 10 grams of desiccated leaves were weighed, combined with 100 milliliters of distilled water, and cooked using a kettle. After filtration, the cooled extract was prepared for synthesis.

A 20 mM solution was generated using 99% pure Sigma-aldrich Na_2SeO_3 (sodium selenite) salt for the purpose of generating SeNPs using green synthesis.

Green Synthesis of SeNPs by Cp Extract

A solution of sodium selenite and Cp extract was mixed in a 5:2 ratio and the reaction took place at 55 °C and pH 6.3 for two hours. Samples were collected periodically from the synthesis medium based on color changes. The samples were diluted by a factor of 10 before being measured. The samples were analyzed for the maximum wavelength absorbance data to monitor the creation of SeNPs by assessing the color change intensity. This was done using a Perkin Elmer One UV-visible spectrophotometer (UV-vis) with measurements made in the range of 300-800 nm.

FTIR Spectra of Cp Extract

A Perkin Elmer One Fourier Transformation Infrared Spectroscopy (FTIR) was utilized to analyze the bioactive functional groups of phytochemicals involved in the bioreduction, coating, and stability of SeNPs. The frequency changes in the FTIR spectra, recorded between 4000-500 cm^{-1}, of both the Cp extract and the liquid media obtained after synthesis were informative.

Characterization of SeNPs

Maximum wavelength absorbance values were collected using UV-vis scanning to detect the production of synthesized SeNPs(. The morphological structures of the synthesized SeNPs were analyzed using a Jeol Jem 1010 Transmission Electron Microscopy (TEM) and a Park System XE-100 Atomic Power Microscopy (AFM). SeNPs' hydrodynamic size distributions were estimated using density-dependent analysis utilising a Marven Dynamic Light Scattering Spectrometer (DLS). The crystal patterns and sizes of the synthesized particles were evaluated using data obtained from a Rigaku Miniflex 600 model. The Debye-Sherrer equation in formula (1) was used to calculate the crystal nanosizes based on the FWHM values obtained from the data (Shirmehenji et al., 2021; Baran et al., 2022;)silver (4.12%

$$D = k\lambda / (\beta \cos\theta) \quad (1)$$

Regarding inequality: D represents particle size, β is half of the FWHM value at the peak, k is a shape factor (0.9), λ is the wavelength of X-ray, and θ is the diffraction angle.

The elemental composition of particles produced using Cp was analyzed using an FEI PQuanta 250 FEG Electron Disperse X-ray (EDX) to measure values. Surface charge distributions impacting stability were studied using density-dependent zeta potential data collected with a Malvern device (DLS).

Examination of the Antibacterial Potential of SeNPs on Pathogenic Strains

Antibacterial properties of SeNPs derived from an extract of Cp leaves were investigated through experimental experiments applying the microdilution method on pathogenic microorganisms. Through the applications, Minimum Inhibition Concentrations (MIC) that effectively suppressed growth were identified. Gram-positive *Bacillus subtilis* ATCC 11774 and gram-negative *Pseudomonas aeruginosa* ATCC27833 bacteria were cultivated and utilised in suitable conditions in the experimental study. Microorganism solutions were created based on the McFarland standard 0.5 turbidity criteria (Zeraatkar et al., 2022). The suitable medium was added to 96-well microplates during the experiment. Microdilution was conducted by adding different amounts of SeNPs, $NaSeO_3$ solution, and specific antibiotics for each strain (colistin for gram-negative and vancomycin for gram-positive bacteria) to the microplates. Microorganisms grown at Macfarland 0.5 turbidity were transferred to microplate wells and cultured at 37 °C for 24 hours to study antimicrobial interaction.

RESULTS AND DISCUSSION

FTIR Spectra of SeNPs and Cp extract

FTIR spectra were utilised to identify the functional groups of the bioactive components in the Cp extract and their role in the bioreduction, coating, and stability of SeNPs. The FTIR spectra of the final synthesis liquid arising from the reaction were analyzed to assess it. The shifts in frequency seen at 3320, 1636, 1425, 1261, 1081, and 695 cm^{-1} in Figure 1's spectra indicate that the groups associated with these locations are linked to bioreduction, stability, and coating. The events include stretching of -OH and -COOH groups at 3320 cm^{-1} due to alcohol and carboxyl groups, as well as N-H stretching and vibration at 1636-1425 cm^{-1} related to amide groups. The peak at 1425 cm^{-1} corresponds to C—O—H stretching, while the peaks at 1261 cm^{-1} and 1081 cm^{-1} are attributed to —S and vinyl group stretching, respectively. The shift at 695 cm-1 is due to —S stretching (Ezhuthupurakkal et al., 2017; Hatzikioseyian et al., 2022; Ramamurthy et al., 2013)while the morphology and the properties of the adsorption beads are studied by SEM, FTIR and rheological analysis. Langmuir isotherm describes the adsorption equilibrium with maximum loading Q0 29.411 mg Ni/g SeNPs and 0.651 mg Ni/g alginate-SeNPs. The adsorption of nickel is fast and equilibrium is attained within one hour. Continuous flow experiments in packed beds reveal early elution patterns and non-symmetric sigmoidal profiles due to the reduced adsorption capacity of the immobilized SeNPs, the short depth of the beds and the apparent non-uniform flow pattern. The Bohart-Adams equation fits adequately the main sigmoidal part of the breakthrough curves, however fails to predict the early breakthrough data. By using the advection–dispersion-reaction equation (ADR.

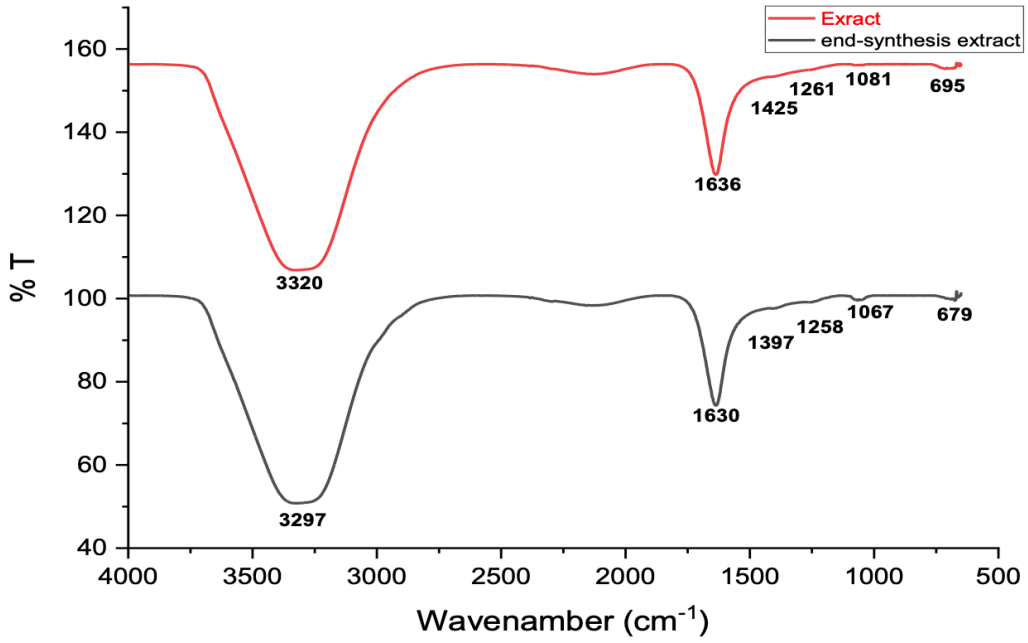

Figure 1. FTIR spectra of Cp extract and liquids obtained as a result of synthesis.

Characterization of SeNPs

UV-vis Data

After 30 minutes of stirring a solution containing Cp and sodium selenite, a red color shift was seen as a result of the reaction. The color change persisted until the 50-minute mark. The UV-vis analysis (Figure 1) determined the maximum absorbance values of 327.8 nm within the wavelength range of 290-400 nm, indicating the synthesis of SeNPs through bioreduction with Cp. The color change occurred because of the creation of SeNPs by reduction and the vibrations on the plasma surface, known as surface plasma resonance (SPR). This was demonstrated by the peak absorbances recorded in UV-vis spectroscopy (Ramamurthy et al., 2013; Perumal et al., 2021; Fadl et al., 2022; Puri & Patil, 2022; Shin et al., 2022; Vundela et al., 2022)respectively, which suggested that C. papaya fruit extract could be a competitive reducing and stabilizing agent during phytofabrication of nanoparticles. UV–Vis and FTIR spectroscopy showed the formation of SeNPs from sodium selenite, which could be related to the reducing and stabilizing activities of C. papaya fruit extract. The SeNPs were found to be stable with a Zeta potential of −32 mV. The average hydrodynamic size of SeNPs was found as 159 nm by dynamic light scattering. The SeNPs showed a broader XRD pattern with no sharp Bragg's peaks and found to be amorphous. SEM showed that SeNPs were spherical in shape and EDX pattern showed that SeNPs were made up of Se (71.81%.

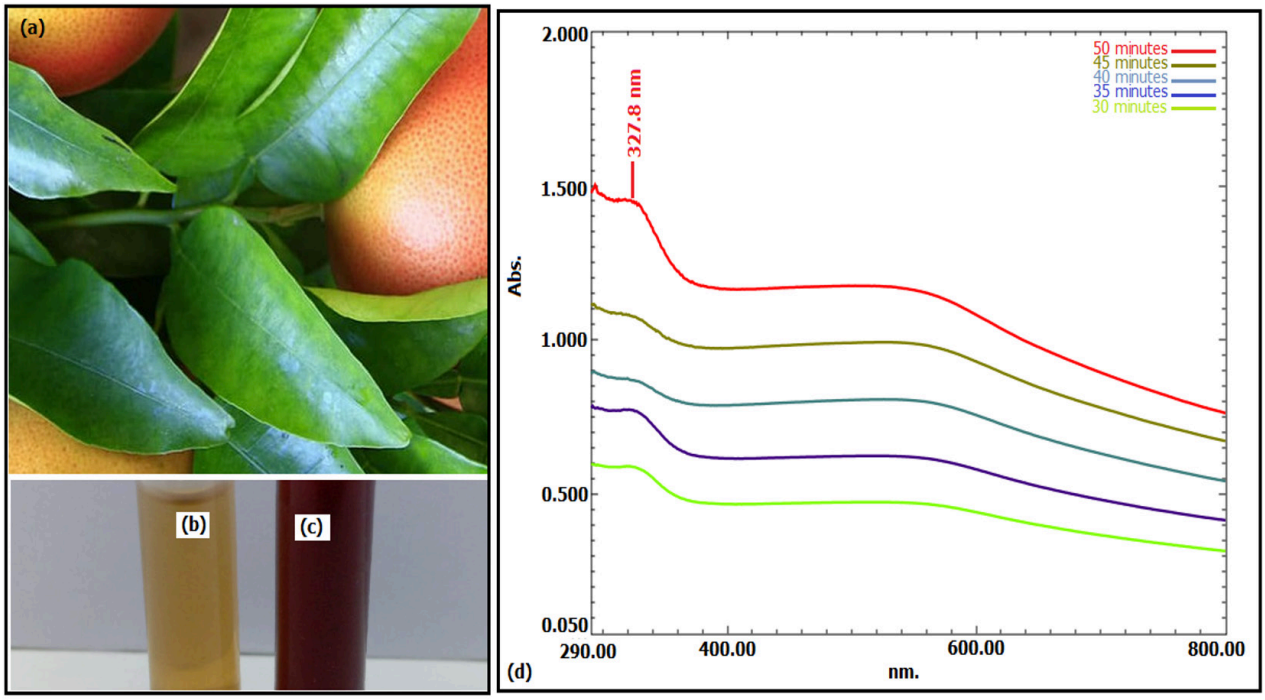

Figure 1. *Citrus paradisi* leaves (a), leaf extract (b), liquid containing colloidal SeNPs at the end of synthesis **(c)**, and UV-vis analysis data showing the synthesis of SeNPs and their presence along their maximum wavelength absorbance bands (327.8) (d)

XRD Data of SeNPs

Selenium nanoparticles were dropped onto a powdered carbon adhesive tape. Then, it was fed into the device and phase analysis was performed.The XRD data was used to analyze the crystal structures of particles synthesized using Cp (20-80 theta). The crystal nanosizes were determined by applying the Debye-Sherrer equation to the FWHM values in the data. Expansions were observed in the Bragg angles of the data obtained at 2-theta on the following planes: (102), (133), (191), (202), (210), (244), (264), (293), and (310). The full width at half maximum (FWHM) values for these angles are 23.50, 29.70, 41.32, 43.56, 45.58, 51.07, 55.62, 61.06, and 65.06, as shown in Figure 2. The results indicated that the crystal structures of the produced SeNPs matched the values specified in the Joint Committee on Powder Diffraction Standards (JCPDS file no 06–0362). Additional modest signals detected in the data were associated with different biomolecules found in the extract. (Fouda et al., 2022). The crystal size was determined to be 42.0 nanometers using the Debye-Scherrer formula. The Debye-Sherrer equation was used to determine that the average size of SeNPs in a study including *Ficus benghalensis* leaf extract ranged from 45 to 95 nm. (Tripathi et al., 2020). SeNPs were determined to have a size of 41 nm in a different green synthesis work (Zeraatkar et al., 2022).

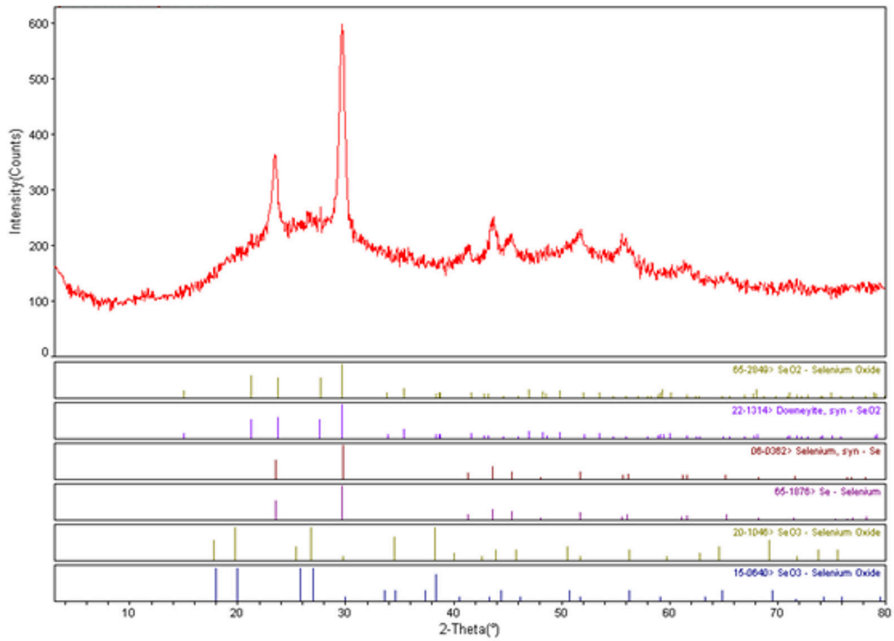

Figure 2. Crystallographic data of SeNPs produced using a certain method was analyzed using X-ray diffraction at a certain angle and reflected on a specific plane.

EDX Profiles of Synthesized Particles

Figure 3 displays prominent peaks in the element profile of the particles collected using Cp, showing a significant concentration of the Se element, attributed to SeNPs (Chitti Kondal Rao et al., 2022). The low signals of oxygen and carbon in the graph were caused by the bioactive components present in the structures of the phytochemicals in the extract. Bioactive compounds are structures involved in coating and stabilising SeNPs (Chitti Kondal Rao et al., 2022; Fouda et al., 2022; Hashem et al., 2022).

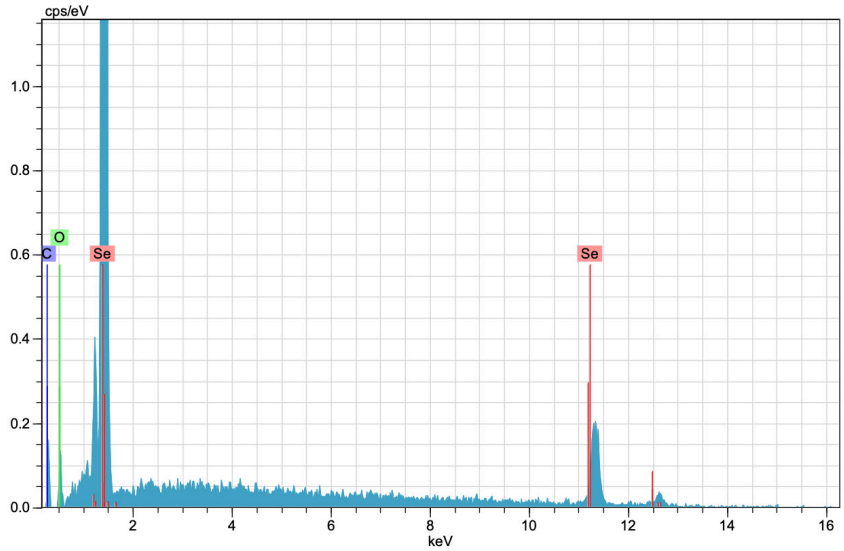

Figure 3. EDX profiles displaying the elemental makeup of particles produced with Cp extract

Morphologies of Synthesized SeNPs

The morphological appearance of SeNPs synthesized using Cp extract source was determined using TEM, AFM, and DLS data provided in Figure 4. The images showed that the SeNPs had a spherical shape, no clustering, and an average size of 45 nm, with some being smaller than 50 nm (Saranya et al., 2022). The green synthesis investigation reported that the synthesized SeNPs exhibited a spherical shape and had an average size of 156.93 nm according to DLS results (Saravanakumar et al., 2022)the SeNPs were synthesized using Trichoderma extracts (TE. SeNPs with a semi-spherical

shape were observed in a green synthesis investigation, with a size distribution of 37.5 nm (Alizadeh et al., 2023). The study reported that SeNPs produced using the extract had a spherical shape and were evenly distributed in the AFM image (Kazemi et al., 2021).

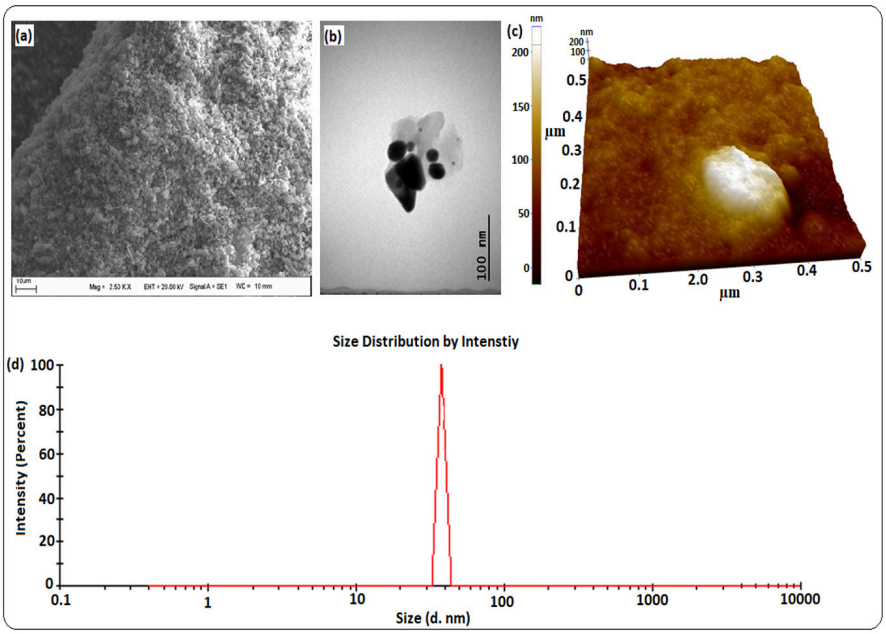

Figure 4. Morphological features of SeNPs produced using Cp extract were analyzed by SEM (a), TEM (b), AFM (c), and DLS (d) techniques

Surface Structures of SeNPs and Mass Loss Points Due to Temperature Change

The density-dependent charge distribution of SeNPs synthesized using Cp was observed to be -20.54 mV, as depicted in Figure 5. The EDX profile in Figure 3 confirmed the existence of bioactive components including carbon and oxygen, aligning with the surface charge distribution findings in Figure 5. The nanoparticles negative surface charge is a crucial characteristic that enhances stability and biocompatibility, which is beneficial for biomedical uses. Having two distinct charges of nanoparticles in the same setting leads to outcomes like aggregation and fluctuation, which have a detrimental impact on stability. SeNPs, having positive qualities in the physiological environment and a negative surface charge distribution, readily interact with biological structures that have these properties in the same environment (Chitti Kondal Rao et al., 2022; Gharbavi et al., 2022; Hatami et al., 2020; Mariadoss et al., 2022; Saravanakumar et al., 2022; Chen et al., 2023;)Bacillus cereus, Salmonella enterica, and Escherichia coli. Zeta potential data from a green synthesis investigation revealed that SeNPs produced using fruit extract had a surface charge of -12.44 mV (Gharbavi et al., 2022).

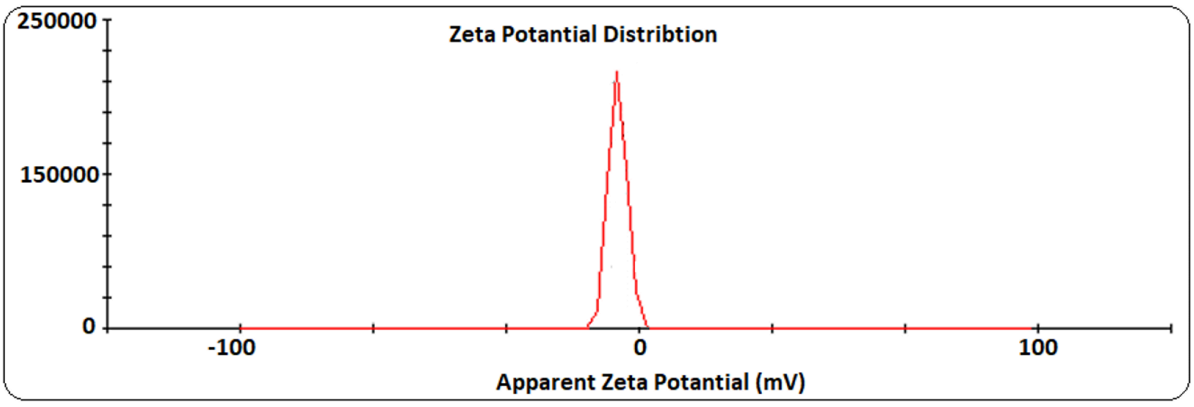

Figure 5. Surface charge distributions of SeNPs synthesized using Cp were graphed based on zeta potential analysis results.

The TGA-DT data presented in Figure 6 was utilised to assess the mass losses of the SeNPs due to temperature variations during the synthesis process. Mass losses of 22.64% and 49.37% were recorded at two distinct temperature ranges. The bulk of the losses occurred due to the degradation of SeNPs. The significant mass reductions were mostly caused by the bioactive elements responsible for the stability of the SeNPs (Baran., 2019; Padalia & Chanda, 2021; Alizadeh et al., 2023; Younas et al., 2023). Furthermore, the data obtained from XRD, EDX, and Zeta potential analyzes in Figure 2, Figure 3, and Figure 5 showed the existence of bioactive components.

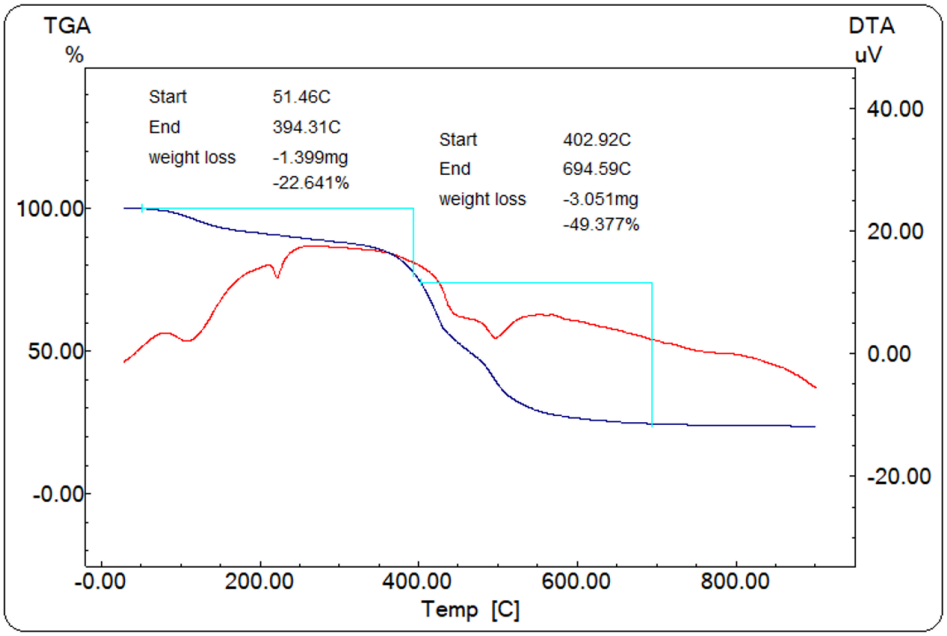

Figure 6. Temperature of mass loss points were determined using TGA-DT for SeNPs synthesized using the Cp extract

Antibacterial Effects of SeNPs

Several characteristics of nanoparticles, including shape, concentration, size, surface charge, and contact duration, play a crucial role in hazardous effects. Nanoparticles interact with cells in physiological contexts due to features such as electrostatic attraction force and hydrophobic contact (Doan et al., 2020; Mehravani et al., 2021; Remya et al., 2015; Webster, 2020). Pores of big circulatory arteries are particularly present in tumour tissues. These regions facilitate the easy passage of things like nutrients and oxygen (Chen et al., 2021; Hosny et al., 2021). Certain biomolecules exhibit an affinity for interacting with nanoparticles. Substances negatively impact the activities of biomolecules involved in metabolic processes as DNA, proteins, and enzymes (Rolim et al., 2019; Barabadi et al., 2020; Donga et al., 2020; Hosny et al., 2022; Webster, 2020).

The emergence of antibiotic resistance in microorganisms hinders the effectiveness of combating infections and leads to more severe issues. Research efforts to find alternative antimicrobial drugs to address this issue continue to be crucial and relevant (Silva et al., 2021; Wongpreecha et al., 2018; Das et al., 2022). SeNPs have the potential to address antibiotic resistance in addition to their effectiveness in other biological processes. Stable SeNPs were quickly synthesized using the Cp extract to examine their antibacterial effects on pathogenic strains by the microdilution method, in order to aid in the hunt for antibiotic agents. The synthesized SeNPs were found to be effective against microorganisms with MICs of 6.00 and 12.00 µg mL^{-1}, as shown in Figure 8 and Table 2. SeNPs exhibited the most favorable impact on Gram-negative Pseudomonas aeruginosa. The impact occurred at a concentration slightly below the Minimum Inhibitory Concentration (MIC) of the Na2SeO3 solution, which was almost equivalent to the concentration of antibiotics utilised. Properties like form, surface charge, size, and contact duration significantly influence the antibacterial capabilities of nanoparticles. Microorganisms and nanoparticles interact in the physiological environment through processes such electrostatic attraction, adsorption, and hydrophobic contact (Ahmed et al., 2016; Ferreyra Maillard et al., 2018; Babu et al., 2020; Mariadoss et al., 2022). Due to this interaction, the morphology of the cell wall and membrane is adversely altered. Furthermore, metabolic activities including energy metabolism in the membrane structure are disturbed, affecting their shape and function. Important biological molecules like DNA and RNA, which have an attraction to these substances, experience dysfunction. Due to these adverse impacts, bacteria are unable to perform their essential functions. Consequently, the mortality of microorganisms occurs due to these consequences (Cui et al.,

2012; Ahmed et al., 2016; Ezhuthupurakkal et al., 2017; Jha et al., 2017; Donga et al., 2020).

The study on green synthesis found that the MIC of SeNPs against Pseudomonas aeruginosa and Staphylococcus aureus bacteria was 100 µg mL^{-1} (Srivastava & Mukhopadhyay, 2015). It was shown that the effective MIC value of SeNPs synthetized through Nepeta extract on P. Aeruginosa was 4 µg mL-1 (Zeraatkar et al., 2022).

Table 2. Effective MIC values of SeNPs synthesized by Cp extract, Na_2SeO_3 solution, and antibiotics on the suppression of bacterial growth.

Organism		Antibiotic* µg $m^{L-}1$	Na_2SeO_3 Solution µg mL^{-1}	SeNPs µg mL^{-1}
Gr (-)	*P. aeruginosa*	4.00	8.00	6.00
Gr (+)	*B. subtilis*	8.00	16.00	12.00

* Colistin for *P. Aeruginosa*, vancomycin for *B. Subtilis.*

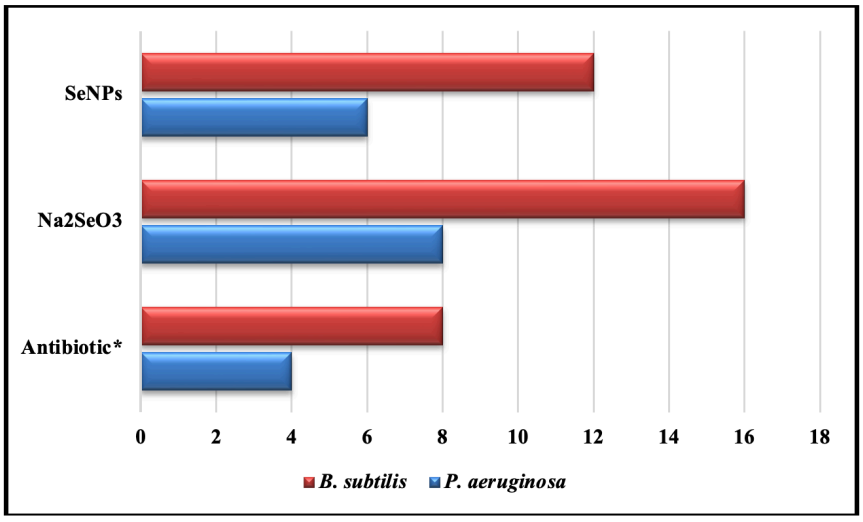

Figure 8. MIC values of SeNPs synthesized by Cp, antibiotics and sodium selenite solution suppressing the growth of bacteria.

CONCLUSION

SeNPs are highly precious materials. Acquiring these elements using green synthesis methods is crucial for ensuring biocompatibility. SeNPs were synthesized using green synthesis using Cp extract in an environmentally friendly, inexpensive, and rapid process. The SeNPs were analyzed using TEM, AFM, DLS, UV-vis, XRD, and EDX data to determine their characteristics. The SeNPs were found to be spherical and uniform, with an average size of 45 nm, a surface charge of -20.54 mV, and a maximum absorbance wavelength of 327.8 nm. SeNPs' suppressive effects on pathogenic strains were investigated via the microdilution method. Concentrations ranging from 4 to 16 µg ml^{-1} were effective in inhibiting bacterial growth.

SeNPs produced with Cp will greatly enhance medical applications as antibacterial agents by streamlining the synthesis process, given their significance in several utilisation areas.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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RESEARCH ARTICLE

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Innovation in the dairy industry: forecasting cow cheese production with machine learning and deep learning models

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Abstract

This study focuses on the use of deep learning and machine learning models to forecast cow cheese production in Turkey. In particular, our research utilizes the LSTM (long short-term memory) model to forecast cow cheese production for the next 12 months by extensively utilizing deep learning and machine learning techniques that have not been applied in this field before. In addition to LSTM, models such as GRU (Gated Recurrent Unit), MLP (Multi-Layer Perceptron), SVR (Support Vector Regression), and KNN (K-Nearest Neighbors) were also tested, and their performances were compared using RMSE (Root Mean Square Error), MSE (Mean Squared Error), MAE (Mean Absolute Error), MAPE (Mean Absolute Percentage Error), and (Coefficient of Determination) metrics. The findings revealed that the LSTM model performed significantly better than the other models in terms of RMSE, MSE, MAE, and MAPE values. This result indicates that the LSTM model provides high accuracy and reliability in forecasting cow cheese production. This achievement of the model offers important applications in areas such as supply chain management, inventory optimization, and demand forecasting in the dairy industry.

Keywords: Deep Learning, Machine Learning, LSTM, Cow Cheese Production, Futurue Prediction

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INTRODUCTION

With the evolution of world conditions, traditional or random eating habits have been replaced by a regular and balanced diet, especially in many developed societies. This means that the focus is on getting the required amounts of energy and nutrients needed by the human body every day. Milk and dairy products, which are vital for the body, are one of the foods that should be consumed at every stage of life, from infancy to old age. Milk and dairy products can be classified into various types. However, when milk and dairy products are mentioned, products such as cheese, yogurt, kefir, buttermilk, butter, milk powder, and cream come to mind (Kahraman, 2012: 49).

The long-term preservation of milk by turning it into cheese through fermentation is a method dating back thousands of years. Today, no matter how industrially advanced cheese production has become, the basic stages of cheesemaking are almost unchanged. However, the variety of cheeses is due to a combination of factors. For example, the type of milk used, the coagulation method, whether the milk is pasteurized or not, fat content, texture, salt content, additives, and ripening time are all important in creating this diversity. Depending on each country's culture and level of development, it offers a wide variety of cheeses. In the case of Turkey, feta cheese, cheddar cheese, and tulum cheese are widely consumed and are accompanied by local products such as curd, cottage cheese,

tongue cheese, Circassian cheese, and herbed cheese (Durlu-Özkaya and Gün, 2007: 488).

Livestock and animal products have become an economic industry and an integral part of economic life. This development emphasizes the importance of recognizing animal husbandry as a strategic sector at the national level. Products of animal origin play an important role in the human diet. They are unique sources of animal protein, containing eight essential amino acids that support the body's health, bone growth, and mental development. It is recommended for humans to have a daily intake of 1 gram of protein per kilogram of body weight, of which approximately 42% should be of animal origin. The protein ratio in animal products is approximately 3-4% in products such as milk (Yıldırım and Altunç, 2020: 138).

Turkey is a country with seas around it such as the Mediterranean, the Black Sea, and the Aegean Sea, where every season is distinctly experienced and where different cultures have met throughout history, with rich vegetation and a strategic location at the intersection of the continents of Asia, Europe, and Africa. These characteristics have undoubtedly provided Turkish cuisine with a wide range of products and influenced cheese diversity. Each region of Turkey is home to its own unique classical and traditional cheese production. This diversity has created a rich variety of cheeses in Turkish cuisine. From the easternmost part of Turkey to the westernmost part, from the north to the south, and even in settlements close to each other, there are similar but different types of cheese (Güllü, 2022: 48). Therefore, cheese is a common foodstuff in Turkey, and estimating production quantities plays a critical role in guiding consumption patterns and food security.

While cow's milk is generally used in cheese and yogurt production in Turkey, sheep, goat, and buffalo milk and their powders are also used in cheese and yogurt production as different milk types. In addition, products containing live microorganisms other than cheese and yogurt, especially fermented milk products such as kefir, are widely produced (Güllü, 2022: 48). Therefore, cheese production in Turkey is considered an important part of the livestock sector and contributes to the country's rural economy. At the same time, Turkey is an important exporter of cheese and dairy products. Therefore, accurately estimating production quantities can influence the foreign trade balance and export strategies. To summarize, cheese production forecasts are of great importance both economically and nutritionally. Especially in countries like Turkey, where agriculture and animal husbandry are the mainstays, these forecasts are seen as an important tool for strategic planning, economic performance, and food security.

Cow cheese production forecasting is a complex forecasting problem with multiple independent variables and a large number of data points. Therefore, deep learning and machine learning methods can be ideal tools to manage this complexity (Bulut, 2024). On the other hand, these predictions require accurate and precise results. Deep learning and machine learning models often have the potential to provide high precision and accuracy, which can improve the reliability of forecasts. Deep learning models are capable of learning complex relationships and can recognize unique patterns using large amounts of data (Şimşek, 2024). This can be advantageous for improving cheese production forecasts. In summary, benchmarking deep learning and machine learning methods is becoming a necessity to obtain more accurate, reliable, and precise predictions. Determining under which conditions these methods perform best can improve the quality of predictions and provide valuable insights. Comparing the performance of multiple models provides the opportunity to make the best prediction.

This study aims to forecast monthly cow cheese production from October 2023 to September 2024. For this purpose, deep learning and machine learning models such as GRU, LSTM, kNN, SVR, and MLP are trained using cow cheese production data from past periods and other independent variables. Then, the model with the best forecasting performance was selected, and future forecasts for cow cheese production were made. Such studies are expected to be of great importance and provide valuable results for dairy producers and other relevant stakeholders. In the rest of the paper, the literature focusing on the forecasting of agricultural and livestock products is reviewed. Then, the methodology of the study is explained, and a conclusion section is presented based on the findings.

Although there are many studies in the Turkish literature on the forecasting of milk, dairy products, livestock, and other agricultural products, there are no studies focusing on the forecasting of cheese production. This is the most important factor that distinguishes this study from other studies. Accordingly, what makes this study important in the literature is that deep learning and machine learning prediction models have not been applied in this sector before.

One of the studies conducted in Turkey is Yıldırım and Altunç (2020). In the study, the ARIMA (Box-Jenkins) model was used to forecast the future milk production of Muş province. The results of the study showed that milk production in Muş province will be approximately 336 thousand tons in 2020 and approximately 368 thousand tons in 2023.

In the study of Goyal and Goyal (2013), feed-forward multilayer artificial neural network (ANN) models were developed to predict the shelf life of processed cheese. These ANN models were used to predict how long processed cheese can maintain its freshness at a given temperature at which it is stored. The input variables used in the study represented the chemical and microbiological characteristics of processed cheese samples. In the study, different ANN model

combinations were used to predict the shelf life of processed cheese. Metrics such as mean square error (MSE), root mean square error (RMSE), coefficient of determination (), and Nash-Sutcliffe coefficient were used to compare the predictive capabilities of these models. The findings of the study showed that the feed-forward ANN model in the combination of 5-16-16-1 gave the best result in predicting the shelf life of processed cheese with a high value. This emphasizes that multilayer machine learning models can successfully predict the shelf life of processed cheese.

Liseune et al. (2021) proposed a model to estimate the time of birth of dairy cows. In this model, they measured the movement behaviors of the cows, such as lying down, standing up, walking, ruminating, and walking, by sensors attached to their necks and feet and recorded them in minutes. To predict the last 24, 12, 6, 3, and 1 hour of calving, they used machine learning methods such as logistic regression (LR) and random forest (RF) and deep learning methods combining long-short-term memory (LSTM), convolutional neural networks (CNN), and a specially developed CNN and LSTM model. As a result, calving was predicted to occur within 24 hours, and the CNN algorithm gave the best result.

Ma et al. (2021) reported in a study that deep learning models have been used for maize yield forecasting with successful results, but existing models do not quantify the uncertainty associated with the forecasts and often require a large training dataset. To address these limitations, this study develops a district-level maize yield forecasting model that incorporates a large number of publicly available data sources. By training the model for forecasts since 2001, the study showed that the developed Bayesian Neural Network (BNN) model achieved an average coefficient of determination () value of 0.77 for late season forecasts in the US Corn Belt during the test years from 2010 to 2019, outperforming five other leading machine learning models.

Li et al. (2021a) conducted research addressing the complexity of industrial cream cheese production. Cream cheese production involves a process that requires the complex scheduling of multiple batches of fermenters and various fluidic units. This study used an artificial neural network (Long-Short Term Memory Network, LSTM) to address this challenge. It is also combined with a mechanistic model that describes changes in biomass, lactose, and lactic acid concentrations. The LSTM network/mechanistic modeling approach showed a difference of 3 minutes for batch durations of 6 to 7 hours compared to the laboratory experiment, and the overall accuracy () was above 0.99.

Keskinbıçak (2023) conducted a study focusing on species classification and yield estimation of chickpea plants in order to increase productivity in the agricultural sector. This study was handled in two stages. In the first stage, the classification of chickpea species was carried out with machine learning methods using the characteristics of chickpea plants. In the second stage, yield predictions for these classified species were made by the regression method. In the classification process, machine learning methods such as decision trees (DT), support vector machines (SVM), and k nearest neighbors (kNN) were used. The accuracy rate was used as a measure of success. The results showed that the highest accuracy rate of 90.6% was achieved by SVM in the classification with raw data. Similarly, the classification success of the dataset with a combination of raw data and synthetic data was recorded as the highest by SVM with 100% accuracy. When only synthetic data was used, the highest success rate was achieved by kNN, with an accuracy rate of 95.4%.

Li and Liu (2023), in their study, aim to detect food fraud to ensure the quality and safety of milk. For this purpose, hyperspectral images of pure and adulterated milk samples were collected using a hyperspectral imaging system (400–1000 nm). Then, the best preprocessing and characteristic wavelength selection methods were selected using the calibration model SVR, and the best combination of data processing was used to process the spectral data. Finally, the LSTM model optimized by the whale optimization algorithm was used to predict the content of additives in milk. Experimental results show that the WOA-LSTM model can accurately predict the content of additives. This study has the potential to provide an effective solution against food fraud and represents an important research area to improve the safety of dairy products.

Gandotra et al. (2023), in a study, examined the performance of different machine learning models for wheat, rice, and maize yield forecasting in the Jammu region. These models are: Long Short-Term Memory (LSTM), Gated Recurrent Unit (GRU), Bi-directional Long Short-Term Memory (Bi-LSTM), classical Deep Neural Network (DNN), and the basic models: Support Vector Regression (SVR), Random Forest Regression, and Ensemble Method AdaBoost. The data used for the study included environmental data such as temperature, precipitation, humidity, solar radiation, and sunshine hours from 2009 to 2019. The results showed that the Long Short-Term Memory (LSTM) model outperformed the other models in this study. This means that it has low values for the error measures RMSE, MAE, and MAPE (0.30, 0.20, and 0.23, respectively).

MATERIALS AND METHODS

This study aims to forecast monthly cow cheese production from November 2023 to September 2024. For this purpose, deep learning and machine learning models such as GRU, LSTM, kNN, SVR, and MLP are trained using cow

cheese production data from previous periods and other independent variables. The dataset used in the study covers a total period of 165 months, from January 2010 to September 2023. In the dataset, monthly cow cheese production data is used as the dependent variable. The independent variables are monthly cheese exports, monthly cattle feed production, monthly average dairy feed price, monthly number of cultured dairy cows, and monthly cow milk production. A total of 966 data entries were provided. Data were obtained through the Central Bank's EVDS system. Table 1 displays a subset of the dataset that was used in this study.

Table 1. A Part of The Dataset Used In The Study

Period	Monthly Cheese Exports (Million Pieces)	Monthly Cattle Feed Production (Thousand Tons)	Monthly Dairy Feed Price (Ton/TL)	Monthly Number of Cultured Dairy Cows (Million)	Monthly Cow Milk Production (Thousand Tons)	Monthly Cow Cheese Production (Thousand Tons)
2010-1	6	294	440	1,9	494	31990
2010-2	8	295	440	1,91	492	32374
2010-3	7	296	460	1,92	594	35296
2010-4	8	297	460	1,93	610	36143
2010-5	7	298	460	1,94	654	38254
…	…	…	…	…	…	…
…	…	…	…	…	…	…
2023-3	18	452	6810	3,48	911	66526
2023-4	16	459	6810	3,49	879	61755
2023-5	17	454	7125	3,5	944	67235
2023-6	15	500	7365	3,6	866	64085
2023-7	15	582	7460	3,5	835	66557
2023-8	16	600	7575	3,6	823	63451
2023-9	16	607	7520	3,6	852	62567

Resource: Turkish Statistical Institute

Furthermore displayed in Figure 1 is the correlation matrix illustrating the relationship between every variable in the study's data set and other factors. Two variables have a positive link when there is a positive correlation between them, and a negative link when there is a negative correlation. There is a complete connection between the variables when the correlation value is 1 or -1.

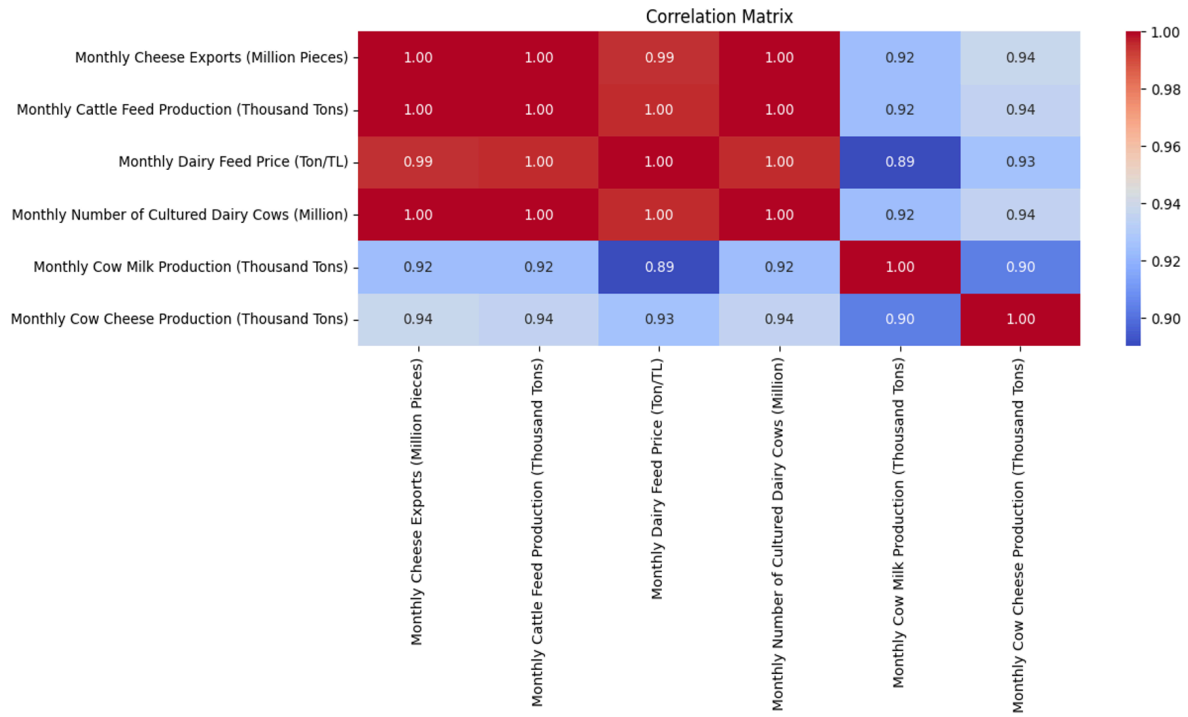

Figure 1. The Correlation Matrix.

As Figure 1 summarizes the results of the correlation matrix, there is a very high positive correlation (0.938506) between "Monthly Cheese Exports (Million Pieces)" and "Monthly Cow Cheese Production (Thousand Tons)". This shows that dairy exports and cow cheese production are highly positively correlated. There are also high positive correlations between "Monthly Cattle Feed Production (Thousand Tons)", "Monthly Dairy Feed Price (Ton/TL)", "Monthly Number of Cultured Dairy Cows (Million)", "Monthly Cow Milk Production (Thousand Tons)", and "Monthly Cow Cheese Production (Thousand Tons)". These results suggest that these variables are strongly correlated with cow cheese production, and these variables can play an important role in your forecasting model. Especially the high positive correlation between "Monthly Cheese Exports (Million Pieces)" and "Monthly Cow Cheese Production (Thousand Tons)" emphasizes the strong relationship between these two variables.

The study forecasts the monthly production of cow cheese using Python software. Importing the required Python libraries is therefore the first step. These libraries enable the creation of models, data processing, and result visualization. NumPy, Pandas, Matplotlib, Scikit-learn, and TensorFlow are the libraries that are used. Pandas was used to load the dataset from an Excel file. Additionally, NaN values were eliminated from the data set using "data.dropna()". Next, the data frame was divided into the independent variables (X) and dependent variables (Y). The min-max scaling method was used to normalize the data. This improves the performance of the model by converting each feature to a value between 0 and 1.

$$x_{scaled} = \frac{x - x_{min}}{x_{max} - x_{min}} \quad (1)$$

Training and test sets made up 80% and 20% of the overall data set, respectively, after data standardization. This allowed evaluation of the model's training efficacy with an alternative data set. A specific random seed (random_state) was utilized to partition the data set. "random_state" is used to make sure that the data set is randomly split in an identical manner each time. This guarantees reproducibility. In other words, the identical data split is produced each time by utilizing the same "random_state" value. When testing hyperparameter settings or assessing the model's performance, this preserves the comparability of the findings. Given that the goal of this research is to compare the performance of different models, it is imperative to ensure that the results are comparable by using the same data split. This will make determining which model performs better more equitable. As a result, 42 was chosen as the "random state" value and applied to every model.

All of the models that were employed in the study had their training loss tracked during the training phase, and the results were displayed in graph form. This made it feasible to see how the loss reduced and how each model learnt.

Next, predictions were generated for each trained model using the test data. Prior to Min-Max scaling, the forecasts were put back on their original scales. The study included five distinct techniques, including LSTM, GRU, MLP, SVM, and kNN models. Using estimated statistical measures such mean absolute percentage error (MAPE), mean squared error (MSE), coefficient of determination (R^2), and mean absolute error (MAE), the models' performance was assessed. These statistical data may be computed using equations 2, 3, 4, 5, and 6.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)}{N}} \quad (2)$$

$$MSE = \frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y}_i)^2 \quad (3)$$

$$MAE = \frac{\sum_{i=1}^{n}|y_i - x_i|}{n} \quad (4)$$

$$MAPE = \frac{\sum_{t=1}^{n}\frac{u_t}{Y_t}}{n} * 100 \quad (5)$$

$$R^2 = 1 - \frac{\sum_i (y_i - \hat{y}_i)^2}{\sum_i (y_i - \mu)^2} \quad (6)$$

The results were released following the calculation of the several error metrics previously created to measure the difference between the actual and projected numbers. A graph was then created using the projected and actual figures. This allowed for a clearer understanding of the models' functionality. This process includes training, predicting, assessing outcomes, preparing data, and building models. A complete list of the hyperparameters utilized in each of these phases may be seen in the Results section. The models' performance is impacted by these hyperparameters, which comprise configurations and optimization techniques. Furthermore, fundamental details on the models employed in the study are provided under the following subheadings.

However, in the next stage of the study, the model with the best training and testing forecast performance was selected to forecast monthly cow cheese production from October 2023 to September 2024.

Long Short Term Memory (LSTM)

Long short-term memory (LSTM) models are a subset of cyclic neural networks (RNNs) that are especially useful for learning chronic addictions. The cell state (C_t), one of the fundamental elements of the LSTM design, is regarded as the "memory" of the LSTM and retains information over extended periods of time (Li et al., 2021b). Each time step's output state is represented by the stored state (h_t), which is then passed on to the following step. What data should be added to the cell state is determined by the input gate (i_t). Typically, it is computed by multiplying a sigmoid (σ) by the tanh function. Which data from the cell state should be removed is decided by the forget gate (f_t). once more computed with the sigmoid function. Which data is moved to the stored state is decided by the output gate (o_t). The sigmoid function often governs this gate as well. The information chosen by the input gate and the data that the forget gate did not delete are contained in the intermediate state ($\hat{C}_t$), which is utilized to update the cell state (Smagulova and James, 2019).

$$f_t = \sigma(W_f \cdot [h_{(t-1)}, x_t] + b_f) \quad (7)$$

The same inputs are used to calculate both the intermediate state ($\tilde{C}_t$) and the input gate (i_t):

$$i_t = \sigma(W_i \cdot [h_{(t-1)}, x_t] + b_i) \quad (8)$$

$$\tilde{C}_t = \tanh(W_c \cdot [h_{(t-1)}, x_t] + b_C \quad (9)$$

After that, the cell status is updated:

$$C_t = f_t * C_{(t-1)} + i_t * \tilde{C}_t \quad (10)$$

Ultimately, the new hidden state (h_t) is formed and the output gate (o_t) is calculated:

$$o_t = \sigma(W_o \cdot [h_{(t-1)}, x_t] + b_o) \quad (11)$$

$$h_t = o_t * \tanh(C_t) \quad (12)$$

In order to provide a mathematical explanation of how an LSTM cell works, let's start by calculating the forget gate (f_t) using the input that is being used (x_t) and the stored state that was previously used ($h_{(t-1)}$).

Gated Recurrent Unit (GRU)

With a more straightforward architecture, GRU is a kind of artificial neural network that is intended to handle sequential input and shares traits with the LSTM (Long Short-Term Memory) model (Athiwaratkun and Stokes, 2017). GRU gets two input vectors (X_t) and the stored state of the previous time step ($H_{(t-1)}$) at each time step. How much of the stored state keeps past data is decided by the update gate. The update gate is computed using the formula and is represented by the symbol Z_t :

$$Z_t = \sigma(W_z \cdot [H_{(t-1)}, X_t]) \quad (13)$$

The sigmoid activation function is denoted by in this case, while the update gate weights are represented by W_z.

How much of the previous data is moved to the new storage state is decided by the reset gate. It is computed as follows and is represented by the symbol R_t (Nosouhian et al., 2017):

$$R_t = \sigma(W_r \cdot [H_{(t-1)}, X_t]) \quad (14)$$

W_r represents the reset gate's weights in this instance.

On the other hand, the input vector and the previous hidden state are combined to compute the new hidden state, $\hat{H}_t$ (Agarap, 2018). The calculation of an intermediate vector, $\hat{H}_t$, is done first as follows:

$$\tilde{H}_t = \tanh(W \cdot [R_t * H_{(t-1)}, X_t]) \quad (15)$$

Tanh stands for the hyperbolic tangent activation function in this case, while W stands for the hidden state weights. Next, the following formula is used to determine the new concealed state:

$$H_t = Z_t * H_{(t-1)} + (1 - Z_t) * \tilde{H}_t \quad (16)$$

Subsequently, the updated stored state is transmitted to the subsequent layer or output at every time step.

Multilayer Perceptron (MLP)

Machine learning and artificial neural networks are built on a sort of artificial neural network called Multi-Layer Perceptrons (MLP). The following is a summary of the fundamental elements that comprise the MLP architecture: The model first accepts information and begins processing it at the input layer. Every node, also known as a neuron, represents a feature in the dataset. The layers that sit between the input and output layers are the hidden layer(s). Weights and activation functions are used in these levels to process the incoming data. Multiple hidden layers are possible in MLP models (Ramchoun et al., 2017). The output layer represents the final layer at which the network generates output. For a classification job, each neuron in the output layer represents a class, and for a regression task, it represents a value. The weights stand for each connection's strength. These weights are updated to allow the network to learn. Each neuron's activity threshold is determined by biases. Biases enable neurons to become more or less responsive to a particular stimulus by shifting the activation function. Lastly, the output of neurons is computed using activation functions. The network may learn several activation functions, including sigmoid, tanh, and ReLU, to overcome non-linear complexity (Desai and Shah, 2021).

The actions at each layer of the network are covered by the mathematical formulation of the MLP, which typically comprises of the successive application of an activation function and a linear transformation (applying weights and biases). These steps may be described in depth mathematically as follows: In a linear transformation, biases () are applied to each layer and inputs (x) are multiplied by weights (W):

$$z^l = W^l x^{l-1} + b^l \quad (17)$$

where W^l is the weight matrix in the 1th layer, $x^{(l-1)}$ denotes the outputs of the (l-1)th layer (or the original inputs for the input layer), b^l denotes the bias vector in the lth layer, and z^l denotes the linear transformation result of the neurons in the lth layer.

The activation function is then applied to the outcome of the linear transformation:

$$a^l = f(z^l) \quad (18)$$

where f stands for the selected activation function and σ^l is the activation result of the neurons in the 1th layer. An activation function that may be used is a sigmoid (σ), tanh, or ReLU (rectified linear unit). After applying the final linear transformation and activation function, the following is the final output generated in the last layer of the network:

$$z^L = W^L a^{L-1} + b^L \quad (19)$$
$$\tilde{y} = f(z^L) \quad (20)$$

where ỹ is the network's anticipated output and L is the total number of layers.

From the input layer to the final output layer, these mathematical procedures are repeated at every layer of the network. The gradient descent approach is used to update the weights and biases at each iteration, while the back propagation technique is used to determine the network's error rate. In order for the network to have "learned" to do the assigned task, this procedure is repeated in order to lower the model's error on the training data set.

Support Vector Regression (SVR)

Support Vector Regression (SVR) is a potent statistical learning model that uses regression issues to apply the idea of Support Vector Machines (SVM). SVR attempts to locate a linear regression line, also known as a hyper-plane, in a high-dimensional feature space created by mapping data points (Qian et al., 2015). The SVR model's fundamental elements and methods of operation can be stated as follows (Hsu et al., 2009):

The margin value, epsilon (ε), establishes the maximum amount of inaccuracy that the model can produce. Errors inside the margin ε are accepted by SVR as zero. The plane that creates a connection between the independent

variables in the data set and the dependent variable that has to be forecasted is known as the hyperplane. SVR attempts to make the most accurate predictions of the data points by locating this hyperplane. In order to linearize a non-linear connection, the function that maps the input data into a higher-dimensional space is known as the feature space. The conventional method for doing this mapping is via a kernel function. The input data is converted into a high-dimensional feature space using the kernel function. Sigmoid, polynomial, linear, and radial basis function (RBF) functions are examples of common kernel functions. The coefficients known as the Langrange multipliers are used in the optimization process to calculate the impact of each data point on the hyperplane's location. The model weights the data points in order to calculate the hyperplane, and this is determined by these multipliers.

Lagrange multipliers are determined to be the most effective in determining the margin ε and the hyperplane's position throughout the SVR model's training process (Akay and Abasıkeleş, 2010). This is accomplished by resolving the subsequent optimization issue:

$$\min_{w,b} \frac{1}{2}\|\mathcal{W}\|^2 + C\sum_{i=1}^{n}(\xi_i + \tilde{\xi}_i)$$
$$\text{subject to} \begin{cases} y_i - \langle \mathcal{W}, \phi(x_i)\rangle - b \le \varepsilon + \xi_i \\ \langle \mathcal{W}, \phi(x_i)\rangle + b - y_i \le \varepsilon + \tilde{\xi}_i \\ \xi_i, \tilde{\xi}_i \ge 0 \end{cases} \quad (21)$$

where the slack variables are ξ and ξ , the normal vector of the hyperplane is , W the y-offset is , b the error penalization parameter is C , the mapping of the input data to the feature space is Ø(X_i), and the actual output values are y_i .

SVR is well-known for being resilient to data noise and can be applied to both linear and non-linear regression applications. SVR's robustness and generalization skills allow it to provide accurate predictions even with high-dimensional data sets.

K-Nearest Neighbors (kNN)

For classification and regression issues, the k-Nearest Neighbors (kNN) technique is a supervised learning model. The "learning" part of the model involves storing data points in a feature space and using the k nearest data points to make a prediction each time a new data point is received (Patwary et al., 2016).

Based on its attributes, every data point is represented as a point in an n-dimensional space. These characteristics include, for instance, the characteristics of the samples for a classification issue. The number of nearest neighbors to take into account when generating a classification or regression prediction is determined by the parameter k, which also gives the procedure its name. K is often selected using cross-validation. The "closeness" of a new data point to old ones is determined using the kNN method using a distance metric. Manhattan, Minkowski, and Euclidean distances are examples of common distance measures. The new data point is allocated to the class with the highest representation in the classification issue, which is decided by a majority vote based on the classes of the chosen k closest neighbors. The simple average or weighted average of the dependent variable values for the k nearest neighbors based on their distances is used to make a forecast for the regression issue (Yu et al., 2015).

The k-Nearest Neighbors (kNN) method finds the closest neighbors using a selected distance metric, then uses that knowledge to produce an output value. This is how it operates theoretically. The distance between a data point and a training sample is usually calculated as the Euclidean or Manhattan distance.

$$d(x, x_i) = \sqrt{\sum_{j=1}^{n}(x_j - x_{ij})^2} \quad (22)$$

For the Euclidean distance:

For the distance to Manhattan:

$$d(x, x_i) = \sum_{j=1}^{n}|x_j - x_{ij}| \quad (23)$$

The jth features of x and xi are represented as xj and xij, respectively, where n is the number of features. The distance measure identifies the k neighbors who are closest to the new data point:

$$NN_k(x) = \{x_i \in X | d(x, x_i) \text{ between the smallest k values}\} \quad (24)$$

where X is the training data set and and $NN_k(x)$ is the k is the closest neighbors to the new data point .

In a regression, the average, or weighted average, of the output values of the closest neighbors is used to calculate the prediction:

$$\hat{y} = \frac{1}{k}\sum_{x_i \in NN_k(x)} y_i \quad (25)$$

or for the weighted average:

$$\hat{y} = \frac{\sum_{x_i \in NN_k(x)} w_i \cdot y_i}{\sum_{x_i \in NN_k(x)} w_i} \quad (26)$$

The weight of the inverse distance can be considered here as W_i :

$$w_i = \frac{1}{d(x,x_i)^2} \quad (27)$$

The actual output value of X_i is represented by Y_i, while the anticipated value is denoted by $\tilde{y}_i$.

The kNN algorithm's method for predicting a data point's categorization or regression is described in these stages. The ability of the kNN algorithm to provide data-driven predictions without requiring the modeling of feature space geometry is one of its benefits. However, the computational cost can be substantial for big data sets, thus selecting the right k number is crucial.

Findings of The Study

The LSTM, GRU, MLP, SVR, and kNN models are employed in this work to anticipate the output of cow cheese. The models employed a large number of independent variables, including the number of cultured dairy cows, the price of dairy feed, the amount of cow milk produced each month, and the amount of cheese exported. Using Python software, the MinMaxScaler technique was utilized to normalize the dependent and independent variables in the dataset. Next, the dataset was split into training (80%) and test (20%) groups. Then, to make sure that every model utilized the same data split, the "randomization" command was employed. This made it possible for the outcomes to be repeatable and comparable. In this regard, the models' predictions on the test data set also fared rather well. 42 was chosen as the random state rate.

Findings Related to The MLP Model

The "Sequential" class was initially used to generate the MLP model once the data set had been prepared to assess the models' prediction skills. There are 300 neurons in the model's first hidden layer, 200 in the second, and 100 in the third. A dasigmoid activation function was employed in each layer. As this is a regression problem, the model's output layer has a single neuron and does not employ an activation function. On the other hand, a learning rate of 0.001 was employed with the "Adam" optimization method. The loss function that was employed was "mean_squared_error". The training loss of the model was recorded and shown on the screen for each of the 500 epochs that it was trained for. To track the model's performance, training and validation losses were recorded at the conclusion of each epoch. The test data served as the basis for the model's predictions. In order to assess the effectiveness of the training, predictions were also generated using the training data. The "MinMax Scaler" was then used to return the normalized forecasts and actual values to the original scale. On the test and training data sets, several error measures are computed, including , RMSE, MSE, MAE, and MAPE. Next, line graphs for the training and test sets are used to depict the actual and anticipated monthly output of cow cheese. Additionally, a graph illustrates how the loss varies throughout the training phase.

The training set was used to train the MLP model for 500 epochs after it was produced. Every epoch's training loss was recorded. The variable "history" was used to monitor the training loss at the conclusion of each epoch. The training loss values recorded throughout the training procedure are displayed on a graph. A graph of the MLP model's training loss values is displayed in Figure 2.

The training loss consistently drops over the course of epochs when the model's training outcomes are assessed using the supplied epoch-wise training loss numbers. From 0.1177 in the first epoch to 0.0037 in the 500th epoch, the loss is reduced. On the other hand, Table 2 displays the MLP model's training outputs' performance.

These results for the training data analysis of the data in Table 2 indicate that the MLP model performs rather well on the training data. Put otherwise, the model predicts the training set with a low error rate. These results, however, don't

show how well the model works with actual data. Its performance on test data is therefore more crucial. Furthermore, Figure 3 displays the MLP model's actual and predicted cow cheese production (CCP) values for the training data set.

Following the MLP model's training, predictions were produced using the trained model on test data. Then, the scales for these forecasts were adjusted to their initial values. Table 3 displays the results of the calculations made using RMSE, MSE, MAE, MAPE, and to determine the errors between the anticipated and actual values on the test data.

The MLP model's performance was evaluated with the test set of data using the error calculations displayed in Table 3. Test data often yields better results for the model than training data does. This shows that the model can reliably provide results in real-world applications and generalize effectively to new and unknown data. However, the actual and expected CCP values for the test data of the MLP model are shown in a graph. Figure 4 shows this graph.

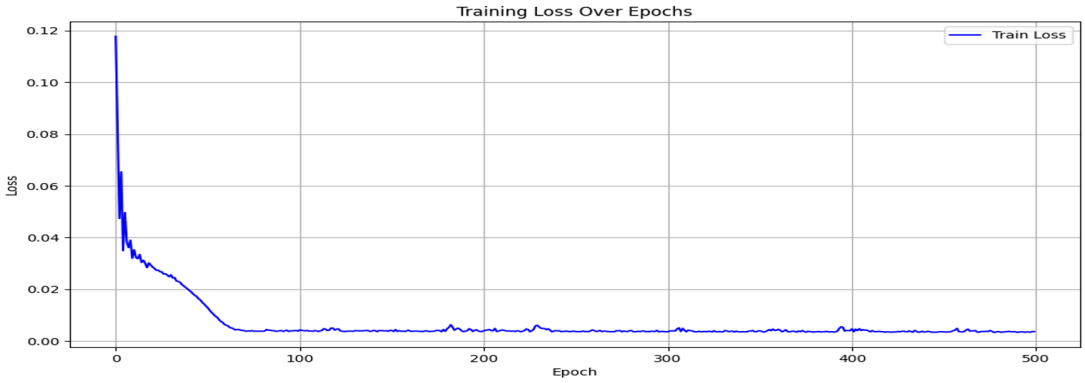

Figure 2. Loss Values of The MLP Model During Training

Table 2. Error Calculations of MLP Model Predictions Using Training Data.

	MLP
RMSE	2.1015
MSE	4.4167
MAE	1.2584
MAPE	0.0278
	0.8917

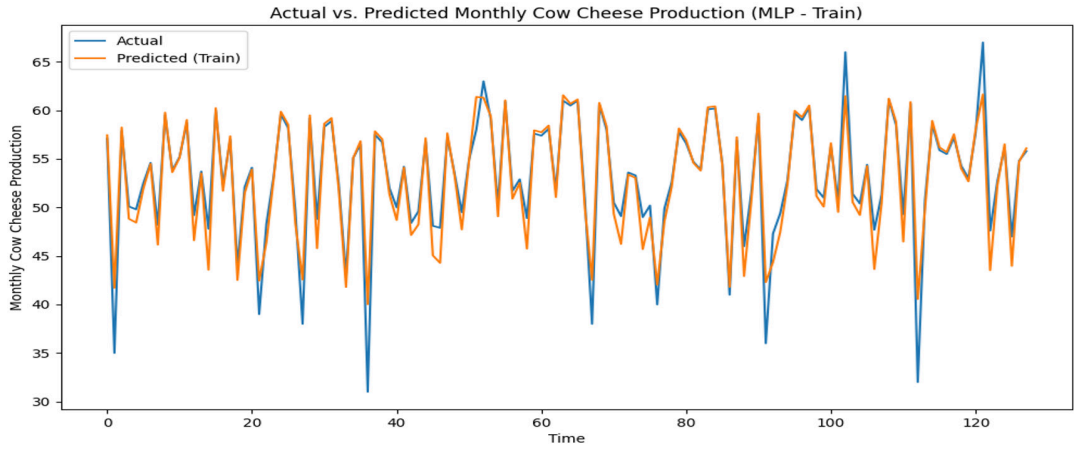

Figure 3. The Actual and Predicted CCP Values of The MLP model on The Training Data

Table 3. Error Calculations of MLP Model Predictions on Test Data

	MLP
RMSE	1.4824
MSE	2.1977
MAE	0.9828
MAPE	0.0200
	0.9076

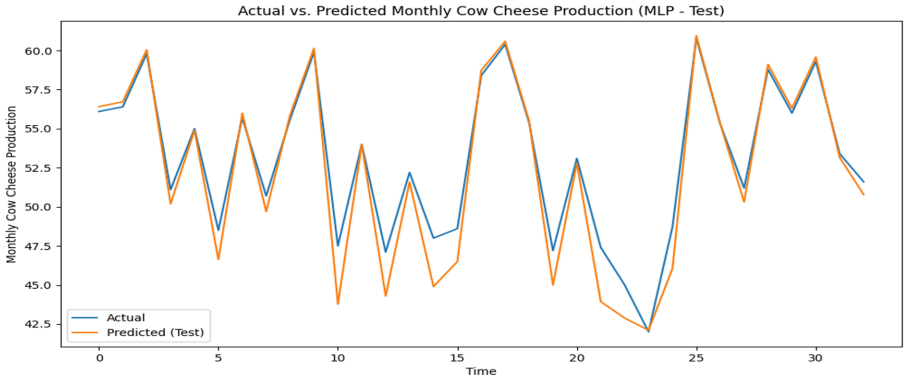

Figure 4. The Actual and Predicted CCP Values of The MLP model on The Test Data

Findings Related to The LSTM Model

The LSTM model is an additional technique employed in the study to estimate CCP values. A sigmoid activation function is applied to the 300 neurons that make up the first layer of the LSTM network. The amount of features in the training data set determines how the input shape is changed. Next, two dense layers with sigmoid activation functions—200 and 100 neurons, respectively—are added. There is just one neuron in the output layer, and regression makes no use of an activation function. Using a learning rate of 0.001, the "Adam" optimization technique was used to assemble the model. The loss function that was selected was "mean_squared_error".

When training machine learning and deep learning models, a hyperparameter known as learning rate is employed. A model's learning rate dictates how much weight is updated while it is being trained. More specifically, the amount by which the weights are changed is determined by the learning rate, which is set at each learning step. This method may require less data because of the enhanced generalization and less overfitting. These settings were designed to prevent overfitting.

The LSTM model was trained on the training set for 500 epochs after it was created. The training loss for each period was recorded. The variable "history" was used to track the training loss at the end of each epoch. The training loss values that were recorded during the training process were displayed on a graph. The training loss value graph of the LSTM model is shown in Figure 5.

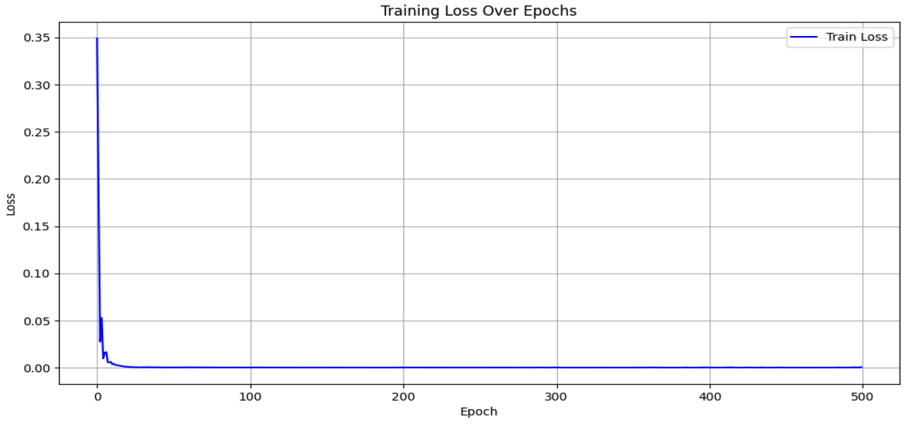

Figure 5. Loss Values of The LSTM Model During Training

The performance of an LSTM model during training is shown by the provided training losses. Given that the model's losses are minimal and often declining with time, its performance during training looks promising. But the model's performance on the test dataset must also be taken into account in order to evaluate its generalization ability. However, Table 4 displays the LSTM model's training outputs' performance.

Table 4. Error Calculations of LSTM Model Predictions Using Training Data

	LSTM
RMSE	0.7675
MSE	0.5890
MAE	0.4609
MAPE	0.0082
	0.9855

Upon analyzing the metrics shown in Table 4, it becomes evident that the model exhibits a strong fit to the training data, resulting in very accurate predictions. Nevertheless, it is crucial to acknowledge that in order to comprehend the model's capacity for generalization, it is imperative to assess its performance on the test data set as well. Furthermore, Figure 6 displays the LSTM model's actual and predicted cow cheese production (CCP) values for the training data set.

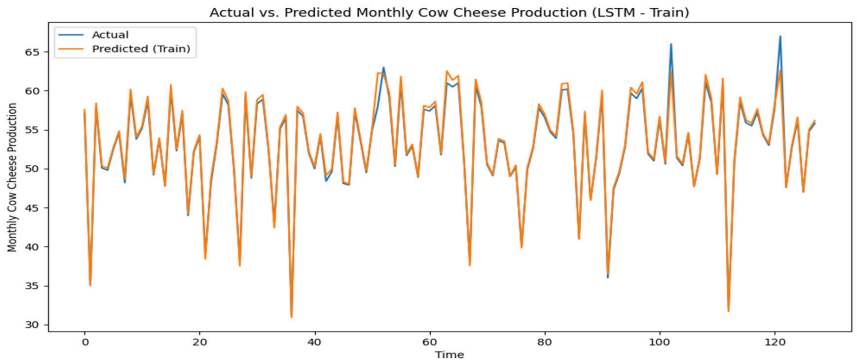

Figure 6. The Actual and Predicted CCP Values of The LSTM Model on The Training Data

Following the LSTM model's training, predictions were produced using the trained model on test data. Then, the scales for these forecasts were adjusted to their initial values. Table 5 displays the results of the calculations made using RMSE, MSE, MAE, MAPE, and to determine the errors between the predicted and actual values on the test data.

Table 5. Error Calculations of LSTM Model Predictions on Test Data

	LSTM
RMSE	0.4387
MSE	0.1924
MAE	0.3852
MAPE	0.0071
	0.9919

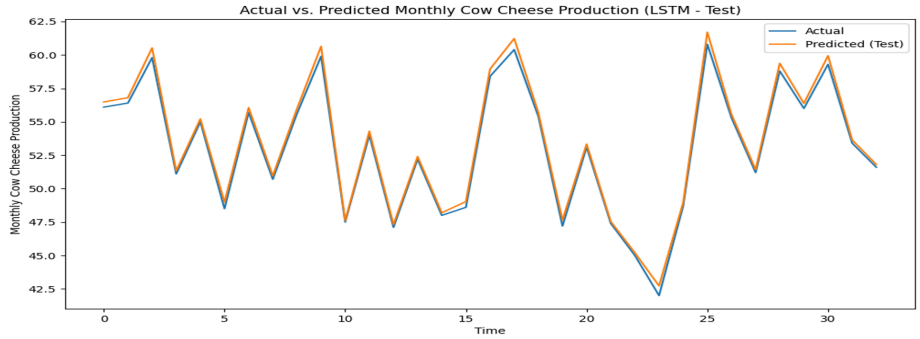

Figure 7. The Actual and Predicted CCP Values of The LSTM Model on The Test Data

Based on the aforementioned criteria, it can be concluded that the model has exceptional performance on the test data set and exhibits the ability to generate accurate predictions on both the test and training data sets. This suggests that the model possesses a strong capacity for generalization, enabling it to effectively extrapolate observed patterns to novel data. On the other hand, a graph displays the LSTM model's actual and predicted CCP values for the test data. In Figure 7, this graph is shown.

Findings Related to The GRU Model

An other method used in the study to estimate CCP values is the GRU model. The first layer of the GRU network consists of 300 neurons that are activated using a sigmoid activation function. The training data set's feature count dictates how the input shape is altered. Two dense layers with 200 and 100 neurons, respectively, having sigmoid activation functions are then added. The output layer consists of a single neuron, and regression does not employ an activation function. The model was put together using the "Adam" optimization strategy with a learning rate of 0.001. "mean_squared_error" was chosen as the loss function.

After it was developed, the GRU model was trained for 500 epochs on the training set. Every period's training loss was noted. At the conclusion of each epoch, the training loss was monitored using the variable "history." A graph showed the training loss values that were noted throughout the training procedure. Figure 8 displays the GRU model's training loss value graph.

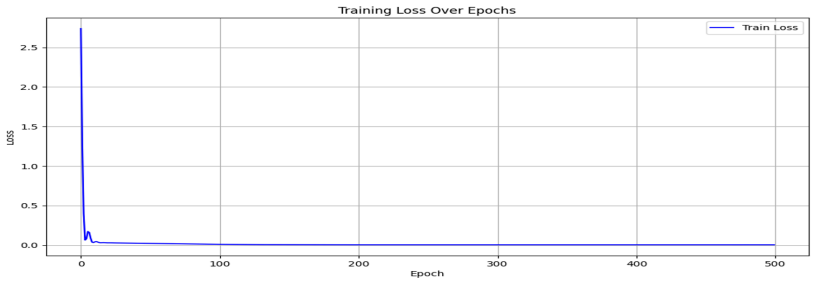

Figure 8. Loss Values of The GRU Model During Training

Figure 8 illustrates how the training loss seems to drop down quickly as training progresses. The training loss dramatically reduces at each epoch after the first, reaching a very low level by the 500th epoch. This demonstrates how effectively the model generalizes and learns the training set of data. Though it also raises the possibility of overfitting, a low training loss suggests that the model does a good job of fitting the training set. However, Table 6 displays the GRU model's training outputs' performance.

Table 6. Error Calculations of GRU Model Predictions Using Training Data

	GRU
RMSE	2.1812
MSE	4.7579
MAE	1.2936
MAPE	0.0286
	0.8833

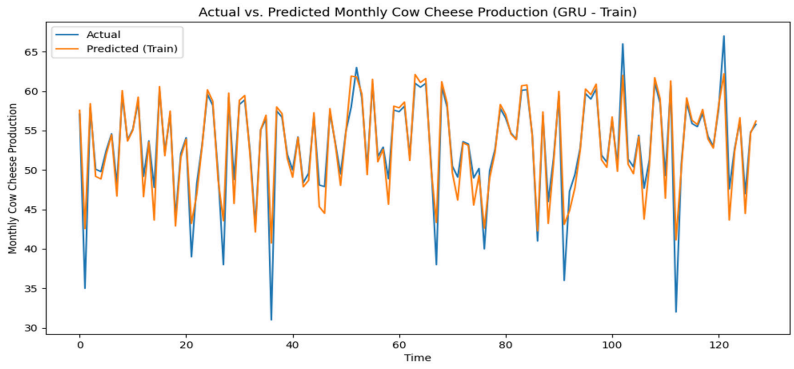

Figure 9. The Actual and Predicted CCP Values of The GRU Model on The Training Data

The table's error statistics suggest that the GRU model does well with training data. To find out how the model will function with real-world data, it is crucial to thoroughly verify and assess the test data. Furthermore, Figure 9 displays the GRU model's actual and predicted cow cheese production (CCP) values for the training data set.

Following the GRU model's training, predictions were produced using the trained model on test data. Then, the scales for these forecasts were adjusted to their initial values. Table 7 displays the results of the calculations made using RMSE, MSE, MAE, MAPE, and to determine the errors between the predicted and actual values on the test data.

Table 7. Error Calculations of GRU Model Predictions on Test Data

	GRU
RMSE	1.3121
MSE	1.7217
MAE	0.9410
MAPE	0.0189
	0.9276

These results demonstrate that the GRU model functions admirably on the test data as well. This indicates that the model can accurately forecast data from the actual world without overfitting the training set. These successes demonstrate that the model was properly trained using the issue context and available data. However, a graph displays the GRU model's actual and predicted CCP values for the test data. In Figure 10, this graph is shown.

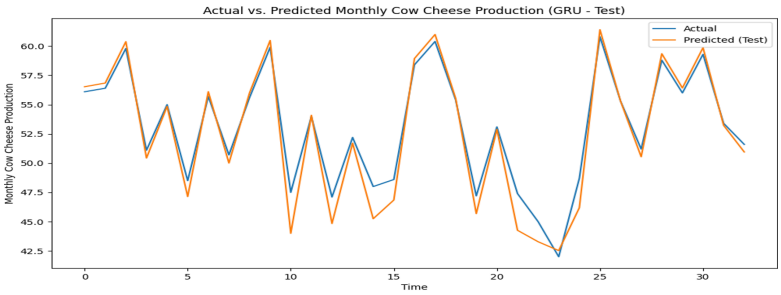

Figure 10. The Actual and Predicted CCP Values of The GRU Model on The Test Data

Findings Related to The SVR Model

An additional method for estimating CCP values in the study was the SVR model. The kernel used to create the SVR model was set to RBF (Radial Basis Function). Additionally, the model made use of the "C" and "Epsilon" parameters. One kind of regularization parameter that is used to manage overfitting is called "C". A margin is an error that is defined by epsilon. The margin determines how near the genuine values should be to the model's predictions. The margin's width is determined by the epsilon value. A larger epsilon number indicates a broader margin, whereas a smaller epsilon value indicates a narrower margin. The model's C value and Epsilon value are respectively set at 5.0 and 0.2. Following the creation of the SVR model, the training set was used to train the SVR model. Table 8 displays the performance of the training outcomes.

Table 8. Error Calculations of SVR Model Predictions on Training Data

	SVR
RMSE	3.6787
MSE	13.5332
MAE	3.3711
MAPE	0.0663
	0.6683

When these results are analyzed, it can be said that the SVR model may need some improvements. The RMSE and MAE values are quite high, and the value is low, indicating that the model cannot fully explain the data. However, the actual and predicted CCP values of the SVR model on the training set are shown in the graph in Figure 11.

Predictions were produced using the trained model on the test data after the training of the SVR model. Then, the scales for these forecasts were adjusted to their initial values. Table 9 displays the results of the calculations made using RMSE, MSE, MAE, MAPE, and to determine the errors between the anticipated and actual values on the test data.

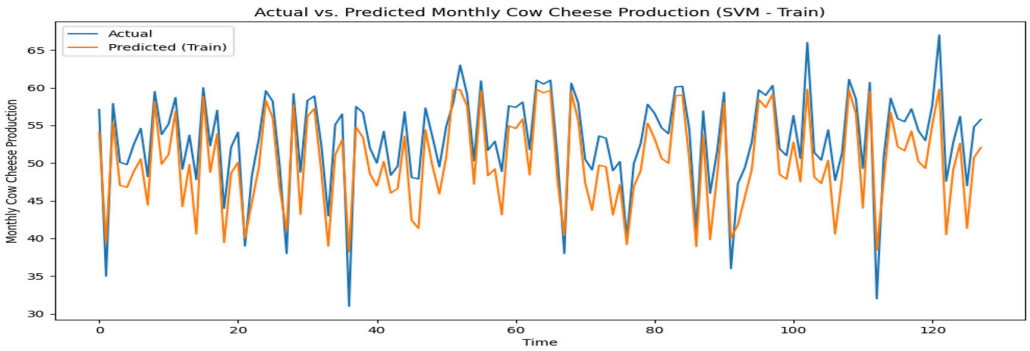

Figure 11. The Actual and Predicted CCP Values of The SVR Model on The Training Data

Table 9. Error Calculations of SVR Model Predictions on Test Data

	SVR
RMSE	3.8132
MSE	14.5406
MAE	3.5418
MAPE	0.0690
	0.3889

The results in Table 9 show that the SVR model does not perform well on the test data. Both RMSE and values are low, and MAE and MAPE values are not at acceptable levels. On the other hand, a graph displays the actual and predicted CCP values of the SVR model based on the test data. In Figure 12, this graph is shown.

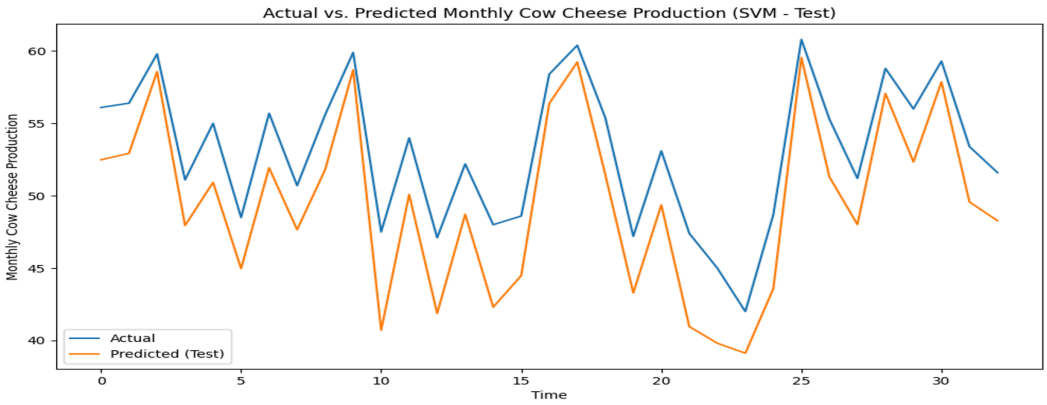

Figure 12. The Actual and Predicted CPI Values of The SVR Model on The Test Data

Findings Related to The kNN Model

The kNN model was another technique used in the research to estimate CCP values. The kNN regression model—the last machine learning model used in this study for CCP prediction—was created using the Python application "KNeighborsRegressor." This model uses the k-NN approach to find the relationship between the independent and dependent variables. The option "n_neighbors" (Number of Neighbors) determines how many neighbors to use in the kNN algorithm. In this model, N_neighbors is equal to 5. This suggests that the five nearest neighbors will be used in each prediction. Conversely, the "weights" parameter determines how the neighbors are weighted in the prediction. Two common choices are uniform and distance. Uniform weighting allocates the same weight to every neighbor, while distance weights are inversely proportional to neighbor distance. The model's weights are "uniform," which means that each neighbor has the same weight. The option "metric (distance metric)" specifies the distance metric to be used for calculating the distance between neighbors. Different metrics may be used, such as Euclidean, Manhatten, and Minkowski distances. In this paradigm, the metric is "euclidean," so Euclidean distance is used. The training set was used to train the k-NN model once the KNN model was created. The performance of the training results is shown in Table 10.

Table 10. Error Calculations of kNN Model Predictions on Training Data

	KNN
RMSE	2.5715
MSE	6.6128
MAE	1.1426
MAPE	0.0269
	0.8379

The results in Table 10 show that the kNN model performs well on the training data. Low RMSE, MSE, and MAE values indicate that the model's predictions on the training data are generally close to their true values. Also, the high value indicates that the model is well fitted to the training data. However, these results are only obtained for training data and do not provide information about the model's performance on test data. In addition to this, the actual and predicted CCP values of the kNN model on the training set are shown in the graph in Figure 13.

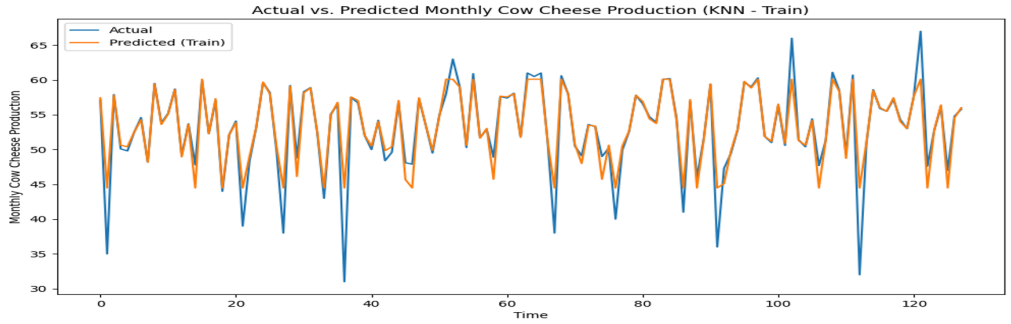

Figure 13. The Actual and Predicted CCP Values of The kNN Model on The Training Data

Following the kNN model's training, predictions were generated using the trained model on the test data. Following that, the predictions' scales were returned to their starting points. The computations of the errors between the expected and actual values on the test data using RMSE, MSE, MAE, MAPE, and are shown in Table 11.

Table 11. Error Calculations of kNN Model Predictions on Test Data

	kNN
RMSE	1.0633
MSE	1.1307
MAE	0.6235
MAPE	0.0129
	0.9524

These outcomes demonstrate the kNN model's excellent performance on the test set. A graph displays the actual and anticipated CCP values of the kNN model based on the test data. In Figure 14, this graph is shown.

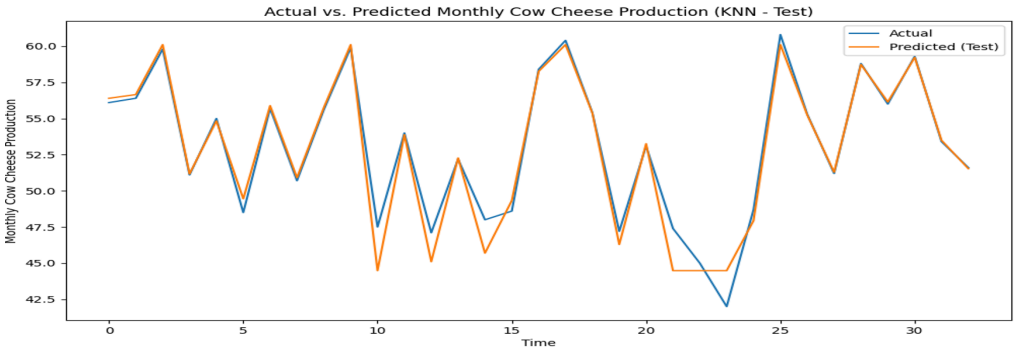

Figure 14. The Actual and Predicted CCP Values of The kNN Model on The Test Data

Table 12 summarizes the test results for each of the forecasting models used in this study.

Table 12. Error Calculations of All Models Predictions on Test Data

	LSTM	GRU	MLP	SVR	KNN
RMSE	0.4387	1.3121	1.4824	3.8132	1.0633
MSE	0.1924	1.7217	2.1977	14.5406	1.1307
MAE	0.3852	0.9410	0.9828	3.5418	0.6235
MAPE	0.0071	0.0189	0.0200	0.0690	0.0129
	0.9919	0.9276	0.9076	0.3889	0.9524

The error estimates of the predictions produced by five different models on the test data are shown in Table 12's findings. From various angles, each error measure aids in assessing a model's performance. The LSTM model performs the best and has the lowest RMSE when it comes to this data. Next in line is the kNN model. The model's predictions are said to be quite accurate if the MSE value is low. Once again, the model with the lowest MSE is LSTM. The model's predictions are said to be reasonably accurate if the MAE value is low. The LSTM model has the lowest MAE once again. The model's predictions are in near percentage agreement with the real values when the MAPE value is low. Once again, the LSTM model has the lowest MAPE. A high value means that a significant amount of the dependent variable's variation can be explained by the model. As the model that most closely matches the test data in this situation, the LSTM model has the greatest .

In light of this, the comparison indicates that, when it comes to test data performance, the LSTM model outperforms the other models. SVR and MLP models perform worse than GRU and kNN models, which nevertheless perform well.

Based on these results, LSTM is selected as the most successful model in terms of both training and testing performance in the forecasting process of CCP data, and then the forecasting process for the next 12 months is performed using this model. The 12-month prediction was produced using a pre-trained LSTM model with the same hyperparameter parameters as previously mentioned for future forecasting. The model predicted the most recent test data point "X_test[-1]" for each forecast. The anticipated values were added to the "future_predictions" list. A new data point, the final predicted value, was added to the "X_test" dataset. This was applied to the subsequent forecast. Following Min-Max normalization, the projected values were transferred back to the original scales. After that, Python software was used to print the forecasts. Table 13 shows CCP values for the next 12 months.

Table 13. CCP Values for the Next 12 Months According to the LSTM Model

Months	Monthly CCP (Thousand Ton)
2023-10	62.09
2023-11	61.80
2023-12	61.36
2024-1	60.79
2024-2	60.07
2024-3	62.01
2024-4	62.72
2024-5	62.39
2024-6	63.05
2024-7	63.73
2024-8	64.48
2024-9	65.33

The monthly output of cow cheese is trending increasing, according to an examination of the projections. This suggests that the output of cheese will probably continue to increase in the next months. Although there is an overall rising tendency, there is monthly uncertainty amongst projections. This variation can be a reflection of variations in output levels over several months. The LSTM model's predictions seem to have a good overall performance in terms of model performance since they are quite close to the actual values. Our understanding of future output and demand for economic sectors like the dairy industry may be improved with further study and improvement of these forecasting models. Ultimately, your LSTM model's monthly cow cheese output projections seem to be a helpful resource for predicting production patterns and variability in the future. However, the graph of the CCP forecast for the next 12 months is shown in Figure 15.

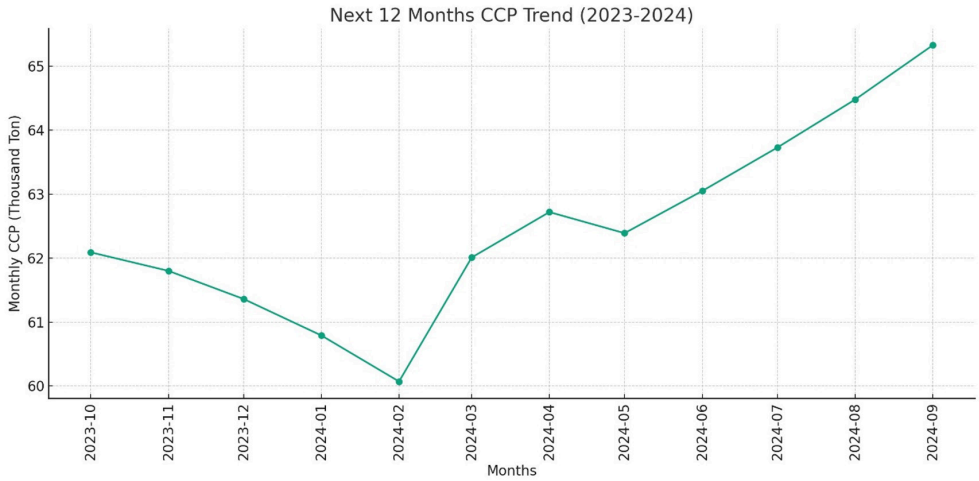

Figure 15. Next 12 Months CCP Trend (2023-2024)

The trend of the Monthly CCP (in thousand tons) from October 2023 to September 2024 is shown in this graphical representation of the data that was given. The CCP value for a given month is represented by each point on the graph, and the line that links these points shows the trend over time.

Conclusion and Recommendations

This study emphasizes how crucial forecasting is to the dairy sector, especially when it comes to predicting the amount of cheese produced from cow's milk (CCP). Precise predictions are essential for effective management of the supply chain, achieving a balance between production and demand, and developing plans to lessen the impact of future market swings.

Because machine learning and deep learning techniques have shown efficacy in handling complicated, non-linear data with several unexpected elements influencing agricultural yield, they were used in this investigation. The goal was to determine which model—LSTM, GRU, MLP, SVR, and kNN—was the most useful for CCP prediction by comparing them.

The results demonstrate that in terms of CCP prediction, the LSTM (long short-term memory) model performs noticeably better than the other models. This advantage is shown in a number of error measures, showing the model's resilience and dependability, including RMSE, MSE, MAE, MAPE, and . The LSTM model seems to be especially useful for forecasting tasks like CCP forecasting, where previous patterns play a significant role in creating future trends, because of its past patterns and its resilience to gap length in the time series.

Nonetheless, there should be a lot of advantages to utilizing the LSTM model to predict cow cheese output for the next year. Production process management is made possible by forecasts. This makes it easier to calculate the precise amount of raw materials needed and to modify production capacity in response to demand. Reducing stock shortages and surpluses is facilitated by knowing future production volumes. This stops waste and lowers expenses. It guarantees that the market will have enough supply to fulfill demand. Customers are happier and brand loyalty is strengthened as a result. Supply-demand balance-based pricing methods may be developed more successfully with the use of production quantity forecasting. It makes it possible to anticipate hazards associated with unforeseen changes or production bottlenecks. This makes it possible to create and put into practice risk management plans. gives details on potential investment possibilities, general market dynamics in the industry, and future production patterns. provide the knowledge required to decrease food waste and make better use of resources, both of which promote environmental sustainability. lays the groundwork for a deeper comprehension of shifts in customer preferences and market demand, which promotes the creation of new goods and the enhancement of current ones.

To further highlight the uniqueness and significance of the research, it would be helpful to compare the cow cheese production forecasting findings achieved in this work using the LSTM model with comparable studies in the literature. When it came to error measures like RMSE, MSE, MAE, MAPE, and , the LSTM model fared better in the research than the other models. This implies that, in comparison to other research approaches, including the ANN model of Goyal and Goyal (2013) and the ARIMA model of Yildirim and Altunc (2020), the LSTM model offers an edge in predicting accuracy. Specifically, the LSTM model exhibits more accuracy when juxtaposed with the values of the ANN models in the Goyal and Goyal research. Nonetheless, this research closes a vacuum in the literature since the majority of earlier studies in the subject of cheese production forecasting concentrated on the forecasting of milk, dairy, and other agricultural

goods. This makes the research more significant in the literature and offers a special contribution. The models used in research works like Li and Liu (2023) and Keskinbıçak (2023) are capable of managing intricate data sets and accounting for diverse data kinds. According to this research, the LSTM model's capacity to handle such intricate and varied data presents a big benefit for predictions that are sector-specific. Research like those conducted by Ma et al. (2021) and Gandotra et al. (2023) shows how machine learning and deep learning models affect real-world data. The findings of this study might have a significant influence on future demand and production projections, particularly in industries like the dairy sector. The research conducted by Liseune et al. (2021) demonstrates the advanced methodological advances and advancements of the LSTM model utilized in this investigation, as shown by the usage of complicated machine learning and deep learning models. Consequently, this comparison demonstrates that our work is among the first to anticipate cheese output using the LSTM model and is a noteworthy methodological advancement in this area. Its unique application and accurate forecasts also constitute a significant addition to the literature.

Future research may benefit from comparison evaluations of various deep learning and machine learning models (such as CNN and GRU) to determine which models are more appropriate for certain circumstances. The general validity and robustness of the model may be strengthened by experimenting with bigger and more varied datasets. The model's potential uses might be increased by using it to predict the production of milk and cheese as well as other food and agricultural products. Real-time data may be included into the modeling to increase prediction accuracy and timeliness. Forecasts may be made more thorough by include external aspects like market movements, climate change, and socioeconomic issues in the modeling. Forecast success may be increased by fine-tuning the LSTM model's parameters and settings. It is feasible to get more detailed information about potential future trends and changes by creating longer-term projections and conducting analysis under various scenarios. These suggestions may improve understanding and applications in this area and be helpful for both academic and industry uses.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Consent to participate

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Consent for publication

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RESEARCH ARTICLE

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Proximal canopy sensing of twenty-two bread wheat genotypes for nutritional quality, yield attributes and grain yield under Mediterranean climate

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Abstract

To ensure nutritional security of rapidly increasing population, research interest has revitalized in determining the nutritional quality traits of staple food crops, especially wheat. Besides higher yield potential, research gaps exist regarding nutritional quality assessment of promising wheat genotypes grown under the Mediterranean climate. A field study was conducted to determine the relationship between yield components and quality characteristics of 22 bread wheat genotypes using the SPAD meter, GreenSeeker (NDVI), and CM-1000 chlorophyll meter at different growing stages (Stem elongation, Heading, Anthesis and Milk stage). The recorded findings revealed that G-41 genotype surpassed the rest of bread wheat genotypes by recording the maximum grain yield, whereas G-60 genotype exhibited the highest protein and wet gluten content. Among response variables, SPAD and NDVI values at the heading stage and CM-1000 values at the milk stage were found to be statistically insignificant. According to the correlation and biplot analysis, a significant positive correlation was found between the SPAD values measured for the stem elongation, anthesis and milk stage and yield and quality characteristics. Significant positive correlations were found between the NDVI values at the stem elongation, anthesis, and milk stages and the yield components, and between the CM-1000 value at the heading stage and the grain yield.

Keywords: Bread Wheat, SPAD, GreenSeeker, CM-1000, Yield, Quality

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INTRODUCTION

Under changing climate and rapidly increasing human population, crop production has become immensely vital to ensure food and nutritional security globally (Siddiqui et al., 2019; Iqbal, 2020; Oakes et al., 2024). Among staple food crops, wheat (*Triticum aestivum* L.) constitutes of one of the most strategic crops by feeding over half of the world's human populace (Arısoy, 2011; Chowdhury et al., 2021; Iqbal et al., 2021; Yildirim et al., 2022; Abbas et al., 2023). There are many wheat species, however, bread wheat is the most produced among wheat species (Iqbal et al., 2018; Choudhary et al., 2021; Sorour et al., 2021). Approximately one-third of the world's population depends on wheat as their primary food as it provides over 20% of daily calories and 55% of carbohydrates in nutrition worldwide (Alghawry et al., 2021; Kizilgeci, 2021; Zahoor et al., 2021). Previously, there has been persistent focus on boosting wheat grain yield under varying agro-climatic and soil conditions, whereas its nutritional quality assessment has got comparatively meager attention. A number of research findings have explicitly established that wheat genotypes differ pronouncedly in terms of nutritional quality traits by virtue of differences in their genetic make-up (Chowdhury et al., 2021; Darwish et al., 2024). Conventional breeding techniques primarily rely heavily on grain yield for identification and selection of wheat

genotypes having superior genetic make-up. Regan et al. (1992), Jansone et al. (2024) and Hassan et al. (2019) opined that destructive sampling techniques traditionally used to sort-out superior genotypes of wheat for desirable traits could be effective only in case when a small number of genotypes is under investigation. However, these sampling techniques tend to be labor-intensive, tedious, expensive and time-consuming. Moreover, it has been reported that sampling errors lead to misleading results in detecting and differentiating crop genotypes (Elliot and Regan, 1993; Ma et al., 2001; Babar et al., 2006; Kiran et al., 2015; Marino and Alvino, 2019; Zsebő et al., 2024).

Recently, proximal canopy sensing techniques hold bright perspectives for assessing wheat genotypes based on desired traits and that too without employing destructive sampling procedures. In breeding studies conducted in recent years, spectral reflection tools such as GreenSeeker and SPAD are preferred by many researchers as important selection tools in determining many parameters monitored in the cultivation of wheat (Yıldırım et al., 2009; Kızılgeçi et al., 2017). The GreenSeeker device is one of the most used spectral reflection instruments. Determination of efficiency parameters in studies using unmanned aerial vehicles is mainly based on NDVI analyzes (Gündoğan, 2018). Normalized difference vegetation index (NDVI) is used to characterize plant green area index, leaf area index, biomass and nutrient content via portable land devices or satellite. NDVI values can be used to determine physiological factors such as growth rate, vegetation area, earliness, plant leaf yellowing and yield. The NDVI technique can effectively determine the efficacy of farm inputs like irrigation water, fertilizers, etc. by determining their effect on plant canopy and photosynthesis rates (Pask et al., 2012).

The correlation between leaf chlorophyll content and SPAD meter measurements might provide valuable information pertaining to growth attributes and nature of association among them. In these studies, it was reported that leaf chlorophyll and leaf nitrogen concentration had a positive correlation with SPAD measurement data in barley, corn, rice and wheat (Schepers et al., 1992; Peltonen et al., 1995; Wienhold and Krupinsky, 1999). CM-1000 Chlorophyllmeter indirectly measures the chlorophyll content in the leaf without contacting the plant. CM- 1000 measurement data is one of the spectral reflection instruments used in recent years to monitor plant development, predict yield, monitor plant health, and detect plant nutrient deficiencies and stress conditions. In this study, the usability of the values measured with the help of GreenSeeker, SPAD and CM-1000 chlorophyll meters in some developmental periods of bread wheat genotypes grown in semi-arid conditions in determining the relationship between yield and quality elements was investigated. However, a significant research gap exists pertaining to nutritional profiling of promising wheat genotypes sown in the Mediterranean climate. Therefore, it was hypothesized that wheat genotypes might vary in their nutritional quality traits based on a typical genetic make-up along with their varying potential to respond to typical agronomic management practices. Thus, the prime aim of this study was to screen out promising wheat genotypes for boosting productivity under semi-arid conditions, while another strategic objective was to comparatively evaluate bread wheat genotypes in terms of their nutritional quality traits.

MATERIAL AND METHOD

The research was conducted in the research area of Teknobiltar R&D company in Diyarbakır province during the growing season of 2021-2022. The planting material of the trial included 22 bread wheat genotypes (8 commercial varieties and 14 advanced bread wheat lines). According to the physical and chemical analysis results of the soil samples taken from the trial area at a depth of 30 cm, the pH value was 7.87, the texture was clayey loamy, the organic matter content was low and the lime amount was moderately calcareous. The average monthly temperature values were above the long-term average during the crop-growing period. While the total amount of precipitation was determined as 319.20 mm, the total amount of precipitation was below the average for the long-term (492.6 mm). The research was set up according to the randomized complete blocks design (RCBD) with 4 replications. The sowing density was calculated as 550 seeds per m^2, and the experimental plots had a net area of 1.2 × 4 m. Sowing was done with a drill machine in 6 rows and the distance between rows was 20 cm. In the research, 60 kg of nitrogen and 60 kg of phosphorus were applied per hectare with planting. During the tillering period, 60 kg of nitrogen per hectare was applied twice during the growing season. Chemical control was carried out against weeds and pests. The study was carried out in rain-fed conditions. Harvesting was done with a combine harvester in the last week of June. Measurements of response variables were made with spectral reflection instruments were carried out during stem elongation, heading, anthesis and milk periods. Chlorophyll content was determined by taking the average of the measurements taken between 10:00 and 14:00 hours in sunny outdoors, in the middle region of the flag leaf of 5 plants in each experimental unit, using the SPAD-502 instrument, in a way that did not coincide with the transmission vein. The NDVI measurements were made with the help of the GreenSeeker (Handheld Crop) device in windless and cloudless weather, when the plant surface was not affected by any precipitation or weather conditions, between 10:00 and 14:00 of the day when sunlight was high. Leaf chlorophyll content was found by averaging the measurements of 5 plants in each parcel using the CM-1000 chlorophyll meter (Field Scout) instrument, which detects light at wavelengths of 700 nm and 840 nm, to estimate the amount of chlorophyll in the leaves. In the study grain yield, thousand grain weight, hectoliter weight, protein content, wet gluten content and sedimentation value were

measured. For quality analysis, samples taken from each plot were measured with Perten Inframatic 9500 NIR device without milling. The data were collected for all response variables under investigation that were subjected to one-way analysis of variance (ANOVA). Correlation analysis was also performed with the JMP Pro (10) statistical package program according to the randomized complete block design. Thereafter, significant difference comparisons between the mean values were determined according to the least significant difference (LSD) test employed at 5% probability level. Relationships among response variables were also determined using a biplot analysis.

RESULTS AND DISCUSSION

Thousand grain weight (g)

The recorded findings revealed that statistically significant ($p<0.001$) differences existed in terms of thousand grain weight among the bread wheat genotypes examined in the study (Table 1). When the average values were examined, it was recorded that the genotypes had thousand grain weights between 23.27 g (G-18) and 40.14 g (Empire Plus). The general average of the genotypes was found to be 33.65 g. These findings corroborate with the conclusions made by many researchers who inferred pronounced effects of environment and genotype on the thousand grain weight of wheat (Aydoğan et al., 2008; Aktar, 2011; Kızılgeçi et al., 2021). Likewise, Yildirim (2005) also inferred after comparatively assessing different bread wheat varieties that thousand grain weight of bread wheat varieties differed significantly and it was attributed to varying genotypic make-up of wheat genotypes which resulted in a typical grain weight. Moreover, Aktaş (2014), in a study conducted on 15 bread wheat varieties under rain-fed and irrigated conditions in Diyarbakır, Mardin and Malatya locations, reported that thousand grain weights varied between 30.4 - 40.8 g.

Table 1. Average yield and quality values of bread wheat genotypes examined in the study

Genotypes	Thousand grain weight(g)	Grain yield (kg ha^{-1})	Hectolitre weight (kg L^{-1})	Protein Content (%)	Wet Gluten Content (%)	Zeleny Sedimen. (ml)
Beyazhan	37.62 abc	5079.2 b-g	78.53 ab	15.48 def	35.98 def	62.50 cde
Ceyhan 99	34.66 fgh	5610.4 abc	77.63 b-e	15.03 e-j	35.03 e-j	54.00 e-i
DZ-20-4	37.53 bcd	4347.9 fg	78.25 abc	15.03 e-j	35.00 e-j	56.50 c-h
DZ-20-6	35.01 d-g	5502.1 abc	77.85 a-d	14.25 hij	33.28 hij	47.75 hi
DZ22-06	37.44 b-e	4795.8 c-g	75.15 fgh	15.23 d-h	35.43 d-h	58.25 c-g
DZ22-10	39.54 ab	4938.5 b-g	75.45 fg	15.38 d-g	35.73 d-g	57.75 c-g
Empire Plus	40.14 a	5563.5 abc	76.40 def	14.65 f-j	34.10 f-j	53.50 f-i
G-12	34.99 efg	4283.3 g	70.28 kl	16.13 cd	37.45 cd	63.25 bcd
G-18	23.27 k	4432.3 efg	71.93 jk	17.20 ab	39.90 ab	75.50 a
G-27	35.86 c-g	4512.5 d-g	79.60 a	15.35 d-g	35.68 d-g	58.50 c-g
G-3	37.14 b-f	4578.1 d-g	76.55 c-f	14.45 g-j	33.63 g-j	51.00 ghi
G-32	33.91 gh	5007.3 b-g	76.73 c-f	15.48 def	36.05 def	57.75 c-g
G-37	27.89 i	5189.6 b-e	76.00 efg	15.18 d-i	35.33 d-i	54.50 d-i
G-41	38.59 ab	6067.7 a	78.60 ab	14.88 e-j	34.68 e-j	53.75 e-i
G-47	24.87 jk	5674.0 ab	73.60 hij	14.88 e-j	34.65 e-j	52.50 f-i
G-48	25.98 ij	5467.7 abc	74.25 ghi	14.13 j	32.98 j	46.00 i
G-54	34.83 fg	5143.8 b-f	72.75 ij	14.18 ij	33.08 ij	47.25 i
G-60	24.19 jk	2962.5 h	65.48 m	18.05 a	41.80 a	79.75 a
G-70	32.27 h	5276.0 a-d	77.88 a-d	14.70 f-j	34.28 f-j	52.75 f-i
Hüseyin Bey	28.17 i	4262.5 g	69.80 l	15.40 d-g	35.80 d-g	60.00 c-f
Tekin	38.34 abc	5459.4 abc	78.22 abc	15.85 de	36.85 de	63.75 bc
Toros 1003	38.08 abc	5125.0 b-f	77.88 a-d	16.93 bc	39.25 bc	71.75 ab
General mean	33.65	4967.2	75.40	15.35	35.72	58.10
Mean square	116.11**	18123.7***	49.76***	3.92***	19.89***	303.56***
C.V.	5.32	11.68	1.66	4.68	4.54	10.66

, * significant at 1% and 0.1% respectively

Hectoliter weight (kg L^{-1})

There were statistically significant ($p<0.001$) differences in terms of hectoliter weight among bread wheat genotypes used in this study (Table 1). It was determined that the average hectoliter weight values of the genotypes varied between 65.48 kg L^{-1} and 79.60 kg L^{-1}. The highest hectolitre weight value was detected for G-27 genotype and the lowest value was detected in the G-60 genotype. The average of all genotypes was found to be 75.40 kg L^{-1}. Many researchers have reported that factors such as variety, ecological conditions and cultural practices impart pronounced influence on hectoliter weight of cereal crops including wheat (Aktar, 2011; Kendal et al., 2011) and it was also inferred that higher grain weight might be utilized as a reliable indicator to predict the grain yield potential of bread wheat genotypes.

Grain yield (kg ha^{-1})

It was observed that there were statistically significant ($p<0.001$) differences between bread wheat genotypes in terms of grain yield. It was recorded that the average grain yield values of the genotypes vary between 2962.5 and 6067.7 kg ha^{-1}. The highest yield value was detected in the G-41 genotype and the lowest value in the G-60 genotype. The average of all genotypes was found to be 4967.2 kg ha^{-1}. The G-60 genotype included in the study had the lowest grain yield due to its late flowering and insufficient rainfall. These findings corroborate with those of Iqbal et al. (2021) who opined that genetic differences among wheat genotypes might be attributed to a typical grain yield recorded for different wheat genotypes. It was also inferred that different genotypes hold varying potential to respond and utilize farm input (irrigation water, fertilizers, etc.) which resulted in pronounced differences in their grain yield potential under similar agronomic management plans and agro-climatic conditions.

Protein content (%)

It was observed that there were statistically significant ($p<0.001$) differences in protein content between bread wheat genotypes. The average protein content values of the genotypes varied between 14.13% and 18.05%. While the highest protein value was obtained in the G-60 genotype, the lowest value was obtained in the G-48 genotype. The general average of genotypes was found to be 15.35%. These findings are in agreement with those of Walsh et al. (2023) who inferred that proximal canopy analysis revealed a significant difference in protein content of wheat genotypes and also opined that these techniques resulted in more precise findings in comparison to destructive sampling techniques and thus these could might be suggested for assessing genotypic difference among wheat genotypes in terms of protein content. It was also inferred that protein content of bread wheat genotypes was a complex quantitative trait that is generally controlled by multiple genes and also get influenced by the interaction effects of genotype and environment. Therefore, non-destructive sampling techniques become vital to evaluate bread wheat genotypes to identify high-performing and stable genotypes in order to recommend them for cultivation in target areas.

Wet gluten content (%)

It was observed that there were statistically significant ($p<0.001$) differences between bread wheat genotypes in terms of wet gluten content. It is seen that the average wet gluten values of the genotypes vary between 32.98% and 41.80%. The highest gluten value was detected in the G-60 genotype and the lowest value was detected in the G-48 genotype. The average of all genotypes was found to be 35.72%. In bread production, the amount and quality of gluten in the structure of the grain is an effective factor in the formation of bread during the fermentation stage. Özen and Akman (2014) stated that the amount of gluten in the grain structure varies according to the variety and ecological conditions.

Zeleny sedimentation (ml)

When Table 1 was examined, it was seen that there were statistically significant differences ($p<0.001$) in terms of sedimentation content among the bread wheat genotypes used. It is seen that the average sedimentation values of the genotypes vary between 46.00 ml and 79.75 ml. The highest sedimentation value was detected in the G-60 genotype and the lowest value in the G-48 genotype. The average of all genotypes was found to be 58.10 ml. The sedimentation value, which determines the protein quality of wheat grain and has a high degree of heritability, provides information about the bread quality of wheat and is an important quality criterion (Koçak et al., 1992).

Normalized differences vegetation index (NDVI)

Based on recorded findings, it was determined that there existed statistically significant differences among bread wheat genotypes in terms of NDVI values measured during stem elongation. The average NDVI values of the genotypes varied between 0.29 and 0.67. The highest NDVI value was determined in the DZ22-10 genotype and the lowest value in the G-60 genotype. The general average of the genotypes was found to be 0.54. G-60 genotype had the lowest

NDVI value because it was a late flowering genotype compared to other genotypes and could not adequately cover the soil surface. However, there was no statistically significant difference between the bread wheat genotypes under investigation in terms of NDVI values measured during the heading stage.

Table 2. NDVI values measured at different developmental periods of bread wheat genotypes examined in the study

Genotypes	Stem Elongation	Heading	Anthesis	Milky
Beyazhan	0.59 a-f	0.73	0.69 b-e	0.63 b-e
Ceyhan 99	0.53 b-g	0.74	0.71 a-d	0.67 ab
DZ-20-4	0.50 c-g	0.74	0.69 b-e	0.61 ef
DZ-20-6	0.50 c-g	0.73	0.71 a-d	0.64 b-e
DZ22-06	0.60 a-e	0.72	0.69 b-e	0.59 f
DZ22-10	0.67 a	0.72	0.66 e	0.60 ef
Empire Plus	0.63 a-d	0.72	0.71 a-d	0.62 def
G-12	0.61 a-e	0.75	0.72 abc	0.64 b-e
G-18	0.43 gh	0.74	0.74 a	0.66 abc
G-27	0.53 a-g	0.75	0.71 a-d	0.63 c-f
G-3	0.61 a-e	0.72	0.70 a-e	0.60 ef
G-32	0.65 ab	0.74	0.70 a-e	0.66 a-d
G-37	0.55 a-g	0.75	0.71 a-d	0.66 a-d
G-41	0.58 a-f	0.74	0.72 a-d	0.65 a-d
G-47	0.48 efg	0.73	0.73 ab	0.69 a
G-48	0.45 fg	0.73	0.71 a-d	0.66 a-d
G-54	0.54 a-g	0.72	0.71 a-d	0.65 a-d
G-60	0.29 h	0.70	0.68 cde	0.64 b-e
G-70	0.50 c-g	0.73	0.74 a	0.66 a-d
Hüseyin Bey	0.49 d-g	0.70	0.68 de	0.63 c-f
Tekin	0.55 a-g	0.74	0.69 b-e	0.63 cde
Toros 1003	0.64 abc	0.74	0.73 ab	0.63 b-e
General mean	0.54	0.73	0.71	0.64
Mean square	0.03	ns	0.0001*	0.0001
C.V.	18.54**	3.67	4.05*	4.51

*, 5% and **, 1% significant. ns: non significant

It was observed that there were statistically significant differences at the 5% level in terms of NDVI values measured during the anthesis stage. It was determined that the average NDVI values of the genotypes varied between 0.66 and 0.74. The highest NDVI value was detected in the G-18 genotype and the lowest value in the DZ22-10 genotype. The general average of the genotypes was found to be 0.71. NDVI values measured during the flowering period were determined to have statistically significant differences at the 1% level. It was determined that the average NDVI values of the genotypes varied between 0.59 and 0.69. The highest NDVI value was detected for G-47 genotype. whereas the lowest value was recorded for DZ22-06 genotype. The general average of the genotypes was found to be 0.64. These results are in concurrence with those of Lopes and Reynolds (2012) and Zsebő et al. (2024) who inferred that NDVI values remained effective in differentiating the wheat genotypes and could be preferred over destructive sampling techniques to sort out the most performing genotypes. Moreover, Swoish et al. (2022), Guan et al. (2019) and Naser et al. (2020) determined the NDVI index by employing red and near-infrared spectra from GreenSeeker sensor measurements and inferred that it remained effective in quantifying the crops' canopies greenness and assessing plant health changes over time.

Chlorophyll content (SPAD)

It was observed that there were statistically significant differences between bread wheat genotypes in terms of SPAD values measured during the stem elongation period. The average SPAD values of the genotypes varied between 45.90 and 53.05. The highest SPAD value was detected in the G-60 genotype and the lowest value in the DZ-20-4 genotype. The general average of the genotypes was found to be 49.96. It was determined that there was no statistically significant difference in terms of SPAD values measured during the heading period. It was observed that there were statistically significant differences at the 0.1% level between genotypes in terms of SPAD values measured during the flowering period. The average SPAD values of the genotypes varied between 50.55 and 56.93. The highest SPAD value was detected in the G-60 genotype and the lowest value in the Ceyhan 99 variety. The general average of the genotypes was found to be 52.82.

Table 3. SPAD average values of bread wheat genotypes examined in the study

Genotypes	Stem Elongation	Heading	Anthesis	Milky
Beyazhan	48.28 c-f	47.43	51.25 ef	51.25 h
Ceyhan 99	50.05 bc	49.85	50.55 f	52.15 gh
DZ-20-4	45.90 f	48.28	51.25 ef	50.90 h
DZ-20-6	51.88 ab	49.10	52.23 def	56.03 bcd
DZ22-06	48.58 cde	49.35	50.98 ef	53.00 e-h
DZ22-10	49.58 bcd	51.00	52.65 def	55.10 b-f
Empire Plus	48.60 cde	49.43	51.35 ef	53.53 d-h
G-12	51.78 ab	48.85	54.38 bcd	56.05 bcd
G-18	51.50 ab	48.38	52.90 cde	54.30 c-g
G-27	52.90 a	53.33	55.18 abc	55.83 b-e
G-3	46.03 ef	48.58	52.83 def	54.30 c-g
G-32	50.30 bc	49.10	52.13 def	52.95 e-h
G-37	51.73 ab	49.18	55.85 ab	55.83 b-e
G-41	49.90 bc	49.68	52.53 def	54.65 c-g
G-47	49.73 bcd	51.70	50.78 ef	51.13 h
G-48	47.23 def	48.30	51.33 ef	52.60 fgh
G-54	51.38 ab	52.10	53.95 bcd	57.93 ab
G-60	53.05 a	48.63	56.93 a	59.78 a
G-70	50.78 abc	50.63	52.88 c-f	53.78 c-h
Hüseyin Bey	48.28 c-f	52.60	55.90 ab	56.58 bc
Tekin	49.85 bc	53.23	52.73 def	55.85 b-e
Toros 1003	51.80 ab	50.78	51.60 ef	52.25 fgh
General mean	49.96	49.98	52.82	54.35
Mean square	16.37***	11.63 ns	13.2***	20.79***
C.V.	3.67	5.86	3.13	3.82

***, significant at 0.1%. ns: non significant

It was observed that there were statistically significant differences at the level of 0.1% in terms of SPAD values measured during the milk maturation period. The average SPAD values of the genotypes varied between 50.90 and 59.78. The highest SPAD value was detected in the G-60 genotype and the lowest value in the DZ-20-4 genotype. The general average of the genotypes SPAD value was 54.35. Carlson and Ripley (1997) reported similar findings whereby spectral techniques remained effective in determining the phenotypic and yield potential difference among genotypes of field crops and also inferred that phenotype measurements using these techniques especially SPAD could produce accurate and precise results free of sampling errors as in case of destructive sampling of bread wheat genotypes.

Leaf chlorophyll content (CM-1000)

The results revealed that statistically significant differences existed among bread wheat genotypes in terms of CM-1000 values measured during the stem elongation period. The average CM-1000 values of the genotypes varied between 384.00 and 605.00. The highest CM-1000 value was detected in the G-54 genotype. and the lowest value was detected in the G-60 genotype. The general average of the genotypes was found to be 487.68.

It was observed that there were statistically significant differences in terms of CM-1000 values measured during the heading period. The average CM-1000 values of the genotypes varied between 400.50 and 689.25. The highest CM-1000 value was detected in the G-47 genotype. and the lowest value was detected in the G-60 genotype. The general average of the genotypes was found to be 526.97.

It was observed that there were statistically significant differences between bread wheat genotypes in terms of CM-1000 values measured during the flowering period. The average CM-1000 values of the genotypes varied between 347.00 and 714.25. The highest CM-1000 value was detected in the G-3 genotype. and the lowest value was detected in the Tekin genotype. The general average of the genotypes was determined as 559.78.

It was also observed that there existed no statistically significant difference in terms of CM-1000 values measured during the milky stage.

Table 4. CM-1000 average values of bread wheat genotypes examined in the study

Genotypes	Stem Elongation	Heading	Anthesis	Milky
Beyazhan	423.50 ef	559.50 b-g	414.50 ef	466.00
Ceyhan 99	504.50 cd	525.25 c-ı	529.75 b-e	530.25
DZ-20-4	413.75 ef	440.25 jk	406.50 ef	421.50
DZ-20-6	453.75 def	525.25 c-ı	501.50 de	457.50
DZ22-06	454.50 def	496.50 f-j	539.00 b-e	405.50
DZ22-10	455.75 def	522.25 d-j	504.50 cde	420.25
Empire Plus	536.50 abc	443.50 ıjk	594.75 a-d	464.75
G-12	468.25 cde	460.25 h-k	607.50 a-d	473.75
G-18	471.50 cde	545.00 b-g	608.50 a-d	500.00
G-27	525.75 bcd	548.50 b-g	634.75 a-d	484.00
G-3	470.50 cde	484.00 f-j	714.25 a	464.25
G-32	506.25 cd	527.75 c-h	593.25 a-d	477.75
G-37	503.00 cd	562.00 b-f	618.00 a-d	525.75
G-41	410.25 ef	478.25 g-k	651.25 abc	473.00
G-47	602.50 ab	689.25 a	639.25 a-d	542.50
G-48	520.50 cd	617.00 ab	573.00 a-d	528.50
G-54	605.00 a	601.75 bcd	568.75 a-d	435.00
G-60	384.00 f	400.50 k	622.75 a-d	475.00
G-70	530.75 a-d	498.00 e-j	498.50 de	486.00
Hüseyin Bey	515.00 cd	605.50 bc	652.75 ab	445.75
Tekin	459.75 c-f	484.00 f-j	347.00 f	440.75
Toros 1003	513.75 cd	579.00 b-e	495.25 de	460.75
General mean	487.68	526.97	559.78	471.75
Mean square	12822***	18052***	32951**	5482^{ns}
C.V.	11.39	11.01	18.65	13.10

, 1% and *, 0.1% significant. ns: non significant

Correlation Analysis

NDVI Correlation Analysis

A significant negative correlation of 5% was determined between the NDVI values measured during the stem elongation period with protein (r= -0.216) and wet gluten content (r= -0.214). A positive significant relationship was determined between NDVI measurement values and grain weight (r= 0.218) between hectolitre (r= 0.375) at the level of 1% and between thousand kernel weight (r= 0.530) at the level of 0.1%. A significant positive relationship was found only between the NDVI values measured during the heading period and the hectoliter (r= 0.245) and no significant relationship was detected between the other examined characteristics. A significant positive correlation was also found between the NDVI values measured during the anthesis period and grain yield (r= 0.246) but no significant correlation was found between the other examined characteristics. A significant negative correlation was found between NDVI values measured during the milky period and thousand grain weight (r= -0.363) and a positive correlation between grain yield (r= 0.234).

Savaşlı et al. (2012) reported that the correlation between NDVI measurement values and grain yield was high, especially in the early period. Sultana et al. (2014) found a positive relationship between NDVI measurement values and grain filling time, ripening time, and yield. Karaman (2017) reported that there is a positive relationship between NDVI measurement values made during milking stages of wheat and grain yield. Kizilgeci and Yildirim (2021) determined a significant correlation between NDVI and grain yield at anthesis stage under rain-fed conditions.

SPAD Correlation Analysis

A significant positive correlation was determined between SPAD values measured during the stem elongation period and sedimentation (r= 0.231) at the 5% level and between protein (r= 0.276) and wet gluten (r= 0.274) at the 1% level. There was no significant positive or negative relationship between the SPAD measurement data at the heading stage and the examined traits. Kızılgeçi et al. (2017) reported that there was a high correlation between SPAD measurement data and thousand grain weight values during the heading period. SPAD values measured during the anthesis stage were significantly correlated with thousand grain weight (r:-0.248) at negative 5% level, with hectolitre (r:-0.410) and grain yield (r:-0.452) at negative 0.1% level. A significant relationship was found between SPAD measurement values

and protein (r= 0.257), wet gluten (r= 0.258) and sedimentation (r= 0.227) at the 5% level.

Debaeke et al. (2006) reported that there was a linear correlation between the SPAD measurement data observed during the anthesis stage of the wheat plant and the grain yield and protein content of the grain. Fotovat et al. (2007) stated that there was a positive correlation between SPAD measurement data and yield values in bread wheat. A significant negative relationship was determined between SPAD values measured during the milky stage and grain yield (r= -0.235) at the level of 5%. and between hectoliter (r= -0.417) at the level of 0.1%. Bahar and Bahar (2016) reported that there was a positive correlation between the SPAD measurement data observed in the anthesis and dough formation stages of the wheat plant and the quality parameters protein, wet gluten and zeleny sedimentation values.

Table 5. Correlation coefficient values showing the relationships of yield and quality characteristics based on data obtained with spectral reflectance instruments

		Protein	Hectoliter	Wet Gluten	Zeleny Sedim.	TKW	Grain yield
NDVI	Stem Elongation	**-0.216***	**0.375****	**-0.214***	-0.186	**0.530*****	0.183
	Heading	0.091	**0.245***	0.099	0.090	0.033	0.154
	Anthesis	-0.048	0.094	-0.045	-0.055	-0.144	**0.246***
	Milky	-0.025	-0.017	-0.022	-0.068	**-0.363****	**0.234***
SPAD	Stem Elongation	**0.276****	-0.147	**0.274****	**0.231***	-0.158	-0.183
	Heading	-0.089	0.053	-0.089	-0.095	0.034	-0.002
	Anthesis	**0.257***	**-0.410*****	**0.258***	**0.227***	**-0.248***	**-0.452*****
	Milky	0.199	**-0.417*****	0.201	0.171	-0.139	**-0.235***
CM-1000	Stem Elongation	-0.176	-0.043	-0.175	-0.196	-0.175	0.197
	Heading	-0.183	0.036	-0.183	-0.194	**-0.279****	**0.294****
	Anthesis	0.132	**-0.347****	0.124	0.087	**-0.348****	-0.174
	Milky	0.095	-0.067	0.096	0.046	**-0.366****	0.192

*,**,*** are significant at 5%, 1%, 0.1% respectively

CM-1000 Correlation Analysis

The results revealed that there was no significant relationship between the CM-1000 values measured during the stem elongation stage and the examined characteristics. A significant negative correlation of 1% was found between CM-1000 values measured during the heading period and thousand grain weight (r= -0.279). A positive significant relationship was found between CM-1000 measurement values and grain yield (r= 0.294) at the 1% level. A significant negative relationship was determined at the 1% level between CM-1000 values measured during the flowering period and hectoliter (r= -0.347) and thousand grain weight (r= -0.348). A negative significant relationship was found at the 1% level between CM-1000 values measured during the milky period and thousand grain weight (r= -0.366).

Biplot Analysis

The Biplot analysis effectively examined and evaluated the relationships between spectral reflection instruments and yield and quality characteristics (Figure 1). In the vector representation of biplot analysis, each feature examined in the research is represented by a vector. It is understood that as the vector length that is the distance to the biplot starting point increases the variation between genotypes increases in terms of the characteristics examined and as the vector length shortens the variation between genotypes decreases (Karaman, 2019). In this regard, it was observed that the variation was generally high in all characteristics under investigation. When the vector representations of the data obtained with spectral reflection instruments were examined, it was elucidated that the variation between genotypes is high in terms of NDVI and CM-1000 values and the variation between genotypes is reduced in terms of SPAD values. Moreover, when quality parameters were examined, it was revealed that the variation between genotypes was recorded to be generally higher. In the biplot analysis technique, each feature examined has been represented by a vector. The cosine of the angle between the vectors indicated the value of the Pearson correlation between these two features. In other words, if the angle between the vectors was less than 90°. it indicated a positive correlation between the examined features. whereas greater than 90°indicated a negative correlation. and an angle of 90° exhibited absences of any correlation (Kendal and Sayar, 2016; Kendal et al. 2016). The biplot analysis performed for all features examined in the research is given in Figure 1. When the biplot chart was examined. PC1 (24.5%) and PC2 (13.2%) constituted 37.7% of the total variation. According to biplot analysis, it was determined that there was a positive relationship between the SPAD value measured during stem elongation. anthesis and milky stages and protein, wet gluten and zeleny sedimentation. According to biplot analysis, there is a positive relationship between the NDVI value measured during the stem elongation period and the hectoliter weight and thousand grain weight;

A positive relationship was determined between the NDVI value measured during the anthesis and milk periods and grain yield. According to biplot analysis, a positive correlation was found between CM-1000 measurement data during the heading period and grain yield.

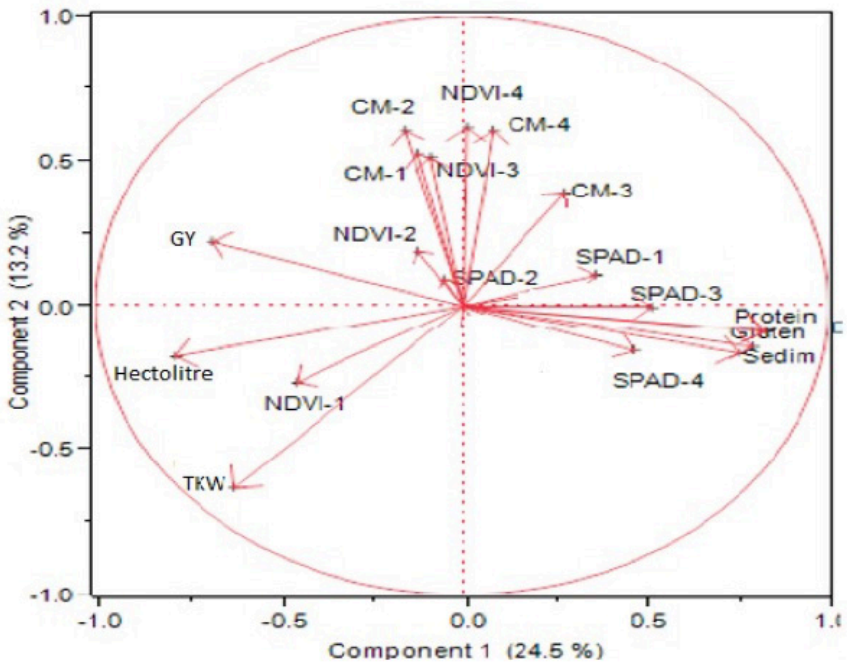

Figure 1. Biplot analysis showing recorded efficiency and quality standards achieved with spectral transmission tool NDVI-1: Normalized vegetation index in the stem elongation stage. NDVI-2: Normalized vegetation index in the heading stage. NDVI-3: Normalized vegetation index in the anthesis period. NDVI-4: Normalized vegetation index in the milk stage. SPAD-1: Chlorophyll content in the stem elongation stage. SPAD-2: Chlorophyll content in the heading period. SPAD-3: Chlorophyll content in the stem elongation stage. SPAD-4: Chlorophyll content in the milk stage. CM-1: Leaf chlorophyll content in the stem elongation period. CM-2: leaf chlorophyll content in heading period. CM-3: leaf chlorophyll content in anthesis period. CM-4: leaf chlorophyll content in milk stage. TKW: Thousand kernel weight. GY: Grain yield

According to correlation analysis and Biplot analysis, a positive correlation was determined between the SPAD value measured during stem elongation, anthesis and milk stages and protein, wet gluten and sedimentation. There is a positive relationship between the NDVI value measured during the stem elongation stage and the hectoliter weight and thousand grain weight; A positive correlation was determined between the NDVI value measured during the anthesis and milky stage and grain yield. It was determined that there was a positive correlation between CM-1000 measurement data during the heading period and grain yield.

CONCLUSION

The recorded findings remained in line with the postulated hypothesis as bread wheat genotypes under investigation varied significantly in terms of yield attributes and nutritional quality traits. It was concluded that the SPAD meter effectively determined the yield and quality characteristics of bread wheat genotypes at varying development periods in semi-arid conditions. Additionally, the NDVI value viably differentiated bread wheat genotypes during the stem elongation. anthesis and milky stage. Moreover. it was inferred that CM-1000 can be used to determine the grain yield of bread wheat during the heading period. At the same time. it has been concluded that these spectral reflection devices can be used as a selection tool in breeding studies. considering the plant development period. Overall. G-41 genotype surpassed rest of bread wheat genotypes under investigation by recording the maximum grain yield that might be recommended to wheat growers for general adaptation under semi-arid climate. Moreover, the GreenSeeker device provided more reliable and accurate winter wheat yield prediction data along with being lesser expensive and simpler device for farmers to use on a large scale.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The arthurs declare no conflict of interest.

Author contribution

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Rhizoctonia species, anastomosis groups, and pathogenicity isolated from common bean in Lake Van Basin, Turkiye

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Abstract
Common bean (*Phaseolus vulgaris* L.) is an important vegetable crop grown in Lake Van Basin. Local genotypes are widely grown in the region. *Rhizoctonia* root rot induced by *Rhizoctonia solani* Kühn is an important soilborne plant disease that leads to global economic losses as well as in Turkey. The present study was conducted to determine anastomosis groups and pathogenicity of *Rhizoctonia* spp. obtained from bean plants in Lake Van Basin in 2013 and 2014. A total of 236 *Rhizoctonia* isolates in 5 anastomosis groups were obtained from bean plant roots. It was observed that AG- 4 (112) was the most isolated group in beans, followed by AG- 2 (41), AG- 3 (28), AG- 5 (33), and binucleate AG- K (22) isolates. Pathogenicity test conducted in thirty isolates in 5 anastomosis groups was analyzed for A64 (Bitlis/ Adilcevaz), TR68557 genotypes, and Gina (cv.) under growth chamber conditions. The study findings demonstrated that all tested isolates could infect the bean plant with different degrees of severity; however, the most virulent group was AG- 4. It was determined that the most virulent isolate was Isolate No. 19 in the A64 genotype, Isolate No. 2 in TR68557, and Isolate No. 18 in Gina cv. in *in vivo* tests. The identification and pathogenicity determination of *Rhizoctonia* isolates are the first steps towards an efficient control strategy for bean diseases caused by *Rhizoctonia* species. In order to obtain quality and productive products in the Van Lake Basin, where intensive bean production is carried out, precautions should be taken by considering the damage caused by *Rhizoctonia* spp. on plants.
Keywords: Anastomosis group, Bean, Pathogenicity, *Rhizoctonia* spp.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.), which is an edible leguminous plant, is significant in local and international agriculture and is commonly consumed in Turkey, as well as in the world. Beans are rich in minerals, vitamins, and protein (18- 31.6%) and highly important for human nutrition (Suárez-Martínez et al., 2016; Ntatsi et al., 2018; Ekincialp and Şensoy, 2018; Gavilanes et. al., 2020). China ranks first in world bean production and Turkey ranks fourth with 630.347 tons, which accounts for approximately 2.6% of the world production (Faostat, 2017). The yield and quality of this legume plant, which is significant for the nation's economy, are adversely affected by various biotic (fungal, bacterial, and viral disease agents) and abiotic factors (Costa-Coelho et al., 2014). *Rhizoctonia* spp., which is one of the most important pathogens in cultivated plants, causes substantial losses in bean production (Yıldırım and Erper, 2017). Disease agent causes yield loss of up to 90% in bean (Palacıoglu et al., 2019). *Rhizoctonia* spp. causes diseases such as wilt and root rot, could emerge in the seedling period as well as in the latter stages of growth and causes early infections that result in plant death (Jaiswal et. al., 2014). It was determined that this pathogen caused

damping-off disease in the seedling phase of plants and root, hypocotyl, capsule, grain rot, and web blight in the latter phases of the plant development (Muyolo et al., 1993; Valentín Torres et. al., 2016).

The number of nuclei in *Rhizoctonia* cells helps with their morphological classification. Those, commonly with two nuclei in their hyphae cells, were called binucleate *Rhizoctonia* and those with multinucleate were called *R. solani*. The other polynuclear species *Waitea circinata* is subdivided into two groups named *Rhizoctonia zeae* and *Rhizoctonia oryzae* (Sneh et al., 1991). Multinucleate *Rhizoctonia solani* (MNR) and binucleate *Rhizoctonia* (BNR) were divided into anastomosis groups (AG) based on the anastomosis reactions between the hyphae of isolates (Oladzad et al., 2019; Nandeesha et. al., 2021). While *R. solani* is divided into 14 anastomosis groups (AG- 1, AG- 13, including AG-BI as a subset of AG-2), several anastomosis groups were also divided into groups in themselves (Sharon et al., 2008; Yıldırım and Erper, 2017). *Rhizoctonia* anastomosis groups with binucleate were divided into 18 groups (AG- A, AG-W) (Sharon et al., 2008; Yang et al., 2015; Dong et al., 2017; Marcou et al., 2021).

Studies reported that both *R. solani* and binucleate *Rhizoctonia* AG were isolated from bean plants. Such isolates exhibited different degrees of virulent properties. *R. solani* AG- 4 was found to be the most common anastomosis group in the world that caused root rot in bean plants (Papavizas et al., 1975; Bolkan and Ribeiro, 1985; Karaca et al., 2002; Kılıçoğlu and Özkoç, 2013). Furthermore, other AGs (AG- 1, AG- 1- IB, AG- 2- 1, AG- 2- 2 and AG- 5) were reported to be prevalent in bean plants and to cause damage in several regions (Muyolo et al., 1993; Valentín Torres et. al., 2016). Binucleate *Rhizoctonia,* which were commonly isolated, were reported to be AG- A, AG- F, and AG- K (Bolkan and Ribeiro, 1985; Muyolo et al., 1993; Nerey et al., 2010).

In Turkey, various AGs were determined in studies conducted on *R. solani* and the anastomosis groups (AG) of the binucleate *Rhizoctonia.* In the Central Anatolia Region, *R. solani* AG- 5 was isolated prevalently from bean plants (Tuncer and Erdiller, 1990). It was reported that BN *Rhizoctonia* AG- K was isolated from hypocotyl in Erzurum province and was followed by BNR AG- A, AG- I, AG- E and MNR AG- 4 and AG- 5, respectively (Demirci and Döken, 1995). A study conducted in Samsun province indicated that *R. solani* AG- 4, AG- 5, and AG- 2- 2 were isolated (Karaca et al., 2002) and another study conducted in the same province concluded that *R. solani* AG- 1IB, AG- 4, AG- 5, AG- 6 and binucleate *Rhizoctonia* as AG- A, AG- Bc and AG- K were isolated from the bean plant and the rhizosphere soil (Erper, 2003). In another study 38 Rhizoctonia isolates were obtained from bean pods (AG-1 IB (1 isolate), AG-2-1 (4 isolates), AG-4 (24 isolates), AG-5 (5 isolates), AG-E (2 isolates) and AG-K (2 isolates). When conducting a pathogenicity test on bean leaves, it was found that the AG-1 IB isolate was the most virulent, followed by the AG-4 and AG-5 isolates, respectively (Akarca and Demirci, 2022). The surveys conducted in the bean plantation sites in Van province by Temizel and Ertunç (1992) indicated that *A. alternata, F. solani, M. phaseolina, Drechslera sorokiniana, R. solani, Stemphylium sp.,* and *Botrytis cinerea* were determined as important disease agents encountered.

Bean cultivation is widely practiced within the Van Lake Basin, encompassing both indigenous and commercial varieties. The utilization of local strains holds particular significance for resource preservation efforts, aiming to minimize harm to plant populations. Particularly in areas afflicted by prevalent diseases, the adoption of specific varieties remains limited. Soil-borne diseases pose a significant threat to bean crops, with root and root collar rot emerging as a primary concern. It can be defined as the most common disease in beans. It was determined as the most common problem in the survey areas visited in the Van Lake Basin. In the study of Temizel and Ertunç (1992), it was stated that *R. solani* was among the disease agents encountered as an important problem in bean plants in Van, but anastomosis groups were not mentioned. It can be taken into account that with the knowledge of anastomosis groups of this pathogen, new sustainable strategies can be developed to determine and control the host status.

The present study was intended to isolate *Rhizoctonia* species from common bean plants cultivated in Lake Van Basin, to identify anastomosis groups, and to determine the plant response of local A64 and TR68557 genotypes and commercial Gina (cv.) cultivars against disease agents. Therefore, in this study, *Rhizoctonia* density and anastomosis groups, their distribution and virulence were determined for the first time in beans in Van basin.

MATERIALS AND METHODS

The A64 bean genotype used in the present study was obtained from the Adilcevaz district in Bitlis province which is located within the boundaries of Lake Van Basin. TR68557 bean genotype was obtained from the gene bank of the Aegean Agricultural Research Institute.

Survey areas and sampling

Field surveys were conducted during the bean cultivating season of 2013 and 2014. Districts of Van (Erciş, Gevaş, Edremit) and Bitlis (Ahlat, Adilcevaz, Tatvan), where bean cultivation was carried out intensively in the basin, were selected as the survey areas. Based on the size of the growing area, 10 to 15 plant samples, which showed symptoms of root rot, were retrieved. The samples were collected randomly through diagonal movements between the corners

of the growing area. The plant samples were placed in polyethylene bags, brought to the laboratory in an icebox, and stored at 5 °C in the refrigerator until the isolation procedure.

Isolation of Rhizoctonia isolates

The roots of the plant samples were washed with tap water and were cleared from soil residues. Pieces were cut from the stem and hypocotyls in sizes of 1 to 2 millimeters, both from the tissues with necrosis and the healthy tissues. The cut pieces were kept in 0.5% sodium hypochlorite (NaOCl) solution for 1 minute for surface sterilization. The pieces were rinsed twice in distilled sterile water and then were retrieved to sterile blotting papers. Subsequently, these pieces were placed in Petri dishes containing 1.5% water agar (WA), to which 50 mg/L of streptomycin sulfate was added, to prevent bacterial contamination (Demirci and Döken, 1993). The Petri dishes were incubated at 23- 25 °C for 48 to 72 hours. Hyphae, which had the general characteristics of *Rhizoctonia* genus, were left to Potato Dextrose Agar (PDA) with hyphae tip isolation to obtain pure culture and were incubated in the dark for 3 to 5 days at 25 °C. The isolates, which were obtained as pure cultures, were stored in test tubes containing PDA at 5 °C.

Determination of anastomosis groups of Rhizoctonia isolates

The isolates obtained in the present study were *R. solani* and binuclear *Rhizoctonia* and were identified based on Ogoshi's (1975) method, depending on the growth, morphological characteristics, sclerotia presence in the isolates incubated in PDA in the dark, at 25 °C, for 7 days and the microscopic characteristics of the isolates incubated in SA, in the dark, at 25 °C for 7 days. The *Rhizoctonia* isolates were identified by utilizing standardized techniques for anastomosis group determination, based on the characteristics of their vegetative hyphae (Ogoshi, 1975), requirement for thiamine (Rovira et al., 1986), and hyphal anastomosis with known tester strains of *Rhizoctonia* AGs. Test isolates used to determine anastomosis groups were obtained from the culture collection of Atatürk University and the Mycology Laboratory of the Plant Protection Department in the Faculty of Agriculture at Yüzüncü Yıl University.

The isolates obtained in this study and test isolates were kept in PDA for 7 days at 25 °C and were paired in 1.5% SA. Therefore, mycelium discs, retrieved from the test isolates and the isolates obtained from the plants with a 5 mm diameter sterile cork borer, were correspondingly placed at a distance of 4 cm, were incubated at 25 °C for 48 to 72 hours, and later were examined directly under light microscopy to determine whether there were the conditions of a cell wall and cytoplasmic interconnection between the hyphae in the aligned colonies (Parmeter et al., 1969). If anastomosis was observed between the hyphae of two paired isolates, these isolates were defined as the same AG (Demirci and Döken, 1992).

Pathogenicity test

Two bean genotypes (A64, TR68557) and a commercial Gina cultivar were used in the pathogenicity tests of *Rhizoctonia* isolate retrieved from different regions. Pathogenicity test was designed with 30 *Rhizoctonia* isolates (3 from AG-K, 5 from AG-2, 3 from AG-3, 15 from AG-4, and 4 from AG-5), to determine the virulence of anastomosis groups. Bean seeds were subjected to surface sterilization for 5 minutes in 1% NaOCl and then were dried.

Pathogen isolates were developed on PDA at 25 °C for one week, to prepare the inoculum. Wheat grains used as the inoculum medium were moistened with pure water and boiled for a while, placed in closed Petri dishes, and autoclaved for 2 days in a row for one hour at 121 °C. Mycelial pieces retrieved from the isolates developed in PDA were inoculated with sterile wheat grains and the Petri dishes were incubated in the dark at 25 °C for two weeks.

Sterilized bean seeds were planted in pots with a mixture of peat and perlite with a 2:1 ratio. The pathogenicity study was carried out by placing 5 wheat grains colonized with isolate around the root collar and 5 sterile wheat grains in the control pots during the first true leaf formation period of the seedlings. Three plants were used for each isolate, and the experiment was repeated twice. For control, 10 plants were used in each experiment (Erper et al., 2011). After keeping the plants at 25 ± 2 °C for 16 hours in the light and 8 in the dark for 6 to 8 weeks, the plants were uprooted, their root lengths were measured and disease severity was rated based on a 0- 4 scale adopted from Muyolo et al. (1993). Each strain was isolated from the plant again and was confirmed by test isolates.

Statistical analysis

The data obtained in the study were evaluated by one-way analysis of variance using the SPSS statistical program with a significance level of $p \leq 0.05$. In the analysis of the data, the differences between the statistically significant means were grouped according to the Duncan Multiple Comparison Test.

RESULTS AND DISCUSSION

Species and anastomosis groups of Rhizoctonia isolates

Distribution of *Rhizoctonia* isolates obtained from the diseased plant samples taken from Erciş, Gevaş, Edremit, Tatvan,

Ahlat, and Adilcevaz districts in Lake Van Basin were given in Table 1. Field studies were carried out during September-October of 2013- 2014. A total of 236 *Rhizoctonia* isolates were obtained from bean plants in Lake Van Basin. According to these results, 48 isolates were obtained from the Erciş district in 2013 and 20 isolates were obtained in 2014; 21 isolates were obtained from Gevaş in 2013 and 11 isolates were obtained in 2014; 16 isolates were obtained from Edremit in 2013 and 15 isolates were obtained in 2014; 22 isolates were obtained from Ahlat in 2013 and 17 isolates were obtained in 2014; 32 isolates were obtained from Adilcevaz in 2013; 34 isolates were obtained from Tatvan in 2013 and 2014.

A total of 236 *Rhizoctonia* spp. obtained common beans were paired with test isolates, it was defined that 112 isolates belonged to *R. solani* AG-4, 41 to *R. solani* AG-2, 33 to *R. solani* AG-5, 28 to *R. solani* AG-3, and 22 binucleate *Rhizoctonia* AG-K.

Table 1. Number of isolates of *Rhizoctonia* species and anastomosis groups isolated from common bean plants according to years and locations

Rhizoctonia spp.	AGs	Districts and Sampling Year												Total
		Erciş		Gevaş		Edremit		Ahlat		Adilcevaz		Tatvan		
		2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	
Binucleate *Rhizoctonia*	K	8	2	2	1	-	2	-	-	5	-	2	-	22
R. solani	2	12	5	6	3	2	-	3	1	4	-	3	2	41
R. solani	3	3	-	2	-	2	3	5	4	3	-	4	2	28
R. solani	4	24	10	11	3	5	10	14	8	15	-	5	7	112
R. solani	5	1	3	-	4	7	-	-	4	5	-	3	6	33
Total		48	20	21	11	16	15	22	17	32	-	17	17	
General Total		68		32		31		39		32		34		236

Pathogenicity test

As a result of the surveys carried out in bean cultivation areas within the Lake Van Basin, pathogenicity tests were performed for 30 isolates obtained from the roots of the diseased plants, the plants were uprooted, and root lengths were measured and evaluated according to 0-4 scale.

The results of the pathogenicity tests, performed for 15 isolates that were selected from the 112 obtained AG-4 isolates, were presented in Table 2. It was determined that there was no statistically significant difference between isolates, based on the effect on plant root length ($p \leq 0.05$). The same case applied to genotypes in terms of root length. AG-4 isolates had significant differences from the control group ($p \leq 0.05$). The highest scale was determined for isolates 19 and 18 and the lowest scale was determined for isolate 20 for the A64 genotype. It was determined that isolate 2 was the most virulent among tested isolates for the TR68557 genotype and isolate 18 was more virulent for the Gina cultivar and they were not much affected by the others.

Of 41 AG-2 isolates obtained, pathogenicity tests were performed with randomly selected 5 isolates considering each district, and the results were presented in Table 3. There was no significant difference between isolates and genotypes in terms of root length values (Duncan Multiple Comparison Tests $p \leq 0.05$). However, the difference in the disease severity scale was significant when compared with the control group ($p < 0.05$). In the A64 genotype, isolate 14 had higher scale values, while isolate 9 was more effective for TR68557 9, and isolates 9 and 27 were more effective for Gina. It was also observed that the highly effective isolate for TR68557 and Gina was found to be the least effective isolate for A64.

Pathogenicity tests were conducted on 33 AG-5 isolates, taking into account each district, four isolates were randomly selected for each test and the results were shown in Table 4. There were no significant differences between the isolates in terms of root length ($P \leq 0.05$). Although there were statistically significant differences between the isolates in terms of scale values, the values were close to each other and the control group. Isolate 10 on A64 and TR68557 and isolate 5 on Gina caused the highest scale values.

Table 2. Root length and scale values of AG-4 isolates according to pathogenicity results in genotypes

Isolates AG-4	Genotypes					
	A64		TR68557		Gina	
	Root length (cm)	Scale*	Root length (cm)	Scale	Root length (cm)	Scale
1	27.5_{NS}±2.18	1.55_{ab}±0.96	36.89_{ab}±8.07	0.78_{ab}±0.39	37.11_{NS}±2.22	0.89_{bc}±0.51
2	38.2±9.06	1.83_{ab}±0.76	40.30_{ab}±10.89	2.11_{a}±1.84	27.05±5.20	0.67_{bc}±0.29
3	41.61±15.51	1.28_{a-c}±1.11	35.05_{ab}±0.67	1.89_{a}±0.77	33.67±5.20	1.44_{b}±1.0
4	31.26±7.89	1.22_{a-c}±0.51	35.55_{ab}±4.14	1.11_{ab}±0.69	33.33±7.51	0.78_{bc}±0.69
7	36.70 ±22.60	1.05_{a-c}±0.82	31.11_{b}±7.35	1.55_{a}±1.26	32.69±12.34	1.39_{b}±1.09
8	33.64±4.30	1.66_{ab}±0.58	37.44_{ab}±6.50	0.88_{ab}±0.69	28.89±0.84	1.16_{bc}±0.60
11	35.6 ± 8.41	1.28_{a-c}±0.75	35.27_{ab}±1.46	1.22_{ab}±0.51	30.61±2.34	1.05_{bc}±0.63
12	34.55±6.05	1.11_{a-c}±0.39	39.38_{ab}±5.09	1.16_{ab}±0.44	37.93±7.87	1.61_{ab}±0.79
13	27.97±13.14	2.11_{ab}±0.84	35.15_{ab}±3.03	1.11_{ab}±0.39	35.16±1.92	0.83_{bc}±0.44
18	37.17±8.98	2.33_{a}±0.67	36.55_{ab}±10.22	1.67_{a}±0.58	39.17±1.89	2.67_{a}±0.58
19	33.61±9.75	2.44_{a}±0.96	36.77_{ab}±4.11	1.44_{a}±0.51	52.39±39.58	0.44_{bc}±0.51
20	37.66±3.18	0.77_{bc} ±0.20	32.48_{b}±6.67	0.99_{ab}±0.34	48.61±28.07	0.89_{bc}±0.20
21	28.66±6.51	1.22_{a-c} ±0.84	39.92_{ab}±6.43	1.39_{ab}±0.98	27.66±6.08	0.10_{bc}±0.34
22	33.39±4.58	1.22_{a-c} ±0.70	38.54_{ab}±3.04	1.22_{ab}±0.19	40.33±25.79	1.22_{bc}±1.17
23	40.88±12.32	1.11_{a-c} ±0.84	36.44_{ab}±1.39	1.89_{ab}±0.19	32.78±8.59	0.94_{bc}±0.42
Control	37.89±6.68	0_{c}	46.27_{a} ±10.74	0_{b}	37.67±8.40	0_{c}

*: Plants were evaluated using the 0-4 scale; There were significant differences among the different letter(s) at P ≤ 0.05 level (according to Duncan's multiple comparison test.); NS: Not Significant (P ≤ 0.05)

Table 3. Root length and scale values of AG-2 isolates according to pathogenicity results

Isolates AG-2	Genotype					
	A64		TR68557		Gina	
	Root length (cm)	Scale*	Root length (cm)	scale	Root length (cm)	scale
6	25.94_{NS}±14.64	1.22_{a}±0.19	36.33_{NS}±2.65	1.66_{b}± 0.88	44.22_{NS}±10.19	0.89_{a}±0.54
9	25.75 ±13.07	0.61_{ab} ±0.67	33.83±0.71	3.0_{a} ± 1.0	35.67±10.07	1.28_{a}±0.25
14	28.83±3.76	1.44_{a} ±0.19	33.05±4.99	1.55_{b} ± 0.51	36.22±7.18	0.83_{a}±0.29
16	34.77±6.77	1.22_{a} ±0.63	34.69±2.47	1.72_{b} ± 0.54	29.78±2.55	1.22_{a}±0.38
27	29.54 ±1.06	1.11_{a} ±0.84	37.66±8.20	1.55_{b} ± 0.51	45.55±8.44	1.28_{a}±0.75
Control	37.89 ±6.68	0_{b}	46.27±10.71	0_{c}	37.67±8.40	0_{b}

*: Plants were evaluated using the 0-4 scale; There were significant differences among the different letter(s) at P ≤ 0.05 level (according to Duncan's multiple comparison test.); NS: Not Significant (P ≤ 0.05)

Table 4. Root length and scale values of AG-5 isolates according to pathogenicity results

Isolates AG-5	Genotype					
	A64		TR68557		Gina	
	Root length (cm)	scale *	Root length (cm)	scale	Root length (cm)	scale
5	35.89_{NS}±14.42	0.67_{ab}±0.29	34.22_{ab}±1.07	0.77_{ab}±0.51	42.67_{NS}±13.05	1.67_{a} ±0.76
10	33.82±7.93	0.99_{a}±0.58	35.11_{ab}±1.26	1.11_{a}±0.51	36.22±5.39	0.94_{ab}±0.63
15	32.95±11.86	0.89_{a}±0.51	33.22_{b}±3.34	0.99_{a}±0.34	27.61±3.46	1.33_{a} ±0.58
17	29.67±7.51	0.89_{a}±0.52	37.55_{ab}±8.18	0.77_{ab}±0.77	41.89±17.48	0.66_{ab}±0.88
Control	37.89±6.68	0_{b}	46.27_{a}±10.74	0_{b}	37.67±8.40	0_{b}

*: Plants were evaluated using the 0-4 scale; There were significant differences among the different letter(s) at P ≤ 0.05 level (according to Duncan's multiple comparison test.); NS: Not Significant (P ≤ 0.05)

Table 5 presents the pathogenicity results of the three AG-3 isolates from 28 AG-3 isolates obtained. The comparison of the A64 genotype root isolates with the control group indicated a significant difference (P≤ 0.05). Although there existed statistically significant differences between the isolates and the control group in terms of scale values, there were no significant differences among the isolates (P≤ 0.05). Isolate 24 was the highest-ranking isolate in terms of both root length and the scale. No significant differences were observed between the isolates and the control group in terms of the root length and scale for TR68557 (P≤ 0.05). A similar condition was observed for the root length for Gina and isolate 26 had the highest scale value.

Table 5. Root length and scale values of AG-3 isolates according to pathogenicity results in genotypes

Isolates	Genotype					
	A64		TR68557		Gina	
AG-3	Root length (cm)	scale *	Root length (cm)	scale	Root length (cm)	scale
24	36.55_{a}±4.69	1.28_{a}±0.25	34.94_{NS}±0.26	1.33_{NS}±1.15	33.93_{NS}±0.81	1.28_{a}±0.75
25	25.10_{b}±4.98	1.33_{a}±0.58	32.99±8.08	1.11±0.39	36.39±18.57	0.78_{b} ±0.48
26	31.11_{ab}±0.39	1.11_{a}±0.69	41.22±3.34	0.99±0.58	31.22±4.44	1.77_{a}±0.51
Control	37.89 a ±6.68	0_{b}	46.27±10.74	0	37.67±8.40	0_{b}

*: Plants were evaluated using the 0-4 scale; There were significant differences among the different letter(s) at P ≤ 0.05 level (according to Duncan's multiple comparison test.); NS: Not Significant (P ≤ 0.05)

A total of 22 AG-K isolates were obtained, and three were used for pathogenicity. The examination of the root length and scale values in Table 6 indicated that there were no statistically significant differences between the isolates, however, the comparison of TR68557 and Gina with the control group indicated a significant difference and they were placed in different groups (P≤ 0.05).

Table 6. Root length and scale values of AG-K isolates according to pathogenicity results in genotypes

Isolates	Genotype					
	A64		TR68557		Gina	
AG-K	Root length (cm)	scale *	Root length (cm)	scale	Root length (cm)	scale
28	37.20_{NS}±1.97	0.89_{NS}±0.84	37.72_{NS}±2.94	1.22_{a}±0.19	30.37_{NS}±2.28	1.22_{a}±0.19
29	36.55±6.15	0.89±0.51	34.11±3.47	1.44_{a}±0.51	36.60±10.91	1.22_{a}±0.48
30	31.27±6.34	1.05±0.63	37.11±5.34	1.11_{a}±0.19	31.54±7.73	1.33_{a}±0.67
Control	37.89±6.68	0	46.27±10.74	0_{b}	37.67±8.40	0_{b}

*: Plants were evaluated using the 0-4 scale; There were significant differences among the different letter(s) at P ≤ 0.05 level (according to Duncan's multiple comparison test.); NS: Not Significant (P ≤ 0.05)

The pathogenicity test for detecting virulence more or less indicated the signs of disease in most applications, except for the control plants. Once the all results were examined, it was determined that the group that caused the greatest damage was AG-4. In the majority of the applications with isolates, there were no significant differences in root lengths with the control group.

The mean root lengths and scale values of all isolates from different anastomosis groups are presented in Table 7. Accordingly, it was determined that the root length was statistically significant between groups and genotypes, except for Gina. In A64 and TR68557, the AG-2 isolate decreased the root length at the highest value when compared to the control group. In scale values, AG-4 caused the highest disease severity on the A64 genotype, AG-2 on TR68557. Gina's genotype was affected by all anastomosis groups.

Table 7. Root length and scale results of all isolates of anastomosis groups according to genotypes

Anastomosis groups	Genotype					
	A64		TR68557		Gina	
	Root length (cm)	scale *	Root length (cm)	scale	Root length (cm)	scale
AG-4	34.56_{ab}±9.54	1.48_{a}±0.79	36.37_{b}±5.38	1.29_{b}±0.71	35.83_{NS}±14.39	1.13_{a}±0.76
AG-2	28.97_{b}±8.67	1.12_{ab}±0.56	35.21_{b}±4.39	1.89_{a}±0.84	38.29±9.19	1.09_{a}±0.45
AG-5	33.08_{ab}±9.51	0.86_{b}±0.43	35.03_{b}±4.18	0.91_{b}±0.49	37.09±11.55	1.15_{a}±0.73
AG-3	31.22_{ab}±5.71	1.24_{ab}±0.48	36.39_{b}±5.74	1.14_{b}±0.69	33.85±9.81	1.28_{a}±0.67
AG-K	35.01_{ab}±5.33	0.94_{ab}±0.59	36.31_{b}±3.89	1.26_{b}±0.32	32.84±7.36	1.26_{a}±0.43
Control	37.89_{a}±5.64	0_{b}	46.27_{a}±9.07	0_{b}	37.67±7.10	0_{b}

*: Plants were evaluated using the 0-4 scale; There were significant differences among the different letter(s) at P ≤ 0.05 level (according to Duncan's multiple comparison test.); NS: Not Significant (P ≤ 0.05)

Bean cultivation is extensive in the Lake Van Basin. Both local and commercial varieties are used for cultivation in the region. The use of local varieties is particularly important in terms of conserving resources. Conservation is also possible by minimizing damage to plants. Specifically, the use of varieties is quite limited in areas where diseases are prevalent. Among all diseases, soil-borne diseases in beans are highly significant. It is possible to state that root and root collar rot is the most prevalent disease in bean plants. It was specified as the frequently encountered problem

in the surveyed areas in Lake Van Basin. The general symptoms in plants can be summarized as the slowing down of development, shortening of the upper parts of the plant and root section, and drying in later phases. The main fungus species causing this disease are *Rhizoctonia*. *Rhizoctonia* spp. is a fungal pathogen that is common and polyphasic throughout the world (Naik et al., 2016; Aydın and Ünal, 2021). A total of 236 *Rhizoctonia* isolates were obtained as a result of survey studies conducted in the basin areas. The identification of the species and anastomosis groups of the isolates indicated that the majority of the isolates were *R. solani* AG-4 (112). Several studies reported that the most common anastomosis group in beans was AG-4. This was followed by *R. solani* AG-2 (41), *R. solani* AG-5 (33), *R. solani* AG-3 (28), and Binucleate *Rhizoctonia* AG-K *(22)*, respectively. Other studies also mentioned that *Rhizoctonia* groups were isolated from beans. Tuncer and Erdiller (1990) reported that they isolated *R. solani* AG-5 in Central Anatolia Region, Demirci and Döken (1995) reported the isolation of AG-4, AG-5 and AG-K from the hypocotyl of bean plants, and Eken and Demirci (2004) stated that they isolated AG-2-1, AG-3, AG-4, AG-5 and AG-K from bean cultivation areas in Erzurum. The isolation from the beans in Samsun indicated that AG-4 was the most widespread group and was followed by AG-5 (Erper et al., 2011). Spedaletti et. al. (2016), reported AG 2-2 from bean in Argentina. Mora-Umaña et. al. (2013) detected different anastomosis groups (AG 1-IA, AG 1-IB, AG 1-IC, AG 1-ID, AG 2-2, AG 2- 2IIIB, AG 2-2IV and AG 4) in Costa Rica. For instance, in Japan, eight of the forty-five Rhizoctonia strains that were isolated from thirty different types of crops, including beans, were BN, while 37 were MN (Misawa and Kurose 2019). From the necrotic roots of 425 symptomatic bean plants that were collected from nine provinces in Turkey, 65 isolates of *Rhizoctonia* spp. were identified. *Rhizoctonia solani* was identified in fifty isolates with multinucleate hyphae. These isolates belonged to seven anastomosis groups: AG-1 (one isolate), AG-1-IA (one isolate), AG-2-1 (six isolates), AG-4-HGI (18 isolates), AG-4-HGII (17 isolates), AG-4-HGIII (five isolates), and AG-5 (one isolate). In contrast, fifteen isolates, AG-F (7) and AG-K (8), were *Rhizoctonia* sp. with binucleate (BN). In pathogenicity tests, three of these anastomosis groups (AG-1, AG-1-IA, and AG-2-1) were found to be non-pathogenic, whereas the remaining groups caused disease severities ranging from 71 to 100% on bean plants (Canpolat et al., 2023). Salman and Boyraz (2023), collected *Rhizoctonia* spp. isolates from plant samples in Konya, Turkey. Ten *R. solani* isolates obtained from beans. Nine of the bean isolates were determined as multinucleic. One isolate from the bean was determined as binucleic. Accordingly, the anastomosis groups of Fa 3.2 (97%), Fa 2.2 (89%) and Fa 1 (86%) in beans were characterized as AG 4HGI.

The anastomosis groups of the isolates identified in the present study are consistent with similar studies. Temizel and Ertunç (1992) indicated that *R. solani* is one of the important disease factors encountered in bean plants in Van however anastomosis groups were not specified. Therefore, the present study determined the *Rhizoctonia* concentration and anastomosis groups and their virulence for the first time in bean plants cultivated in the Lake Van Basin.

As a result of the surveys carried out in bean cultivation areas within the Lake Van Basin, pathogenicity tests were performed for 30 isolates obtained from the roots of the diseased plants. Total of 15 from 112 AG-4 isolates, 5 of 41 AG-2 isolates, 4 of 33 AG-5 isolates, 3 of 28 AG-3 isolates, and 3 of 22 AG-K isolates were used for pathogenicity testing. In selecting the isolates to be used in pathogenicity testing, the distribution of each group in districts was taken into consideration. The results indicated no statistically significant difference between the isolates and genotypes in terms of root lengths; however, there existed decreased root lengths in plants with the disease. It was also determined that the scale values of the isolates were statistically significantly different ($p \leq 0.05$). An overall wilt, growth deficiency, yellowing, and drying in the upper parts of the plants were observed in the plants affected by the disease. The uprooted plants indicated symptoms such as shortness of the roots, black roots, and darkened color of the root collar. The pathogenicity test for detecting virulence more or less indicated the signs of disease in most applications, except for the control plants. The examination of the average of the results indicated that the group causing the greatest damage to plants was AG-4. Correspondingly, Eken and Demirci (2004) stated that the most virulent AG group in beans were the isolates that belonged to AG-5 and AG-4 groups. In Samsun, AG-4 was determined as the most virulent isolate in pathogenicity tests (Erper et al., 2011). In another study, AG-2, AG-4 and AG-5 isolated from faba beans had high virulence, while AG-K was found to be weak (Genç Kesimci et al., 2022). In addition, Omar et al. (2021), obtained from the bean cultivation areas were *Fusarium* (62.5%) followed by *Rhizoctonia* (27.5%) and *R. solani* AG-4 were ranging from 26.7%–50% in the pathogenicity test. It was stated that *R. solani* AG-4 was the most common group that caused bean root rot in the world (Papavizas et al., 1975; Bolkan and Ribeiro, 1985; Karaca et al., 2002; Matloob and Juber, 2013).

The examination of the average root lengths and scale values of all isolates belonging to anastomosis groups, of which the pathogenicity tests were performed, indicated that the root lengths were statistically significant between groups and genotypes except Gina. Scale values suggested that the A64 genotype was mostly affected by AG-4, TR68557 genotype was mostly affected by AG-2, and Gina was affected by all isolates. Based on these outcomes, it was possible to suggest that Gina cultivar is sensitive to *Rhizoctonia* spp. Cankara (2019) study was to determine the reactions of some bean varieties commonly grown in Turkey to *R. solani*. There were significant differences between the pathogen isolates in terms of theirs symptoms and virulence. The reaction of bean varieties against the disease

varied significantly depending on the pathogen isolate. In another study examining the reactions of some bean cultivars against *R. solani* AG-4, 13 cultivars were evaluated as moderately resistant and 17 as susceptible (Palacıoğlu et al., 2019). In a study in which anastomosis groups of *R. solani* isolates obtained from different provinces of Turkey were identified according to sequence analysis of the rDNA-ITS region, it was determined that the most common group was AG-4. In the pathogenicity tests performed with different bean varieties, it was determined that there were significant differences between the variety reactions depending on the pathogen isolates. When all the results were analyzed, no cultivar showed a resistant reaction against all isolates (Palacıoğlu et al., 2024).

CONCLUSION

The study aimed to isolate *Rhizoctonia* fungi from bean plants in the Lake Van Basin, identify anastomosis groups (AGs), and assess their virulence through pathogenicity tests. Five AGs were isolated, with AG-4 being the most virulent and AG-5 the weakest, indicating severe disease factors in the basin. Due to its soil origin, *Rhizoctonia* disease in beans is difficult to control, with cultural methods showing limited effectiveness (Conner et al., 2014; Gossen et al., 2016). Therefore, integrated management strategies, including antagonistic microorganisms or chemical control, are necessary. However, chemical control poses environmental and health risks and can lead to resistance in microorganisms. Breeding studies are a crucial aspect of disease management in Turkey, but they are influenced by the presence of various *Rhizoctonia* AGs on beans. Effective resistance breeding and rotation strategies depend on identifying *Rhizoctonia* AGs and subgroups, as their host suitability varies. Screening AGs among isolates and selecting resistant cultivars are essential for managing *Rhizoctonia* disease in common bean production, emphasizing the importance of understanding *Rhizoctonia* diversity and pathogenicity.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Data availability

Not applicable.

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RESEARCH ARTICLE

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Metal bioaccumulation in some Auchenorrhyncha (Hemiptera) species in apple orchards

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Abstract

Traffic is an important pollution factor causing environmental damages such as soil, water and atmospheric pollution, greenhouse effect, and climate change. Effects of traffic pollution on various organisms enlightened with various studies. In this study, it was aimed to examine the effects of traffic-based pollution on Auchenorrhyncha (Insecta: Hemiptera) species in apple orchards and their potential as biomonitor for heavy metal pollution. The Auchenorrhyncha specimens were collected from the apple orchards near the Amasya-Samsun motorway in Türkiye. The heavy metal concentrations were determined by ICP-OES. Five Auchenorrhyncha species were determined from three sites from each of three different distance. *Empoasca decipiens* specimens were collected only from 0 m while others found in all localities. Heavy metal concentrations in insect specimens tended to decrease with the increasing distance from the motorway. These differences were clearly indicated in *Psammotettix provincialis* and *Phlepsius intricatus*, which were found in all localities. Except for Ni, Fe and Mn for *Phlepsius intricatus,* all examined heavy metals significantly varied in both species. Results showed that heavy metals tended to accumulate in the body of Auchenorrhyncha specimens and because of this they may be evaluated as a biomonitor for heavy metal pollution.

Keywords: Auchenorrhynca, Apple orchard, Metal, Insect, Air pollution, Bioindicators

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INTRODUCTION

Traffic is an important pollution factor in terrestrial ecosystems. Traffic-based pollutants tend to accumulate on the surface and tissues of organisms, causing metabolic changes and damages. Additionally, dispersal and diversity of organisms are also affected by pollution factors. Previous studies presented a negative effect of motorways on some groups of animals (Trombulak and Frissell, 2000; Forman et al., 2003; Muñoz et al., 2015). Traffic-related pollutants such as HC, CO, NOx, PM, SO_2 and heavy metals may change the nutrient status of plants and affect herbivore feeding indirectly. Although there are several studies on effects of traffic-based pollution on organisms, still there is a little knowledge about insects.

In general, traffic pollution affects insects by two ways (external environmental pollution and through contaminated foods). It is known that insects usually feed on plants and traffic pollution have an important impact on plants. Previous studies reported that impacts of pollution on plant-feeding insects may be in three types (1) affecting habitat quality, (2) plant quality, or (3) the life of natural enemies (Zvereva and Kozlov, 2006; Butler and Trumble, 2008).

In the literature, studies about the effect of traffic pollution on insects were usually carried on aphids because of ease of collection and feeding monophagous on plants (Devkota and Schmidt, 2000; Viskari et al., 2000; Boyd, 2009). Other studies focused on Aranea, Coleoptera Odonata, Lepidoptera and Hymenoptera (Samways et al., 1997; Severns, 2008; Hayward et al., 2010; Melis et al., 2010; Soluk et al., 2011). Karavin (2024) investigated the heavy metal concentrations accumulated in the bodies of Auchenorrhyncha species depending on the distance from the road in cherry orchards and found that the heavy metal concentrations in the specimens increased as the distance from the motorway decreased.

In the case of aphids, it was observed that plants exposed to air pollutants were usually preferable hosts. For example, it was found that aphids feeding on plants exposed to traffic pollutants exhibited higher relative growth rates and population density (Dohmen et al., 1984; Warrington et al., 1987; Houlden et al., 1990; Heliövaara and Vaisanen, 1993; Summers et al., 1994; Gao et al., 2008). Furthermore, the effects of metal pollution on the immune system of ants and night butterflies were studies by Sorvari et al. (2007) and van Ooik et al. (2008). A study on usage of insects as bioindicators for metal pollution were carried out by Nummelin et al. (2007). Negative effects were reported in weight, growth, survival, reproduction and hatching success for insects due to heavy metal pollution in the previous studies (Boyd and Martens, 1998; Kramarz and Stark, 2003; Scheirs et al., 2006; Noret et al., 2007; van Ooik et al., 2008; Butler and Trumble, 2008).

Some of the predatory insects, which can accumulate high amounts of metals in their bodies can be used as bioindicators for the environments (Nummelin et al., 2007; Riaz et al., 2023). Proper usages of insects as bioindicators for heavy metal pollution were indicated with the previous studies (Parikh et al., 2021; Adelanwa et al., 2016; Girotti et al., 2020; Riaz et al., 2023). Adelanwa et al. (2016) reported that *Salvinia molesta* is a good phytomediator for copper (Cu) and lead (PB) (Riaz et al., 2023).

Because of their morphological characteristics, high reproduction rate, and great mobility range, butterflies, spiders and honeybees are used as biomonitors for environmental pollution (Girotti et al., 2020; Murashova et al., 2020; Riaz et al., 2023).

This study aimed to determine the impacts of traffic pollution on bioaccumulation of heavy metals in Auchenorrhyncha species in apple orchards and their potential as biomonitor for heavy metal pollution. Auchenorrhyncha species can have impacts on plants by feeding and, in some cases, transmitting plant pathogens. Some of them are important pests for economically important plants (Guglielmino, 2000; Rizwan et al., 2020). Because the Auchenorrhyncha species have various niches in urban, agricultural land and forestland. Therefore, assessing their role of bioindicator of heavy metal could be important because they can be found in very different environments.

MATERIALS AND METHODS

The study was carried out in apple orchards near the motorway in Amasya (40°45'17.3"N 35°44'00.7"E), Türkiye. Sweeping nets were used to collect Auchenorrhyncha specimens. Samplings were performed in three sites from each of three different distances from the motorway, 0 m, 50 m, 100 m in the apple orchards. Before preparation, the samples were kept in 5% acetic acid solution. Specimens were examined in detail under stereo-microscope. Genital parts of the specimens were removed with the help of a dissection needle. Species were identified by comparing with the descriptions and figures given in Ribaut (1936), Kalkandelen (1974), Ossiannilsson (1981) and Holzinger et al. (2003).

In order to determine the heavy metal contents of specimens, microwave extraction method was applied to dried and milled specimens (Naccarato et al., 2020; Karavin, 2024). Heavy metal contents were measured by using ICP-OES as mg.kg -1 dry weight. SPSS 20 was used for statistical analyses. Normality tests were performed for data set and homogeneity of variance was tested by the Levene's test. One-way ANOVA (analysis of variance) was used to compare the means. Differences between the means were analyzed with the Tukey test when the variances were homogeneous, and with the Welch test where they were not homogeneous.

RESULTS AND DISCUSSION

Five Auchenorrhyncha species: *Empoasca decipiens* Paoli, 1930; *Laodelphax striatella* (Fallén, 1826); *Arboridia versuta* (Melichar, 1897); *Psammotettix provincialis* (Ribaut, 1925) and *Phlepsius intricatus* (Herrich-Schaffer, 1838) were identified in the apple (*Malus domestica* Borkh.) orchards and examined for heavy metal bioaccumulation (Table 1). *E. decipiens* specimens were collected only from 0 m while other species found in all sites.

According to the results, there was a decreasing trend in the heavy metal concentrations in Auchenorrhyncha species in apple orchards with the increasing distance from the motorway and it is suggest that likely this is due to the traffic pollution. The maximum heavy metal concentrations were determined in *A. versuta* while the minimum values were measured in *P. provincialis* and *P. intricatus*. The average heavy metal concentrations determined in the examined species are compatible with the literatüre. The heavy metal concentrations of the specimens collected in this study were similar to the values determined in apple leaves by Karavin and Ural (2016). Karavin and Ural (2016) reported same decreasing trend in the heavy metal concentrations in the apple leaves with the increasing distance from the motorway.

Because *P. provincialis* and *P. intricatus* were collected from more than one plots in all sites, the variations in the heavy metal concentrations in specimens due to distance from the motorway were indicated for them (Figure 1, 2). Except nickel (Ni), iron (Fe) and manganese (Mn) for *P. intricatus*, all the examined heavy metals were significantly varied in both species.

Similar decreasing trends in the heavy metal concentrations in *A. versuta, P. intricatus, L. striatella* and *P. provincialis* in the cherry orchards with the increasing distance from the motorway were also found in the study of Karavin (2024). *E. decipiens* could only be collected at 0 m in the apple orchards, similarly, it was found more commonly at 0 m, and in only one plot at 50 m in cherry orchards. However, none were collected at 100 m in either orchard. Therefore, these results suggest that *E. decipiens* may prefer areas closer to the roadsides in both orchards.

Most Auchenorrhyncha species feed on plant sap, so they directly absorb the heavy metals found in plant sap. Cadmiun (Cd), zinc (Zn), copper (Cu), arsenic (As), Fe, mercury (Hg) and lead (Pb) are usually reported in superworms, yellow mealworms, termites, locusts, black soldier fly larvae and grasshoppers (Malematja et al., 2023). In some previous studies conducted with grasshoppers, heavy metals such as Pb and Cd were found in insect bodies (Handley et al., 2007; Zhang et al., 2009; Poma et al., 2017).

It has been reported that the amount of heavy metals in some insects used as food, such as grasshoppers, ants and locusts, are above the levels recommended by the World Health Organization (Muhammad et al., 2022). As in soil and plants, the heavy metal with the highest concentration in the examined Auchenorrhyncha species is Fe. Similarly, Denloye et al. (2015) and Mézes (2018) reported high concentrations of Fe in termites, grasshoppers and locusts. Azam et al. (2015) reported that the most accumulated heavy metal in insect species such as *Crocothemi servilia*, *Oxya hyla hyla* and *Danaus chrysippus* was Cd and it was followed by Cu, Chromium (Cr) and Ni, respectively (Riaz et al., 2023). It was explained that heavy metal concentrations in insects varied in accordance with the pollution level of the area. It was reported that Cu concentration in worker termites found in southwestern Nigeria was 0.076 mg/L (Idowu et al., 2019), in termites collected from natural habitats was 0.08-0.18 mg/L (Kapaale et al., 2021) and in housefly larvae was 9.06 mg/kg (Gao et al., 2019). The accumulation of heavy metals in insects varies depending on the pollution status in the habitat, insect species and heavy metal type (Ng'ang'a et al., 2021; Malematja et al., 2023).

According to the results of the study, the number of species did not vary according to the distance from the motorway. This means that traffic-related pollution did not have a deterrent effect on the species in apple orchards established close to the motorway. However, the high accumulation of heavy metals in specimens collected from the plots near the motorway suggested that there may be heavy metal contamination in apples grown close to the motorway. In fact, in the study conducted by Karavin and Ural (2016) in apple orchards, it was found that the concentration of heavy metals in apple leaves was higher in the plots near the motorway. The findings of both studies showed that for plant and human health, fruit orchards should be established in the areas far from the motorways to avoid heavy metals.

CONCLUSION

Although there is also metal pollution in agricultural lands caused by pesticides, fertilizer applications, vehicles and tools which are used in agricultural activities, the results obtained in this study reflect the effects of traffic pollution since sampling was done according to the distance from the highway and heavy metal concentrations were evaluated according to the distance from the highway. Results clearly indicates significant variations in heavy metal concentrations in the specimens based on traffic pollution. Decreasing trends were determined in the heavy metal concentrations in Auchenorrhyncha species in apple orchards with the increasing distance from the motorway and it is suggest that likely this is due to the traffic pollution. Results of this study showed that heavy metals tended to accumulate in the body of Auchenorrhyncha specimens and because of this they may be evaluated as a biomonitor for heavy metal pollution, especially *A. versuta*. It is thought that similar studies to be conducted in different areas are needed to clearly reveal the heavy metal accumulation in Auchenorrhyncha species due to pollution and the use of these species as biomonitors.

Table 1. Means metal concentrations in specimens.

Empoasca decipiens			**Metals (mg.kg^{-1} ± SD)**							
m	Plot	n	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb
0	1	21	0.59 ±0.0002	1.23 ±0.0008	0.37 ±0.0017	2.69 ±0.0052	8.24 ±0.0013	0.56 ±0.0013	0.08 ±0.0022	3.19 ±0.0025
	2	8	2.84 ±0.0071	4.88 ±0.0236	2.28 ±0.0326	10.72 ±0.0759	63.92 ±0.5116	1.32 ±0.0015	0.44 ±0.0329	15.00 ±0.0314
	3	10	1.39 ±0.0022	2.91 ±0.0033	0.67 ±0.0016	4.32 ±0.0045	7.64 ±0.1476	0.45 ±0.0001	0.12 ±0.0190	6.64 ±0.0679
Laodelphax striatella										
0	1	9	0.81 ±0.0006	1.70 ±0.0002	0.41 ±0.0001	2.15 ±0.0071	12.59 ±0.0355	0.25 ±0.0015	0.07 ±0.0021	3.40 ±0.0039
	2	10	0.72 ±0.0002	1.47 ±0.0005	0.45 ±0.0013	2.77 ±0.0027	8.68 ±0.0293	0.48 ±0.0007	0.13 ±0.0074	4.10 ±0.0019
50	2	13	0.01 ±0.0000	0.02 ±0.0001	0.09 ±0.0002	0.87 ±0.0045	4.36 ±0.0546	0.11 ±0.0010	0.05 ±0.0018	0.73 ±0.0087
100	1	12	0.03 ±0.0009	0.05 ±0.0012	0.05 ± 0.0003	0.50 ±0.0063	2.14 ±0.0193	0.3 ±0.0025	0.18 ±0.0018	0.88 ±0.0175
	2	8	0.04 ±0.0014	0.07 ±0.0002	0.07 ±0.0008	0.61 ±0.0029	2.22 ±0.0180	0.13 ±0.0006	0.23 ±0.0007	1.21 ±0.0220
Arboridia versuta										
0	1	7	3.57 ± 0.0034	7.57 ±0.0168	2.28 ±0.0048	14.71 ±0.0245	42.46 ±0.1901	13.42 ±0.1403	0.55 ±0.0031	18.58 ±0.0336
50	1	15	0.98 ±0.0022	2.07 ±0.0043	0.69 ±0.0009	3.79 ±0.0009	14.53 ±0.0275	0.32 ±0.0004	0.10 ±0.0004	4.72 ±0.0104
	2	9	1.50 ±0.0006	3.17 ±0.0030	1.06 ±0.0008	3.41 ±0.0057	11.79 ±0.0227	0.65 ±0.0032	0.23 ±0.0075	7.23 ±0.0741
100	1	7	0.05 ±0.0019	0.16 ±0.0119	0.53 ±0.0014	4.69 ±0.0326	31.20 ±0.3935	3.36 ±0.0045	0.96 ±0.0442	4.96 ±0.0535
Psammotettix provincialis										
0	1	8	0.59 ±0.0002	1.20 ±0.0015	0.41 ±0.0026	2.59 ±0.0063	9.28 ±0.1671	0.51 ±0.0041	0.10 ±0.0072	3.33 ±0.0137
	2	7	0.57 ±0.0005	1.18 ±0.0057	0.36 ±0.0065	2.26 0.0005	7.80 ±0.1391	0.35 ±0.0051	0.08 ±0.0022	2.95 ±0.0651
	3	9	0.55 ±0.0010	1.16 ±0.0006	0.30 ±0.0007	1.91 ±0.0156	6.10 ±0.0376	0.18 ±0.0008	0.05 ±0.0010	2.55 ±0.0028
50	1	28	0.20 ±0.0004	0.42 ±0.0007	0.10 ±0.0001	0.72 ±0.0014	2.12 ±0.0304	0.12 ±0.0002	0.02 ±0.0012	0.94 ±0.0044
	2	83	0.16 ±0.0001	0.34 ±0.0001	0.08 ±0.0002	0.57 ±0.0025	1.69 ±0.0166	0.09 ±0.0008	0.02 ±0.0012	0.75 ±0.0086
	3	62	0.12 ±0.0001	0.25 ±0.0001	0.11 ±0.0001	0.50 ±0.0007	1.07 ±0.0067	0.10 ±0.0003	0.01 ±0.0005	0.56 ±0.0044
100	1	18	0.02 ±0.0004	0.030 ±0.0017	0.04 ±0.0005	0.43 ±0.0001	2.57 ±0.0043	0.39 ±0.0078	0.11 ±0.0004	0.51 ±0.0051
	2	12	0.03 ±0.0010	0.05 ±0.0016	0.06 ±0.0018	0.58 ±0.0067	4.01 ±0.0789	0.51 ±0.0121	0.20 ±0.0024	0.72 ±0.0426
Phlepsius intricatus										
0	2	7	0.31 ±0.0009	0.63 ±0.0001	0.23 ±0.0005	1.53 ±0.0086	5.34 ±0.0176	0.31 ±0.0011	0.05 ±0.0040	2.22 ±0.0111
	3	7	0.52 ±0.0001	1.11 ±0.0022	1.20 ±0.0050	2.30 ±0.0067	12.27 ±0.0482	0.90 ±0.0028	0.33 ±0.0064	2.74 ±0.0250
50	1	10	0.09 ±0.0001	0.20 ±0.0001	0.04 ±0.0001	0.39 ±0.0033	1.19 ±0.0021	0.10 ±0.0004	0.02 ±0.0003	0.44 ±0.0013
	2	21	0.01 ±0.0002	0.04 ±0.0004	0.07 ±0.0001	0.33 ±0.0001	0.83 ±0.0073	0.09 ±0.0010	0.01 ±0.0003	0.13 ±0.0011
	3	19	0.02 ±0.0001	0.05 ±0.0003	0.01 ±0.0002	0.36 ±0.0010	1.25 ±0.0097	0.16 ±0.0005	0.07 ±0.0007	0.16 ±0.0022
100	1	12	0.01 ±0.0001	0.02 ±0.0001	0.07 ±0.0001	0.18 ±0.0015	0.57 ±0.0027	0.06 ±0.0080	0.01 ±0.0005	0.10 ±0.0021
	2	7	0.01 ±0.0001	0.03 ±0.0009	0.10 ±0.0009	0.45 ±0.0021	1.86 ±0.0073	0.14 ±0.0004	0.04 ±0.0016	0.34 ±0.0125

(m: distance from motorway; Plot: Sampling plot; n= Number of specimens)

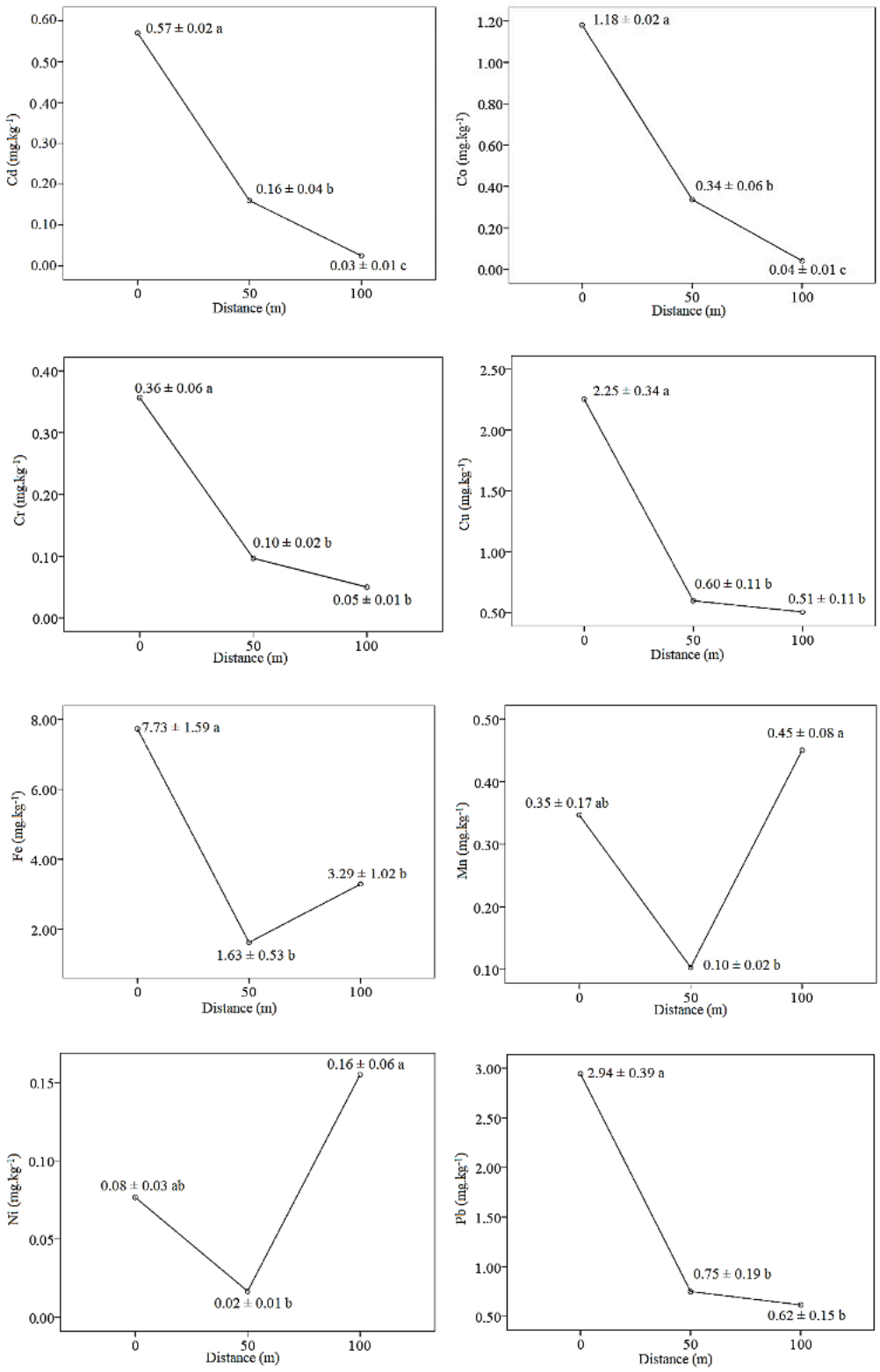

Figure 1. Metal contents of *Psammotettix provincialis* according to distance from the motorway.

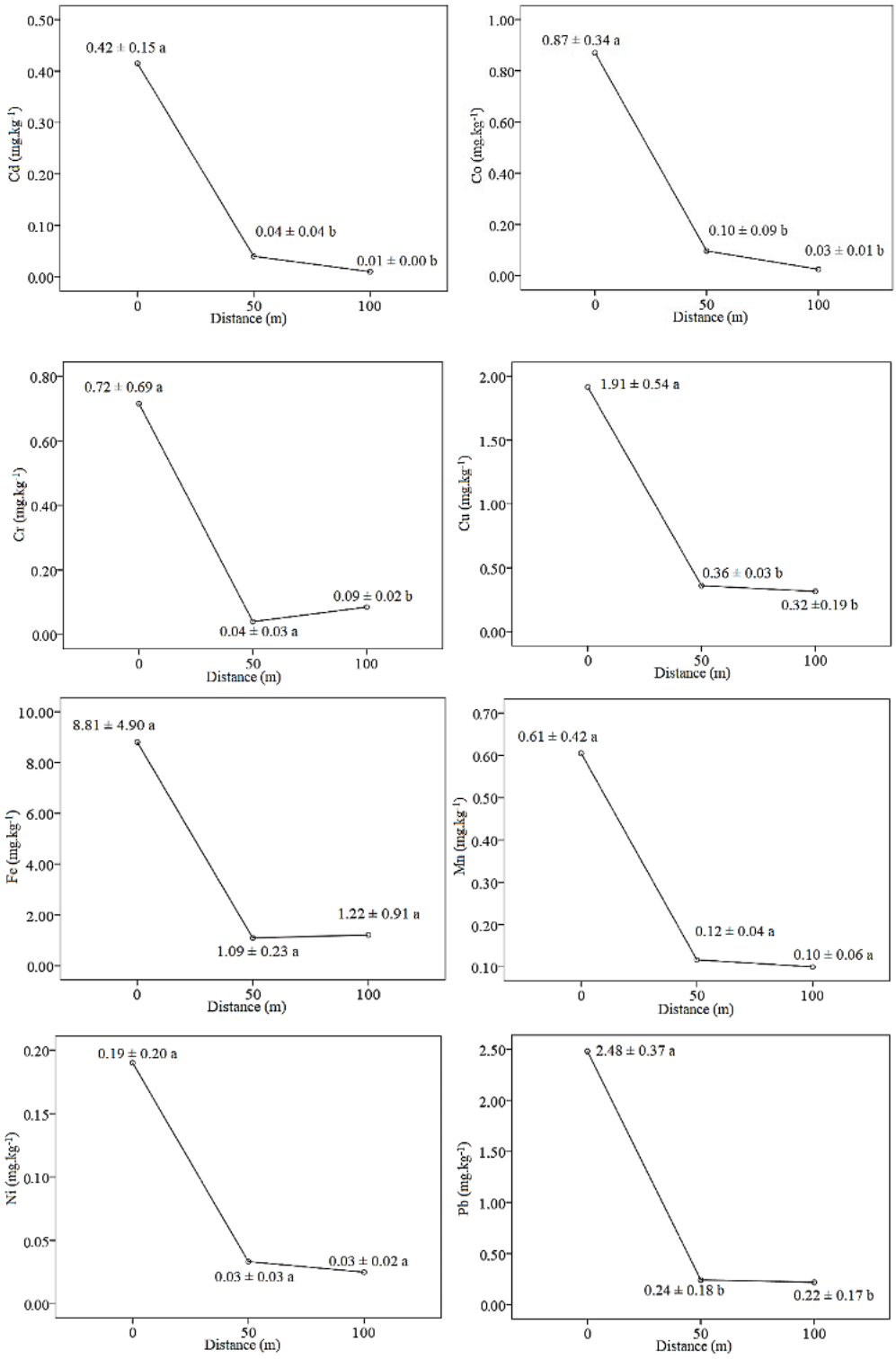

Figure 2. Metal contents of *Phlepsius intricatus* according to distance from the motorway.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declare no competing interests.

Author contribution

The author read and approved the final manuscript. The author verifies that the text, figures, and tables are original and that they have not been published before.

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Data availability

All relevant data generated or analyzed during this study are included in this document.

Consent to participate

Not applicable.

Consent for publication

The author consent for publication.

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RESEARCH ARTICLE

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Evaluation of the environmental sustainability performance of Eastern European countries with integrated MCDM methods

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Abstract

Especially in recent years, the environmental problems of countries have been increasing due to the acceleration of industrialization, increasing population, continuous increase in the consumption and energy requirements of the society and the development of technology. In order to eliminate these problems, countries take many measures and precautions. This study aims to compare Eastern European countries by evaluating their positions in the environmental sustainability performance index (EPI). For this purpose, the importance levels of the criteria were found with ENTROPY, which is in the objective category of multi-criteria decision making (MCDM) methods, and then the ranking of the countries in the environmental sustainability performance index was determined with Complex Proportional Assessmnet (COPRAS) and Weighted Aggregated Sum Product Assessment (WASPAS) methods. The data used in the evaluation of the environmental sustainability performance index "The 2022 Environmental Performance Index (EPI)" ranking of Eastern European countries is the data prepared by Yale University and obtained from the relevant web address. According to the results obtained, the ranking of the criteria in terms of their importance levels were ranked as water resources, waste management and agriculture, and the rankings obtained according to COPRAS and WASPAS methods differed in the environmental sustainability performance ranking of the countries, and it was determined that the WASPAS method gave more consistent results.

Keywords: Environmental Performance Index (EPI), ENTROPY, COPRAS, WASPAS

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INTRODUCTION

The increase in population, the acceleration of industrialization and urbanization, and the increase in the need for energy with increasing production and consumption lead to environmental problems. With the increase in environmental problems, the necessity to take environmental measures has emerged and studies on environmental protection have been initiated. In 1972, the United Nations Conference on the Human Environment and in 1992 the United Nations Conference on Environment and Development (Earth Summit) were convened. In 2000, 2001, 2002 and 2005, studies on the Environmental Sustainability Index (EPI) were conducted in cooperation with the Yale Center for Environmental Law and Policy and the Center for International Earth Science Information Network (CIESIN) in partnership with the World Economic Forum and the European Commission Joint Research Center. Environmental Sustainability Index studies have been carried out regularly every two years since 2006 (Savaş, 2012: p. 135).

Organized in 11 sub-categories under the main criteria of "climate, environmental

health and ecosystem vitality", the EPI ranks the environmental sustainability performance of 180 countries. This ranking shows the extent to which countries are achieving their environmental policy goals at the national level. The EPI provides guidance to countries that want to move forward for a sustainable future, showing leading countries and other countries according to their environmental performance (Enviromental Performance Index, 2020). The EPI is not only an average ranking of data, but also provides separate quantitative assessments and measurements according to specific issues (Karaman, 2018: p. 80). The data and analysis can enable government officials to develop policies, facilitate communication with stakeholders, and maximize returns on environmental investments. Overall EPI rankings also present who best addresses the environmental challenges faced by countries (Uca and Yüncü, 2020: p. 302).

The aim of this study is to make a comparison by evaluating the environmental sustainability performance indices of Eastern European countries. In the selection of the countries to be compared with Turkiye; Eastern European countries were chosen due to the fact that there are very few studies on this subject in the literature. In addition, since the environmental sustainability performance evaluation subject is suitable for the use of methods that can evaluate a large number of criteria together, the study utilized MCDM methods. The importance levels of the criteria were found using ENTROPY, and then the countries were ranked according to the environmental sustainability performance index using COPRAS and WASPAS methods. Although different MCDM methods have been used in the literature on the environmental sustainability performance index ranking of countries, this is the first paper to combine ENTROPY based COPRAS and WASPAS models. In the Turkish literature, only one study (Akandere and Zerenler, 2022) was found on the environmental sustainability performance index ranking of Eastern European countries using MCDM methods. However, in this study, the environmental sustainability performance and economic performance of Eastern European countries are considered together. It is thought that this study, which deals only with the environmental sustainability performance index ranking of Eastern European countries, will contribute to the literature.

The study consists of five chapters: after the introduction, the second chapter presents the literature review, the third chapter presents the materials and methods, the fourth chapter presents the findings, and the fifth chapter presents the conclusions and recommendations.

LITERATURE REVIEW

When the literature is examined, there are different studies on environmental sustainability performance. Among these studies, the studies on the environmental sustainability performance of countries using the MCDM method are summarized in Table 1. When the studies in the literature on environmental performance related to Turkiye, which do not use MCDM methods, are analyzed; Savaş (2012) aimed to evaluate Turkiye's environmental performance according to the index; Karaman (2018) aimed to reveal Turkiye's environmental performance against the EU. Bek (2019) analyzed the environmental performance of Switzerland and Turkiye and compared the two countries. Uca and Yüncü (2020) analyzed the ecological performance and sustainability competitiveness of the countries bordering the Mediterranean Sea by using the environmental performance index with multidimensional scaling analysis. Yiğit (2020) investigated the impact of globalization on the environmental performance of countries.

When the studies on countries other than Turkiye are examined in the literature; Färe et al., (2004) aimed to develop a method to measure the environmental performance of OECD countries for 1990. Zhou et al., (2007) used Data Envelopment Analysis (DEA) based model to compare the multilateral environmental performance of OECD countries and Malmquist index of environmental performance to model the change in environmental performance. Ave and Babolsar (2010) aimed to estimate and evaluate the relationship between EPI and economic growth in selected developing countries. Djoundourian (2012) examined environmental performance in developed countries and analyzed the differences between regions using ANOVA test. Chandrasekharan et al., (2013) aimed to develop a methodology to rank states based on EPI scores. Olafsson et al., (2014) developed a theoretical model to measure the environmental sustainability performance of countries and tested the model on Iceland. Sima and Gheorghe (2014) aimed to make a comparison of the EPI results calculated for 2014 between Romania and Switzerland. Bucher (2016) aimed to measure the EPI in Europe. Zuo et al., (2017) used the EPI to assess China's environmental performance at the provincial level between 2006 and 2011. Topal and Hayaloğlu (2017) examined the economic development levels of 124 countries by using data from the years 2000-2014 and evaluated how institutional quality affects environmental performance with Panel Data Analysis. Chowdhury and Islam (2017) investigated whether the relationship between EPI and Gross Domestic Product (GDP) growth rate is valid in BRICS countries. Pimonenko et al., (2018) conducted a study to analyze the relationship between environmental performance and ecological, social and economic welfare. Botetzagias et al., (2018), economic the impact of the crisis on the environmental performance of EU member states impact of environmental quality indicators and environmental policy Hierarchical Linear Modeling under the indicators by using the same method. Tunçarslan (2018) compared the climate and environmental policies of BRICS countries using the Climate Change and EPI data. Chandrasekharan and Srinivasan (2020) aimed to rank Indian

states according to the EPI for 2020. Liu et al., (2021) applied the proposed method to evaluate the environmental performance of 30 provincial administrative regions of China. Nguyen et al., (2022) aimed to measure the progress of Vietnam's provinces towards achieving national environmental performance targets. Ding and Beh (2022) evaluated the effectiveness of regional efforts of ASEAN countries on climate change and sustainability. Ha et al. (2022) used the EPI data of 25 European countries for the years 2015-2020 to measure the impact of digitalization on environmental performance. Signes et al. (2022) aimed to measure the relationship between environmental performance and risk scores of 163 countries with regression analysis.

Table 1. Literature review

Author(s)	Objective	Method	Finding(s)
Altuntaş ve Kaya (2023)	Comparing the sustainable development of the European Union member states and the sustainable performance of enterprises constitutes the theme of the study.	ENTROPY, TOPSIS	There is no significant relationship between country sustainability level and corporate sustainability level.
Akandere and Zerenler (2022)	The aim of this study is to evaluate the EPI of Eastern European countries with the help of MCDM techniques.	CRITIC, TOPSIS	According to the CRITIC method, the most important criterion is ecosystem services; the least important criterion is ecosystem vitality; and according to the assessment of environmental and economic performance, Romania was the most successful and Bosnia and Herzegovina was the least successful.
Alkaya (2022)	OECD countries with DEA relative effectiveness in terms of their environmental performance.	DEA	The efficiency score for Denmark, Finland, Iceland, Colombia, Lithuania, Luxembourg, Latvia, Iceland, Colombia, Lithuania, Luxembourg, Latvia and Sweden is determined as 1; these countries are OECD countries that are efficient according to environmental performance.
Doğan (2022)	To measure the environmental performance of OECD and EU member countries using CRITIC and MABAC methods, taking into account the criteria included in the Climate Change Performance Index (CCPI) and EPI.	CRITIC, MABAC	It was determined that the criterion with the highest importance was ecosystem services. Among the selected countries, Denmark, Sweden and Finland perform better than other countries.
Akandere (2021)	It is aimed to evaluate ENTROPY and TOPSIS methods according to logistics performance index (LPI) and EPI criteria in Belt and Road countries.	TOPSIS, ENTROPY	Air quality was identified as the most important criterion in 2014, water and sanitation in 2016, water resources in 2018, and efficiency of customs control procedures as the least important criterion in 2014, 2016 and 2018.
Altıntaş[a] (2021)	It is aimed to measure the environmental performance of the G7 group countries in 2018 with CODAS and TOPSIS methods using EPI data.	CODAS, TOPSIS	According to the CODAS method, the environmental performance ranking of the countries is determined as UK, France, Japan, Germany, Canada, Italy and USA; according to the TOPSIS method as UK, France, Germany, Japan, Canada, Italy and USA.
Altıntaş[b] (2021)	For 2020, it is aimed to calculate the significance levels of the EPI components of the G20 countries with the ENTROPY method and to measure the environmental performance of the countries with ENTROPY based ROV, ARAS and COPRAS methods.	ENTROPY, ROV, ARAS, COPRAS	It has been determined that the most important criterion in environmental performance for countries is water resources and the countries with the best environmental performance are Germany, Japan, the UK, France and Japan.
Baloch et al. (2020)	It is aimed to calculate the environmental performance efficiency of the BRICS group countries according to their EPI values between 2011-2016 with DEA.	DEA	It was found that all countries achieved environmental performance efficiency and the ranking was determined as Brazil, Russia, South Africa, China and India.

Matsumoto et al. (2020)	This study evaluated the environmental performance of European Union (EU) countries using DEA approach and the global Malmquist-Luenberger index.	DEA	The empirical results revealed that the trends in the environmental performance of the entire EU and its individual countries were similar under all examined models. Environmental performance was indeed negatively affected by the financial crisis of 2007-2008; this impact was mainly observed in eastern EU countries.
Ayçin and Çakın (2019)	It is aimed to introduce a model that measures the environmental performance of countries with the integrated use of MCDM methods.	ENTROPY, GİA, MOORA, Fuzzy Logic	Forests, agriculture and water resources were identified as the criteria with the best importance level, and Austria, Denmark and France as the countries with the best performance.
Aksu and Gencer (2018)	It is aimed to analyze the environmental performance of OECD countries according to the EPI data.	DEA	According to the results, Iceland was the most efficient country, followed by Sweden and Estonia.
Ozkan and Ozcan (2018)	Environmental performance of OECD countries in selected environmental according to the indicators with DEA evaluation was aimed.	DEA	It has been determined which countries should be taken as an example for increasing efficiency in OECD countries and making those with inefficient efficiency scores efficient.
Sözen et al. (2016)	It is aimed to examine the correspondence between the effectiveness of tourism indicators and environmental performance.	DEA	Luxembourg was found to be the most efficient country, while the improvement in total factor productivity of the 27 selected countries, including Turkiye, remained limited.
Ab-rahim (2015)	It is aimed to measure the environmental performance of Southeast Asian countries.	DEA	According to the results of the study, smaller economies such as Laos, Cambodia and Brunei were found to be environmentally efficient.
García Sánchez et al. (2015)	It is aimed to calculate the integrated EPI of countries between 2004-2009.	CRITIC, SAW	The criteria with the best level of importance are urban population growth, fertilizer use, agricultural area and protected coasts, while the best countries in terms of performance are Iceland, Norway and Sweden, and the worst countries are Nigeria, Burundi and Bangladesh.
Bilbao-Terol et al. (2014).	It is aimed to evaluate the countries' Adjusted Net Savings (ANS), Ecological Footprint (ECF), EPI and Human Development Index (HDI) data with TOPSIS method.	TOPSIS	France, Italy and the Netherlands were identified as the most successful countries.
Ismail and Abdullah (2012)	Analytic Hierarchy Process (AHP) was used to determine the EPI ranking of ASEAN countries.	AHP	The analysis revealed that Brunei has the highest EPI ranking among ASEAN countries, followed by Singapore.
Roggea (2012)	For 2010, the EPI components of Finland, Brazil, Canada, Guinea, Costa Rica, Mexico, Indonesia and the United Arab Emirates were used to measure the environmental performance of these countries by DEA.	DEA	Finland, Canada, Costa Rica and the United Arab Emirates are found to be efficient in terms of their environmental performance; Brazil is close to environmental performance efficiency and Guinea and Indonesia are found to be inefficient in terms of environmental performance efficiency.

When the studies in Table 1 are examined, it is seen that different MCDM methods are used in the evaluation of the environmental sustainability performance of countries. However, it is noteworthy that DEA is used more than other methods in the studies conducted. In addition, the studies were conducted on different country groups "OECD, BRICS, G20, G7, South East Asia and Eastern European" countries. Among these country groups, OECD countries have been addressed in more studies.

MATERIALS AND METHODS

In this section, information on the data set used, the analysis methods used, the criteria used in the analysis and the countries where the comparisons were made are provided.

Data set used

The data for the study was obtained from the web address where "The 2022 Environmental Performance Index (EPI)" is presented, Environmental Performance Index 2023. The most recent data belongs to 2022 and the data for 2023 has not been published yet. In addition, due to the lack of data on the "fisheries" criterion, which is a sub-criterion of the "ecosystem vitality" criterion, this sub-criterion was not included in the analysis.

Analysis methods used

In the study, MCDM methods were used. In the EPI ranking of the Eastern European countries and the comparison of the countries, the importance levels of the criteria were first found with the ENTROPY method, and then the ranking of the countries was obtained with the COPRAS and WASPAS methods. In the ENTROPY method, the data in the decision matrix are used to calculate the weights of the criteria in the decision problem. The method is very easy to apply since there is no need for any other subjective evaluation. This is the most powerful aspect of the method. Objective results are obtained by using data on decision alternatives without the need for evaluations by decision makers. When comparing decision alternatives, the COPRAS method indicates in percentage terms how much better or worse one alternative is than the other. The method can perform multi-criteria evaluation in order to maximize the values of the criteria if it is a maximization (benefit) criterion and minimize the values of the criteria if it is a minimization (cost) criterion. The COPRAS method, which can address both quantitative and qualitative criteria, is a method that allows the full ranking of decision alternatives to be obtained. The ability of the WASPAS method to provide more accurate results compared to other methods has led to its acceptance in the literature in recent years as an effective MCDM method. The most important advantages of the method are that the application process is shorter and easier compared to other MCDM methods and that it does not require specific computer programs to perform the calculations. The methods and application steps are given briefly below.

ENTROPY method

The ENTROPY method is one of the objective methods for calculating the weights of the criteria (Ayçin, 2019: p. 122). The application steps of the method are given in 5 steps.

1. First, a decision matrix with all alternatives and criteria is created. The decision matrix is given in equation 1:

$$E = [Z_{ij}]_{m\times n} = \begin{bmatrix} z_{11} & z_{12} & \cdots & z_{1n} \\ z_{21} & z_{22} & \cdots & z_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ z_{m1} & z_{m2} & \cdots & z_{mn} \end{bmatrix} \quad (1)$$

2. The values in the decision matrix are standardized using equation 2 (benefit-side criteria) and equation 3 (cost-side criteria). The r_{ij} values in the equations are the standardized version of the Z_{ij} value in the decision matrix.

$$r_{ij} = \frac{z_{ij}}{max_j(z_{ij})} \quad (2)$$

$$r_{ij} = \frac{min_j(z_{ij})}{z_{ij}} \quad , \quad min_j\,(z_{ij}) \neq 0 \quad (3)$$

3. Using Equation 4, the standardized values are normalized. The value of t_{ij} in Equation 4 is the normalized value of r_{ij}.

$$t_{ij} = \frac{r_{ij}}{\sum_{i=1}^{m} r_{ij}} \quad (4)$$

4. The entropy values of the criteria (H_j) are calculated by equation 5.

$$H_j = -\frac{\sum_{i=1}^{m} t_{ij} \ln(t_{ij})}{\ln(m)} \quad (5)$$

5. In the last application step, the weight of each criterion (w_j) is found by equation 6.

$$w_j = \frac{1-H_j}{\sum_{j=1}^{n}(1-H_j)} \quad (6)$$

COPRAS method

The COPRAS method is one of the methods for ranking decision alternatives (Ayçin, 2019: p. 122). The implementation steps of the COPRAS method consist of 6 steps.

1. In the first stage, the decision matrix consisting of x_{ij}, denoted by D, is created as shown in Equation 1.

$$D = [Z_{ij}]_{m\times n} = \begin{bmatrix} x_{11} & x_{12} & \cdots & x_{1n} \\ x_{21} & x_{22} & \cdots & x_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ x_{m1} & x_{m2} & \ldots & x_{mn} \end{bmatrix} \quad (1)$$

2. The normalization process is created using Equation 2.

$$x_{ij} = \frac{x_{ij}}{\sum_{i=1}^{m} x_{ij}} \quad , \forall_j = 1,2,\ldots\ldots n \quad (2)$$

3. The normalized decision matrix (D′) is obtained using Equation 3.

$$D' = \begin{bmatrix} d_{11} & d_{12} & \cdots & d_{1n} \\ d_{21} & d_{22} & \cdots & d_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ d_{m1} & d_{m2} & \ldots & d_{mn} \end{bmatrix} \quad (3)$$

4. Equation (4) is used to weight the normalized decision matrix.

$$d_{ij} = x_{(ij)^*} . w_j \quad (4)$$

5. The sum of the values of the weighted normalized decision matrix for maximization-based criteria is given by "S_{+i}" and the sum of the values of the weighted normalized decision matrix for minimization-based criteria is given by "S_{-i}". Equations (5) and (6) are used to calculate these values.

$$S_{+i} = \sum_{j=1}^{k} d_{+ij} \; ; J = 1,2, \ldots\ldots, k \quad (5)$$

$$S_{-i} = \sum_{j=k+1}^{n} d_{-ij} \; ; J = k+1, k+2, \ldots\ldots, n \quad (6)$$

6. The relative importance of the decision alternatives, Q_i, is calculated using Equation (7).

$$Q_i = S_{+i} + \frac{S_{-min} \sum_{i=1}^{m} S_{-i}}{S_{-i} \sum_{i=1}^{m} \frac{S_{-min}}{S_{-i}}} \quad (7)$$

In terms of the Q_i values found by Equation (7), the decision alternative with the largest Q_i value is determined as the alternative with the highest relative importance (Q_{maks}).

7. In the final stage, the performance index values (P_i) of the decision alternatives are calculated using Equation (8).

$$P_i = \frac{Q_i}{Q_{maks}} .100 \quad (8)$$

The decision alternative with a performance index of 100, symbolized as () in Equation (8), is identified as the best alternative.

WASPAS method

The WASPAS method is one of the first MCDM methods presented to the literature by Zavadskas et al. (2012). The method is a method developed with the integrated use of the weighted sum model and the weighted product model (Ayçin, 2019: p. 254). The solution steps of the WASPAS method are as follows:

1. The decision matrix consisting of X_{ij} and denoted by X is obtained using Equation (1).

$$X = [X_{ij}]_{mxn} = \begin{bmatrix} x_{11} & x_{12} & \cdots & x_{1n} \\ x_{21} & x_{22} & \cdots & x_{2n} \\ \cdots & \cdots & \cdots & \cdots \\ x_{i1} & x_{i2} & \cdots & x_{in} \\ \cdots & \cdots & \cdots & \cdots \\ x_{m1} & x_{m2} & \cdots & x_{mn} \end{bmatrix} \quad (1)$$

2. The normalization process is based on Equation (2) for benefit-based criteria and Equation (3) for cost-based criteria.

$$x_{ij}^{*} = \frac{x_{ij}}{maks_i x_{ij}} \quad (2)$$

$$x_{ij}^{*} = \frac{min_i x_{ij}}{x_{ij}} \quad (3)$$

3. In terms of the Weighted Sum Method, the total relative importance of alternative i is obtained as the weighted sum of the criteria values using Equation (4).

$$Q_i^{(1)} = \sum_{j=1}^{n} x_{ij}^{*} w_j \quad (4)$$

4. In terms of the Weighted Multiplication Method, the total relative importance of alternative i is calculated by calculating the power of the normalized value of an alternative with respect to the criterion by the weight of the criterion and multiplying the obtained values for each alternative using Equation (5).

$$Q_i^{(2)} = \prod_{j=1}^{n} \left(x_{ij}^{*}\right)^{w_j} \quad (5)$$

5. Equation (6) gives the weighted overall criterion value Q_i.

$$Q_i = 0.5Q_i^{(1)} + 0.5Q_i^{(2)} \quad (6)$$

6. Equation (7) is used to find the total relative importance of alternatives. Alternatives are ranked in terms of their Q values. The best alternative is the one with the highest Q value. Equation (6) transforms the WASPAS method into WPM when λ is set to 0 and into WSM when λ is set to 1. Decision makers can use the value of λ as they wish.

$$Q_i = \lambda Q_i^{(1)} + (1-\lambda) Q_i^{(2)} \quad (7)$$

In order to determine the final ranking of the alternatives, Q_i values are ranked in descending order. The most suitable alternative is ranked first.

7. Sensitivity analysis is performed using different λ values and the ranking of the alternatives is determined.

8. Using Equation (8), the optimal λ is calculated to determine whether the ranking is correct.

$$\lambda = \frac{\sum_{i=1}^{m} Q_i^{(2)}}{\sum_{i=1}^{m} Q_i^{(1)} + \sum_{i=1}^{m} Q_i^{(2)}} \qquad (8)$$

Criteria used

The EPI data consists of three main criteria and eleven sub-criteria. The relevant decision criteria are given in Table 2. Information about the criteria was obtained from EPI 2023. The sub-criterion "fisheries", which is a sub-criterion of the "ecosystem vitality" criterion, was not included in the analysis due to lack of data on this sub-criterion. In the study, "mitigating climate change" was used as a sub-criterion of the "climate" criterion, "air quality, water sanitation, heavy metals, waste management" as sub-criteria of the "environmental health" criterion and "ecosystem services, biodiversity, acid rain, agriculture and water resources" as sub-criteria of the "ecosystem vitality" criterion.

Table 2. Environmental sustainability performance index criteria

Main Criteria	Sub-Criteria	Sub-Criterion Codes
Climate (C1)	Mitigating Climate Change	C11
Environmental Health (C2)	Air quality	C21
	Water Sanitation	C22
	Heavy Metal	C23
	Waste Management	C24
Ecosystem Vitality (C3)	Ecosystem Services	C31
	Biological Diversity	C32
	Acid Rain	C33
	Agriculture	C34
	Water Resources	C35

Determination of alternatives used in the analysis

The scarcity of studies in the literature guided the identification of Eastern European countries to be compared with Turkiye in the study. In this context, 19 Eastern European countries were included in the study. Countries are considered as decision alternatives according to the MCDM methods. The codes related to the countries are given in Table 3.

Table 3. Eastern European countries for comparison

Countries	Alternative Code
North Macedonia	A1
Slovenia	A2
Latvia	A3
Croatia	A4
Cyprus	A5
Slovakia	A6
Czech Republic	A7
Albania	A8
Montenegro	A9
Estonia	A10
Romania	A11
Greece	A12
Bulgaria	A13
Hungary	A14
Lithuania	A15
Bosnia-Herzegovina	A16
Serbia	A17
Poland	A18
Turkiye	A19

RESULTS AND DISCUSSION

After determining the criteria used in the environmental sustainability performance index ranking and the alternatives to be compared, the data on the countries shared by Yale University in the EPI 2023 was obtained from the relevant web page for the year 2022. This data is the decision matrix given in Table 4. The benefit-oriented sub-criteria in this matrix are shown as "maximization (max)" and the cost-oriented sub-criteria are shown as "minimization (min)".

Table 4. Environmental sustainability performance index decision matrix

	C11 (max)	C21 (max)	C22 (max)	C23 (min)	C24 (max)	C31 (max)	C32 (max)	C33 (min)	C34 (max)	C35 (max)
North Macedonia (A1)	69,8	22,6	61,1	46,1	42,1	24	57,9	24	41,9	0,8
Slovenia (A2)	62,9	55,1	74,7	87,2	66,7	34,1	84,5	34,1	55	92,2
Latvia (A3)	58,6	51,1	59,1	77,5	63	15,8	84,3	15,8	64,4	90,7
Croatia (A4)	56,6	45,8	70,3	74,2	55,3	34,4	81,5	34,4	68,9	69
Cyprus (A5)	53,8	68,3	94	68,6	58,9	32,5	78,3	32,5	13,9	50
Slovakia (A6)	53,5	50,9	71,9	68,4	62,2	19,9	82,7	19,9	68	44,7
Czech Republic (A7)	52,8	53,3	76,5	75,5	74,9	19,1	83,3	19,1	37,4	61,5
Albania (A8)	52,5	37,5	54,1	45,5	13,4	24,2	63,9	24,2	28,9	1,9
Montenegro (A9)	52,3	30,7	65,6	64,4	15,5	36,7	52,6	36,7	34,7	8,4
Estonia (A10)	52	74,6	61,9	86,5	66,7	15,8	86	15,2	61,8	70,4
Romania (A11)	51,3	39,2	56	50,8	45,6	35	81,1	35	53,8	25,7
Greece (A12)	50,8	62	98,2	68,6	59,9	28,1	69,1	28,1	38,9	81,7
Bulgaria (A13)	49,8	28,6	68,4	45,2	58,8	37,4	75,1	37,4	55,8	13,9
Hungary (A14)	48,1	38,2	62,2	67,4	43,4	28	78	28	53	55,3
Lithuania (A15)	47,1	58,4	58,4	83	67,4	21,9	84,4	21,9	65,6	52,3
Bosnia-Herzegovina (A16)	45,1	27,8	61,5	42,3	30,9	45,4	34,1	45,4	21,3	1,1
Serbia (A17)	41,7	29,4	65,6	50,4	40,3	39,7	46,7	39,7	45,3	0,7
Poland (A18)	38,8	40,4	71,8	64,5	63,7	17,7	87,3	17,7	42,7	61,5
Turkiye (A19)	21,5	44,6	52,7	60,8	40,6	22	7,5	22	39,1	30,5

Using the ENTROPY method, the importance levels of the ten criteria given in Table 2 were found. The importance levels of the criteria are shown in Table 5. In terms of the results obtained, the most important criteria are water resources (C35), waste management (C24) and agriculture (C34).

Table 5. Importance levels of the criteria

C11	C21	C22	C23	C24	C31	C32	C33	C34	C35
0,0278	0,0674	0,0198	0,0367	0,0895	0,0652	0,0816	0,0710	0,0818	0,4586

According to the importance levels calculated in Table 5, the environmental sustainability performance index ranking of the countries was calculated using the COPRAS method. The ranking is given in Table 6. Accordingly, five countries shared the first highest score in the environmental sustainability performance index of Eastern European countries, namely Slovenia, Latvia, Greece, Poland and Turkiye.

Table 6. Ranking of alternatives according to COPRAS method

Alternatives	Pi	Ranking
Greece (A12)	1	100
Turkiye (A19)	1	100
Slovenia (A2)	1	100
Poland (A18)	1	100
Latvia (A3)	1	100
Lithuania (A15)	2	98,5016
Estonia (A10)	3	95,3029
Hungary (A14)	4	94,1021
Croatia (A4)	5	93,7709
Czech Republic (A7)	6	85,3863
Cyprus (A5)	7	75,9589
Slovakia (A6)	8	75,0473

Bulgaria (A13)	9	60,9348
Romania (A11)	10	58,2443
Serbia (A17)	11	42,5583
Bosnia-Herzegovina (A16)	12	37,8637
Montenegro (A9)	13	37,1809
Albania (A8)	14	30,9096
North Macedonia (A1)	15	30,2575

Table 7. Ranking of alternatives according to WASPAS method

Alternatives	Qi	Ranking
Latvia	0,8609	1
Slovenia	0,8604	1
Estonia	0,7849	2
Greece	0,7805	3
Croatia	0,7411	4
Czech Republic	0,6993	5
Poland	0,6839	6
Lithuania	0,6713	7
Slovakia	0,6292	8
Hungary	0,6278	9
Cyprus	0,5954	10
Romania	0,4746	11
Turkiye	0,3982	12
Bulgaria	0,3961	13
Montenegro	0,2704	14
North Macedonia	0,2083	15
Albania	0,2063	16
Serbia	0,1994	17
Bosnia-Herzegovina	0,1836	18

Table 8. Comparison of countries' environmental sustainability performance in terms of methods

Countries	EPI Values	Ranking	COPRAS	Ranking	WASPAS	Ranking
Slovenia	67,3	1	100	1	0,8604	1
Estonia	61,4	2	95,3029	3	0,7849	2
Latvia	61,1	3	100	1	0,8609	1
Croatia	60,2	4	93,7709	5	0,7411	4
Slovakia	60,0	5	75,0473	8	0,6292	8
Czech Republic	59,9	6	85,3863	6	0,6993	5
Cyprus	58,0	7	75,9589	7	0,5954	10
Greece	56,2	8	100	1	0,7805	3
Romania	56,0	9	58,2443	10	0,4746	11
Lithuania	55,9	10	98,5016	2	0,6713	7
Hungary	55,1	11	94,1021	4	0,6278	9
North Macedonia	54,3	12	30,2575	15	0,2083	15
Bulgaria	51,9	13	60,9348	9	0,3961	13
Poland	50,6	14	100	1	0,6839	6
Albania	47,1	15	30,9096	14	0,2063	16
Montenegro	46,9	16	37,1809	13	0,2704	14
Serbia	43,9	17	42,5583	11	0,1994	17
Bosnia-Herzegovina	39,4	18	37,8637	12	0,1836	18
Turkiye	26,3	19	100	1	0,3982	12

According to Table 8, the EPI values of the countries and the results of the environmental sustainability performance values found by WASPAS and COPRAS method are compared. Slovenia ranked 1st according to all methods. Estonia ranked 3rd according to COPRAS method and 2nd according to WASPAS and EPI values. Latvia ranked 3rd according to EPI value and 1st according to COPRAS and WASPAS methods. Bosnia and Herzegovina ranked last according

to EPI values and WASPAS method. Turkiye's ranking is almost similar in terms of WASPAS method and EPI values. Turkiye ranked 19th according to the EPI value and 12th according to the WASPAS method. When the environmental sustainability performance ranking of the countries according to the ENTROPY based COPRAS method is compared with the EPI values, it has shown consistency for 3 countries. These countries are Slovenia, Czech Republic and Cyprus. Slovenia ranked 1st, Czech Republic ranked 6th and Cyprus ranked 7th. On the other hand, when the environmental sustainability performance ranking of the countries according to the ENTROPY based WASPAS method is compared with the EPI values, it has shown consistency for 6 countries. These countries are Slovenia, Estonia, Croatia, Serbia and Bosnia and Herzegovina. Slovenia ranked 1st, Estonia 2nd, Croatia 4th, Serbia 17th and Bosnia and Herzegovina 18th. According to the results obtained, it was observed that the EPI values of the ENTROPY based WASPAS method and the EPI values gave more consistent and similar results than the ENTROPY based COPRAS method in the environmental sustainability performance ranking of countries.

CONCLUSION

Especially with the recent increase in environmental awareness, countries have been developing various policies to solve their environmental problems. In order to evaluate the effectiveness of the policies developed and to analyze the situation of countries against other countries, environmental performance evaluations are regularly conducted. According to the results of the analysis conducted in this study, the criteria are ranked as "water resources", "waste management" and "agriculture" according to their importance levels. When we look at the main criteria to which the sub-criteria are linked, it is observed that the "ecosystem vitality" criterion stands out in the environmental performance index, followed by the "environmental health" criterion.

In a similar study in the literature, Akandere and Zerenler (2022) conducted to evaluate both the environmental and economic performance of Eastern European countries, firstly, the importance levels of the criteria were found by CRITIC method, and the most important criterion was found as "ecosystem services" and the least important criterion as "ecosystem vitality". When the importance levels of the criteria were compared, it was determined that the importance levels of the criteria obtained in this study differed with the studies of Akandere and Zerenler (2022). In this study, in which the environmental sustainability performance index ranking of countries was made, it was determined that the "water resources" criterion under the main criterion of "ecosystem vitality" was the most important criterion. In addition, in the study of Altıntaş[b] (2021), in which the environmental performance index of the countries in the G20 group was evaluated, the most important criterion was determined as "water resources" according to the ENTROPY method in environmental performance according to countries. The results of this study in the literature also support each other with this study. This is thought to be due to the fact that countries have realized the importance of this issue for future generations in ensuring the efficiency of water resources.

On the other hand, according to the environmental and economic performance assessment of Akandere and Zerenler (2022), Romania was found to be the most successful and Bosnia and Herzegovina the least successful countries. Turkiye ranked 8th among 19 European countries in the study. In our study, Turkiye ranked 1st according to the ENTROPY based COPRAS method and 12th according to the ENTROPY based WASPAS method in the environmental sustainability index performance ranking. Turkiye ranked 19th in the ranking according to the EPI value. Turkiye has obtained the closest ranking to the EPI value in the environmental sustainability index performance ranking with the WASPAS method. According to this result, it is seen that the result of Turkiye's environmental sustainability index performance ranking obtained by ENTROPY based WASPAS method is more consistent than the result obtained by ENTROPY based COPRAS method. Although there has been an increase in the installation of renewable energy sources, progress in wastewater management, increase in environmental taxes and increasing environmental investments of the private sector in Turkiye in recent years, it can be said that it is still not at the desired level. In order to eliminate the disadvantageous situations of countries, it is obvious that it is necessary to use the country's resources with an environmentalist perspective, to make more use of renewable energy sources such as solar, wind and biomass, and to work on recycling waste through zero waste studies. In order to prevent environmental destruction and protect the environment, it is very important to cooperate within and between countries, to create environmental public awareness in the international arena and to carry out environmental policies and activities.

In future studies, new and different studies can be conducted by using integrated forms of different MCDM methods. The environmental sustainability performance of countries can be determined by using methods such as CODAS, OCRA, ROV, MABAC and MOOSRA, which are not included in the literature summary given in Table 2. For the ten criteria used in this study, 2022 data which is the most recent data based on years, was utilized. However, since regular data on the "fisheries" criterion in the main criterion of "ecosystem vitality" could not be obtained for 2022, this criterion was not included in the study. In future studies, comparisons can be made with previous studies by using the data for 2023, which will be published.

On the other hand, in this study, sub-criteria related to all three dimensions, namely "climate", "environmental health"

and "ecosystem vitality", were included for environmental sustainability performance index ranking. In addition, objective methods were preferred in this study. In addition to these three dimensions, the literature can be enriched by adding dimensions related to countries' economic performance, innovation index and tourism indicators ranking. In addition, the results obtained by using subjective methods where expert opinions are taken can be compared with the results obtained in the studies conducted.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

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Effects of organic amendments on tomato yield and electrochemical properties of soilless growing media

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Abstract
This research sought to investigate how using organic amendments derived from readily accessible materials affects both tomato production and the electrochemical characteristics of soilless growing media. A greenhouse experiment using six (6) different locally accessible and underutilized composted organic materials [cocoa peat, rice husk, ground Doum palm (*Hyphaene thebaica*) fruit, Iroko (*Milicia excelsa*) saw dust, mahogany (*Khaya senegelensis*) saw dust, and Sapele saw dust (*Entandophragma cylindricum*)] was carried out. Standard procedures were used to determine the physical, chemical, and electrochemical characteristics of the modified materials.The results revealed that the highest water retaining capacity of the media varied from 51.11% to 85.56%. Iroko palm has the highest bulk density (0.94 g cm-3) while Doum palm has the highest particle density (0.81 g cm-3). The results of the study showed that the pH of the medium in KCl ranged from 6.32 to 7.81 and 7.36 to 8.37 for pH in water. The electrical potentials for the different media ranged from -52.01 to -93.38. The point zero charge of soils was shown to be positively correlated to the properties of the medium. The pH, electrical conductivity (ECe), and cation exchange capacity (CEC) of the media all increased when the media was modified. It is recommended that rice husks and cocopeat be used as growing given their superior performance compared to the other tested media in tomato production. However, because of the cost of producing cocopeat media, rice husk can serve as an alternative to cocopeat as a growing medium. Despite a slight delay in germination in the rice husk media, a high yield was attained at the end of production.
Keywords: Soilles Media, Composting, Cation Exchange Capacity, Point of Zero Charge, Tomato

INTRODUCTION

Due to high temperatures, leaching, loss of surface soil due to erosion, an array of pH changes, low organic matter content and plant nutrient availability are critical challenges in agricultural soils in semi-arid Northern Nigeria. Furthermore, typical topsoil contains soil-borne pests and diseases, as well as a significant amount of certain heavy metals, which will ultimately lead to a decline in quantity and quality (Ibeawuchi *et al.,* 2015). This inadvertently affects the nature and composition of soil colloids (Ibrahim *et al.,* 2014). Negative charges predominate on the surfaces of soil colloids, aiding in cation attraction and retention in soils. These charges have a substantial influence on soil cation exchange capacity. These charges results from the adsorption and desorption potentials of the ions, notably H^+ and OH; hence, colloids are referred to as pH dependent (Zhang *et al.,* 1991). Because of the aforementioned losses associated with semi-arid soils, developing a more effective method to enhance nutrient use efficiency is critical (Gruda, 2019).

Recent technological developments in agriculture which involves various organic and inorganic soilless growing media have been developed as topsoil substitutes to improve greenhouse technology utilization, protect crops from soil-borne pathogens, and control environmental pollutants such as nitrate, pesticides, and other toxic chemicals (Ahmad *et al.,* 2011). However, the basic resources used to make these media are costly. Barrett *et al*. (2016), for example, named cocopeat as the best soilless media for greenhouse technology because of its unique physical and chemical features, and it has been shown to boost crop quality and output. However, the cost of production and raw material availability persist to be a major barrier limiting its application in many geographical regions.

Tomato (*Solanum lycopersicum* l.) is one of the most significant vegetable crops cultivated in NorthernNigeria. The total area under tomato cultivation worldwide is 47.82 million hectares, with a yield of 1770.93 MT (Anonymous 2016). Tomato cultivation is often done in open fields where the crop is vulnerable to biotic and abiotic stressors, the most significant of which is soil health, which restricts its growth, productivity, and quality. As a result, producing tomatoes under protected conditions is a viable option for enhancing output and quality.

A practical way to solve this persistent challenge is to explore other materials that are economically viable, environmentally friendly, socially acceptable and technically adaptable for use as substitutes for cocoa peat as greenhouse growing media. Hence, this study aimed to investigate different common but underutilized materials found in our surroundings, like rice husk, sapele, doum palm, iroko, and mahogany, with the goal of transforming them into sustainable growing mediums. The objective was to assess their electrochemical properties and their effect on tomato yield. This research seeks to mitigate soil-related risks and offer an alternative to traditional soil for agricultural production, thereby enhancing food security.

MATERIALS AND METHODS

Study Area

The research was carried out at the Centre for Dryland Agriculture's Research and Training Farm at Bayero University Kano, which is located at latitude 11° 59"N and longitude 8° 25"E, with an altitude of 458 m above sea level. The area's vegetation is the Sudan Savanna, with a tropical wet and dry climate. The trees in the area are comprised of numerous species and are typically no taller than 20 m. The temperature in the region is usually high all year, with seasonal variations, with a progressive increase from January to April, when the maximum value reaches 43°C (Mohammed *et al.,* 2015). The rainfall amount of the area is slightly variable between years, with a mean annual rainfall of 897.7 mm (Mustapha *et al.,* 2014).

Soilless Media Preparation and Modification

Six (6) different locally available and less utilized organic materials were collected, including cocoa peat, rice husk, ground Doum palm (*Hyphaene thebaica*) fruit, Iroko (*Milicia excelsa*) saw dust, mahogany (*Khaya senegelensis*) saw dust, and Sapele saw dust (*Entandophragma cylindricum*) across many parts of Kano State. These materials were modified via compositing. The materials were soaked in water and then transferred into plastic containers to provide adequate heat for composting. Regular sprinkling of water was maintained during the process. The materials were turned every week to supply adequate air for the aerobic microbial activities to occur. This process was maintained for 90 days before harvesting for final curing in a well-aerated place.

Soilless Media Characterization

The physical and chemical properties of the media were determined using the standard analytical procedure as follows:

Physical Characterization

Water retention capacity

30g of the materials (w) were weighed into a conical flask, and then 50 ml of water was added (V1). After 10 min, the samples were filtered using Whatman no. 1 filter paper. The filtrate was collected and measured using a measuring cylinder (V2). The percentage water retention capacity was measured using the following:

$$\% \text{ WRC} = \frac{V1-V2}{W} \times 100$$

Particle Density

The particle density of the materials was measured using a Pycnometer. The mass of the empty Pycnometer (*Mp*) was first measured. Afterward, it was filled with distilled water and measured (*Mpw*). The mass of water (*Mw1*) was obtained by subtracting *Mp* from *Mpw* and then divided by the density of water (0.99753 g cm^{-3}) to obtain the volume

of the Pycnometer (*Vp*). About half of the Pycnometer was filled with each of the materials, and its weight (*Mpm*) and *Mp* were subtracted from it to obtain the mass of the media material (*Mm*), while its volume was obtained by adding distilled water to the media in the Pycnometer (*Mpmw*), and then *Mpm* was subtracted from it to obtain the mass of water in the media (*Mw2*) and then divided by the density of water to obtain its volume (*Vw*), then *Vw* was subtracted from *Vp* to obtain the volume of the media *Vm*. Finally, the density of the material was determined using the following relation:

$$\rho m = \frac{Mm}{Vm} \ldots\ldots\ldots\ldots 1$$

Bulk Density

The bulk density of the media was determined by weighing the oven-dried media removed from the holes of the seed trays, which was then divided by the volume it occupied.

$$\rho B = \frac{Wm}{Vm} \ldots\ldots\ldots\ldots 2$$

Chemical Analysis of the Media

The electrical conductivity (EC) and pH of the materials were determined via 10g of the media with 10 ml of distilled water. The solution was agitated with a glass rod before being allowed to settle for 10 min before measuring the pH using a pH meter and EC using a conductivity meter. In addition, Organic carbon and nitrogen levels were determinedcalorimetrically using a micro plate reader. The C: N ratio was then calculated using the organic carbon and nitrogen levels. Finally, 30g of the media was weighed into the conical flask, and 50 mL of deionized Water was introduced to the flask. After 24 h, the solution was filtered to obtain a filtrate for elemental analysis of Ca, Mg, K, Na, Zn, Cu, Mn, Fe, and Al using a microplasma atomic emission spectrophotometer (MP-AES 4200).

Determination of the Point of Zero Charge

The point zero charge (PZC) of the soil treatment combinations was evaluated using the equation adopted by Onokebhagbe *et al*. (2021) to determine PZC and surface electrical potential.

$$\mathrm{PZC} = (2\,\mathrm{x}\,\mathrm{pHKCl}) - \mathrm{pHH2O} \ldots\ldots\ldots 3$$

The pH values and ΔpH of the media were also calculated using the following formula:

$$\Delta\mathrm{pH} = \mathrm{pHKCl} - \mathrm{pHH2O} \ldots\ldots\ldots\ldots 4$$

The surface electrical potential (Ψ0) in mV was estimated using the Nernst equation, which Chaves *et al*. (2016) reduced as follows:

$$\Psi_0 = 59.1(\mathrm{PZC} - \mathrm{pHH2O}) \ldots\ldots\ldots\ldots 5$$

Experimental Design

The study was carried out in a greenhouse using a completely randomized design (CRD) with six soilless media treatments and two replications.

Data Analysis

JMP® 15 edition was used for descriptive statistics and one-way analysis of variance (ANOVA). To identify significant differences between the individual treatments, the post hoc Tukey's HSD test was performed. Pearson correlation analysis was utilized to examine the relationship between the soilless media's electrochemical properties and PZC.

RESULTS AND DISCUSSION

Physical and Chemical Properties of the Media

The properties of the media are summarized in Tables 1 and 2. The composted rice husk and composted Sapele media had a high water holding capacity, while composted Doum had the lowest moisture content (51.11%). The water retention capacity of the media could be affected by changes in surface area, density, and porosity after modification,

as well as microbial activities of the composted materials. Azim *et al*. (2018) found that composting may alter water retention capacity due to microorganisms consuming water for their metabolic activities. The particle density was highest in Doum palm at 0.80 g cm^{-3}, and lowest in composted Sapele sawdust at 0.21 g cm cm^{-3}. However, the bulk density of all media was low, with the highest in Iroko at 0.91 g cm cm^{-3} and lowest in Sapele at 0.12 g cm^{-3}. Low bulk density may be due to material properties such as strength, porosity, and compaction ease. Mahogany palm had the highest pH (H_2O) of 8.37, and Iroko sawdust had the lowest pH (H_2O) of 7.06. pH (KCl) showed a similar trend to pH (H_2O). The highest EC value (0.50 dSm^{-1}) was found in Iroko, and the lowest value (0.10 dSm^{-1}) was found in cocoapeat.A similar result was observed by Azim et al. (2018).

Table 1. Physical and Chemical Properties of the Media

Media	WRC (%)	PD (gcm^{-3})	BD (gcm^{-3})	pH(H_2O)	pH(KCl)	EC ($dS\ m^{-1}$)	N %	OC%	C: N
Cocopeat	83.33^{c}	0.46^{c}	0.23^{d}	7.90^{c}	7.21^{c}	0.10^{d}	0.014^{a}	0.50^{a}	35.01^{fg}
Doum Palm	51.11^{f}	0.81^{a}	0.30^{b}	8.17^{b}	7.73^{b}	0.27^{b}	0.013^{b}	0.48^{bc}	34.75^{g}
Iroko	80.00^{d}	0.26^{d}	0.94^{a}	7.06^{f}	6.32^{ef}	0.50^{a}	0.012^{c}	0.47^{cd}	36.55^{bcd}
Mahogany	67.78^{e}	0.25^{e}	0.18^{e}	8.37^{a}	7.81^{a}	0.17^{c}	0.012^{c}	0.46^{de}	36.22^{cde}
Rice Husk	84.44^{b}	0.60^{b}	0.26^{c}	7.36^{e}	6.57^{e}	0.23^{b}	0.013^{b}	0.49^{a}	38.34^{a}
Sapele	85.56^{a}	0.21^{f}	0.12^{e}	7.70^{d}	7.10^{d}	0.21^{bc}	0.013^{b}	0.46^{e}	35.72^{ef}
SE±	0.00001	0	0.0004	0.0008	0.0003	0.0009	0.0008	0.002	0.175
P-Value	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**

SE = Standard Error of Mean **Significance at 1% level of Probability Levels not connected by the same letters are significantly different. WRC denotes Water Retention Capacity; PD denotes Particle Density; BD denotes Bulk Density.

The nitrogen content of Cocopeat was found to be the highest at 0.014%, while the lowest amount was 0.012% (shown in Table 1). The variation in the nitrogen content of the media can be attributed to the release of mineralized N into ammonium (NH_4^+) and nitrate (NO_3^-) during nitrification. The percentage of organic carbon in the media was low and the variation among the media is likely due to microbial activity and the presence of anaerobic sites in the compost that may result in methane (CH_4) emissions during the fermentation process, which is consistent with the findings of Thiyageshwari *et al*. (2018). The highest C: N ratio was found in rice husk (38.34) and the lowest value of 34 was found in Doum. The slight decrease in the C: N ratio of the media after modification methods could be due to the increase in the humification of organic matter during the composting process, which is consistent with the findings of Qasim *et al*. (2018). However, the variation in the C: N ratio in the media indicates that the compost is not fully matured as no additional N source was added during composting. The highest calcium content was found in rice husk, while the lowest value was found in cocoapeat (16.26 mg kg^{-1}). The concentrations of the remaining exchangeable bases were all within range, with potassium being slightly higher than the other metals (Table 2). The micronutrients were also within range, with rice husk having the highest concentrations of Mn, Cu, and Fe, while Iroko had the highest Zn and Al concentrations (Table 2). The variation in the mineral compositions of the exchangeable bases can be attributed to the mineralization of minerals by microbial activity during the composting process. Another factor that may influence the availability of nutrients is the change in pH of the media. This result is consistent with earlier literature that found that the mineralization of compost may alter the concentration of nutrients due to microbial activities(Cappuyns and Swennen, 2008).

Table 2. Chemical Composition of the Media

Media	Ca Cmol kg^{-1}	Mg Cmol kg^{-1}	K Cmol kg^{-1}	Na Cmol kg^{-1}	Zn mg kg^{-1}	Cu mg kg^{-1}	Mn mg kg^{-1}	Fe mg kg^{-1}	Al mg kg^{-1}
Cocopeat	16.26^{d}	6.74^{c}	27.72^{e}	36.02^{ab}	0.06^{b}	0.08^{cd}	0.06^{ef}	0.21^{c}	0.10^{c}
Doum Palm	17.70^{cd}	7.58^{bc}	223.72^{bc}	40.34^{a}	0.08^{a}	0.18^{ab}	0.10^{cd}	0.18^{cd}	0.11^{c}
Iroko	19.08^{cd}	7.71^{b}	307.29^{a}	14.90^{cd}	0.08^{a}	0.11^{b}	0.12^{bcd}	0.30^{b}	0.48^{b}
Mahogany	17.63^{cd}	6.77^{c}	168.99^{cd}	8.98^{d}	0.06^{b}	0.09^{cd}	0.04^{f}	0.11^{e}	0.06^{d}
Rice Husk	48.35^{a}	19.79^{a}	39.24^{e}	36.00^{ab}	0.02^{c}	0.20^{a}	0.34^{a}	0.54^{a}	1.29^{a}
Sapele	20.97^{b}	5.79^{d}	128.92^{cde}	21.46^{bcd}	0.07^{ab}	0.09^{cd}	0.09^{cde}	0.18^{cd}	0.11^{c}
SE±	1.3	0.79	25.14	3.14	0.005	0.003	0.01	0.008	0.007
P-Value	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**

SE = Standard Error of Mean **Significance at 1% level of Probability. Levels not connected by the same letters are significantly different.

Electrochemical properties of the media

The charge properties of the different media are shown in Table 3. There were significant ($p<0.0001$) variations in the ECe means obtained from the media. Treatments containing composted Iroko had the highest EC with value of 0.51dSm^{-1}, with the lowest being cocoapeat with value 0.10 dSm^{-1}. The PZC of the media was within the same range. In comparison to the natural pH values, the medium produced higher pH values. The high pH levels obtained from the medium were comparable to the pH range found by Azim *et al*. (2018). This contradicts the conclusions of Chen *et al*. (2022), who found that the pH of soils increased with the addition of compost. Citak and Sonmez (2011) found that increasing compost rates resulted in an increase in pH. The increase in pH values of the media might be attributed to the oxygen containing functional group's dissociation reactions on the surfaces of the organic material, which is consistent with the findings of Marta et al. (2019). Furthermore, the liming effects of full compost may have contributed to a rise in soil pH, which may have reduced cationic attraction and mobility due to less competition between H^+/metal cations for exchange sites on the media (Beesley *et al.,* 2011). Similarly, the negative electric potential (Ψ0) values observed in the current study were caused by an increase in compost media pH as well as low PZC values (Table 3).Rice husk, Iroko and cocoapeat produced more negative charges. It is reasonable to believe that the study's negative potential values were directly impacted by the composting of the media content. This is consistent with Chaves *et al.* (2016)'s discovery that "this negative sign and magnitude of Ψ0 were directly influenced by the related magnitude of the ΔpH." The increase in negative charges during composting might be directly related to the dissociation of functional groups and the action of potential determining ions (e.g., H^+ and OH^-).The negative ΔpH values indicated that the media had a higher concentration of negative charges. Under altered pH conditions, the cation exchange capacity (CEC) exceeded the anion exchange capacity (AEC). However, as seen in Table 3, the magnitude of pH decreased with varied media, indicating a decrease in CEC. The increase in CEC is mostly due to the negative charge on the organic media's outer surface, which results from the dissociation of functional groups (Cheng *et al.* 2006).

Table 3. Electrochemical Properties of the Media

Treatment	pH (KCl)	pH (H_2O)	ECe (dSm^{-1})	ΔpH	PZC	Ψ_0 (mV)
Cocopeat	7.21^{c}	7.90^{c}	0.10^{e}	-0.69	6.52	-81.56
Doum Palm	7.73^{ab}	8.17^{b}	0.27^{b}	-0.44	7.29	-52.01
Iroko	6.32^{f}	7.06^{e}	0.50^{a}	-0.74	5.58	-87.47
Mahogany	7.81^{a}	8.37^{a}	0.17^{d}	-0.56	7.25	-66.19
Rice Husk	6.57^{e}	7.36^{d}	0.23^{c}	-0.79	5.78	-93.38
Sapele	7.10^{d}	7.70^{cd}	0.21^{cd}	-0.6	6.5	-70.92

Levels not connected by the same letters are significantly different at the 1% level of probability.

Correlation Coefficient between PZC and Selected Media Properties

Table 4 shows the correlation coefficient between PZC and several media characteristics. The results showed that pH and Ψ0were significantly correlated with the PZC of the medium in the experiment, with an*R* value of 0.91 suggesting that ΔpH and Ψ0 influenced 91% of the change in PZC. Table 4 shows that electrical conductivity (ECe) has no influence on the PZC of the media.

Table 4. Correlation between PZC, pH and Ψ0 of the Media.

Media		
Factors	**R**	**Significance level**
Ece	-0.54	NS
pH	0.91	***
Ψ0	0.93	***

, **, * are significance levels of < 0.05, < 0.01 and < 0.001, respectively; NS: not statistically significant.*

Effects of Media on Tomato Root Volume (cm^3)

The effect of media on root volume of tomato test crop is shown in Fig 1, where there was a highly significant difference ($\rho < 0.0001$) among the media with the highest volume in cocopeat, mahogany, and rice husk (0.03) followed by Iroko and Sapele (0.2) and lowest in Doum Palm (0.01). The variation observed in the root volume between the media could be attributed to the difference in the density of the media, which is the weight of the media per unit volume, which has a negative effect on root penetration and, in turn, retards its volume. Another factor that may influence root volume is nutrient availability, especially nitrogen, which directly affects root growth (Best *et al.,* 2014).

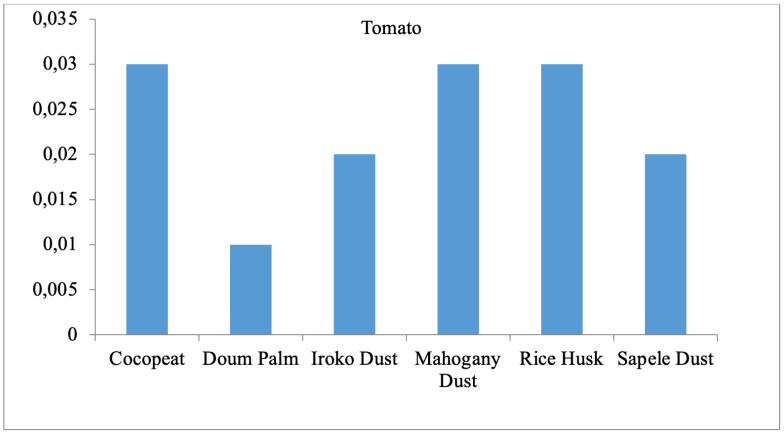

Figure 1. Effects of Media on Root Volume (cm^3)

Effect of Media on Root Density

The effect of media on root density is shown in Fig. 2, where a significant difference ($p < 0.0001$) among the media was observed. The variation observed in the root density between the media can be attributed to the difference in the density of the materials. Furthermore, since soilless media mostly maximize space utilization, allowing for higher plant densities in a given area. This close spacing can stimulate competition among plants, leading to increased root density as plants vie for available nutrients and space (Best *et al.,* 2014). However, nutrient availability, particularly nitrogen, which directly affects root growth may influence root density.

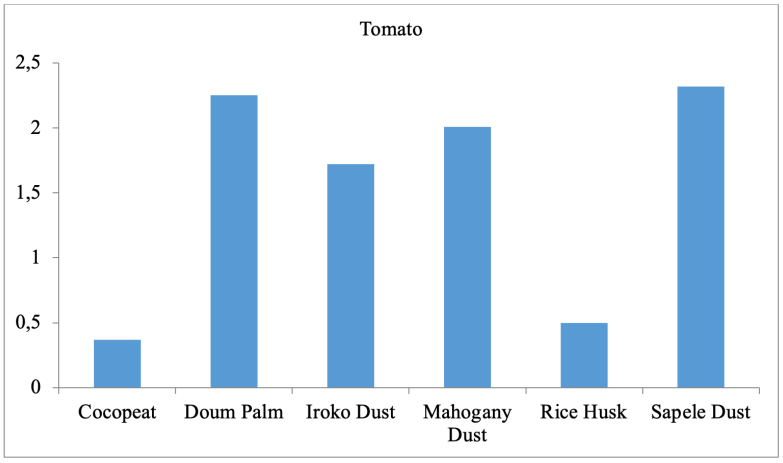

Figure 2. Effects of Media on Root Density ($g\ cm^{-3}$)

Effects of Media on Days to Flowering

Table 5 shows a significant ($p<0.0001$) effect of media on days to flowering of the test crop. The lowest number of days to flowering was observed in rice husk (20.67), followed by cocopeat, mahogany, Sapele, and Iroko dust. The Doum palm has the highest number of days to flowering (30), with slight variation observed between the media. The variability observed in the number of days to flowering could be attributed to the germination of the media and the initial plant height and root volume of the test crop. The capacity of the media to supply adequate moisture, nutrients, and proper aeration amongst many other physical and chemical attributes needed by the crop to grow might attribute to the delay in days to flowering (Ahmad *et al.,* 2009).

Table 5. Effects of Media on Days to Flowering

Media	Tomato
Cocopeat	21.33[a]
Doum Palm	30.17[d]
Iroko Dust	26.17[b]
Mahogany Dust	21.50[a]
Rice Husk	20.67[a]
Sapele Dust	22.83[bc]
SE±	0.58
P- Value	<0.0001**

SE = Standard Error of Mean **Significance at 1% level of Probability Levels not connected by the same letters are significantly different.

Effect of media on number of tomato fruits (yield)

Table 6 illustrates the tomato yield data. A highly significant difference ($p < 0.0001$) was observed in the number of fruits per hectare. The highest tomato yield was recorded in cocopeat media (4,604,444), followed by rice husk, Sapele, mahogany, and Iroko dust. Doum palm exhibited the lowest fruit yield (1,175,556), with minor variations observed among the media. The variance in fruit yield could be attributed to factors such as the absence of transplanting shock during transplantation and the initial height of the plants. Robust seedlings tend to establish quickly and vigorously due to their efficient absorption of water and nutrients through developing roots, resulting in higher fruit yield and quality. Additionally, nutrient content, particularly the high phosphorus content in rice husk, may influence fruit yield (Olle *et al.,* 2012). Moreover, Ahmad et al. (2011) reported that rice husk media possess exceptional physical, chemical, and biological properties, translating to optimum growing condition, which aligns with the findings of this study (as presented in Table 1 and 2). This similarity in properties could explain the higher yield observed in rice husk media compared to other media types.

Table 6. Effect of media on number of fruits (yield) per hectare

Media	Tomato (ha^{-1})
Cocopeat	4,604,444[a]
Doum Palm	1,175,556[d]
Iroko Dust	1,593,333[cd]
Mahogany Dust	2,120,000[bcd]
Rice Husk	3,420,000[ab]
Sapele Dust	2,606,667[bc]
SE±	253,636
P- Value	<0.0001**

SE = Standard Error of Mean **Significance at 1% level of Probability Levels not connected by the same letters are significantly different.

Effect of Media on Fresh Weight of Tomato

Table 7 shows the effect of the media and modification methods on fresh weight (t ha^{-1}) in the test crops. The highest fresh weight in tomato was observed in cocopeat (69.30), followed by rice husk, Sapele, Iroko dust and mahogany. Doum palm has the lowest yield (17.10), with slight variation observed between media. The differences noticed in fresh weight can be attributed to various factors such as the germination process in different growing media, the initial height of plants, root volume, and the duration until flowering. These aspects vary across different media types due to the distinct materials they contain.

Table 7. Effect of Media on Fresh Weight (kg ha^{-1})

Media	Tomato kg ha^{-1}
Cocopeat	69.30[a]
Doum Palm	17.10[d]
Iroko Dust	22.67[cd]
Mahogany Dust	21.70[bcd]
Rice Husk	51.63[ab]
Sapele Dust	39.10[c]
SE±	4.41
P- Value	<0.0001**

SE = Standard Error of Mean **Significance at 1% level of Probability Levels not connected by the same letters are significantly different.

CONCLUSION

This study revealed that composted rice husk and cocopeat were the best growing medium as they showed more promise in terms of yield both in number of fruits and weight. Furthermore, the study shows that the modification (composting) of the growing media decreased ΔpH and CEC levels, however the media's PZC and pH was increased. This study also demonstrated the significance of the function performed by composted media owing to the rise in negative charges that will aid in plant nutrient (cations) adsorption and retention, hence boosting the soil's fertility status. It is recommended that rice husk and cocopeat be used as the growing media. However, due to the cost of producing cocopeat media, rice husk can serve as an alternative to cocopeat as a growing medium. Despite a slight delay in germination shown by rice husk media, a high yield was attained at the end of the production.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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The nexus between information sources, gender and adoption of fall armyworm management practices in Southern Ghana

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Abstract

The impact of Fall Army Worm (FAW) infestation on the livelihood outcomes of farmers in Africa is an issue of critical concern. Specificities of information sources and their efficacy in the management of Fall Armyworm remain crucial. Yet still, the nexus between farmers' information sources and the management of Fall Armyworm appears scarce in the related literature pertaining to the global south. This article answers the research question: What is the relationship between specific information sources and the management practices of Fall Army Worm in Ghana? Using cross-sectional data on 340 smallholder farmers, the findings showed that information derived from peer farmers, Agricultural extension officers, and the media related to the adoption of fall armyworm management practices. Additionally, information from agricultural extension agents has a significant relationship with the use of pesticides, handpicking, and frequent weeding. Generally, the majority (97%) of smallholder farmers remained aware of the presence of FAW and had been negatively affected. We recommend that peer-to-peer extension be harnessed and scaled up in the dissemination of useful agricultural information given the shortfall in adequate agricultural extension officers in Ghana and most countries in Sub-Saharan Africa.

Keywords: Fall Armyworm, Information sources, Management practices, Gender, Ghana

Type: Research Article

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INTRODUCTION

This article addresses the research question: what is the relationship between information sources and the management practices relating to FAW? We premise this question within the context of maize farmers in southern Ghana. In Sub-Saharan Africa (SSA), maize remains the leading and most widely grown staple food crop. It serves as a food source and a secured livelihood for over 208 million people, covering over 36 million hectares of land (Macauley, 2015). In Ghana, maize constitutes a vital staple crop, accounting for half of the cereal production, and it is grown in all agroecological zones (Acquah et al., 2020). However, Ghana's maize yield (1.95Mt/ha) falls below the expected (5.5Mt/ha) (FAOSTAT, 2022.). Deutsch et al. (2018) attributed the destruction caused by insect pests as a significant cause of low maize production. Koffi et al. (2020) indicated that the FAW (Spodoptera frugiperda) constitutes one of the most destructive insect pests. For instance, The Center for Agricultural and Bioscience International (CABI)) in 2017 indicated losses of about 22% - 65% and 25%- 50% were recorded in Ghana and Zambia. In 2018, the value of maize loss resulting from FAW infestation in Ghana was extrapolated to USD 177 million (Day et al., 2017; Kansiime et al., 2019). That same year, the Government of Ghana spent over GHC 15 million (USD 1.2 million)

in various attempts to curb FAW infestation. Its destruction in Africa was initially reported in Benin, Nigeria, Togo, and the island of Sao Tome in 2016 (Goergen et al., 2016). Between 2016 and 2018, the FAW spread to 44 African countries, including Ghana (Longari, 2019). The spread and destruction caused by the FAW have rendered millions of subsistence farmers food insecure and continue to threaten future livelihood outcomes in Africa (Tambo et al., 2020). It can therefore be concluded that the impact of FAW infestation on the economy of Ghana and the livelihood of the individual maize farmers are crucial and hence cannot be ignored. However, this impact on livelihood can also be related to other crop pests; therefore, tackling FAW means tackling other crop pests.

The availability and timely access to sources of information needed to fight the FAW remains essential in managing FAW and yet crucial in the fight against any other crop pest. Adekambi et al. (2020) and Mittal and Mehar (2015) indicated that specific information sources affect farmers' attitudes and practices differently; however, it is unclear how different information sources relate to farmers' attitudes and, consequently, their FAW management practices. Farmers in different geographical zones have different relevant sources of information in Ghana. It is, therefore, relevant that research is conducted on the different geographical zones to identify information sources available to farmers and how this influences their management of FAW infestation. Studies have found that information sources relate to farmers' practices differently in different situations and geographical zones. Adekambi et al. (2020) and Mittal and Mehar (2015) found that access to AEAs relates to farmers' adoption decisions differently in different locations. To this end, the importance of information sources and how it relates to farmer practices differently cannot be over-emphasized.

However, access to agricultural resources, including information and information sources, is differentiated based on gender in developing countries, including Ghana. Many researchers (Anaglo et al., 2020; Anaglo1 et al., 2014) have argued that women farmers have less access to agricultural resources including information. Anaglo1 et al., (2014) found that in Northern Ghana, women farmers had less access to agricultural information compared to their male counterparts. This differentiation was also identified for other productive resources such as labour, land and credit in Northern Ghana. On the order hand, it was found that in southern Ghana, these differentiated access to agricultural resources does not exist (Ankrah et al., 2020). Yet they further argued that the gendered and ungendered access to productive resources in southern Ghana intersects with class, age, education and socio-cultural norms in shaping access to and control over resources. From these, one can argue that geographical differences plays a vital role in access to resources including information by both men and women farmers. It is, therefore, logical that initiatives targeted at delivering resources, including information to farmers, should be gender sensitive.

Despite the relevance of information sources to farmers' management practices, few studies have been conducted on FAW management practices adopted by farmers and information sources available to farmers in Ghana. The few studies (Asare-Nuamah, 2020; Tambo et al., 2020) that looked at management practices adopted by farmers to curb FAW infestation have not considered the information sources available to farmers on FAW and its control and how access to information from these sources affect farmers' decision to adopt the management practices. Neither have these studies also considered the role of gender in accessing information and how this further influences the adoption of management practices. Moreover, knowledge of how farmers manage FAW can be applied to other crop pest infestations that may occur in the future. This study, therefore, assessed management practices adopted by farmers to fight FAW, information sources available to farmers on FAW and its management practices, and how access to information from these sources affects farmers' decision to adopt management practices. It also considers the role of gender in farmers' ability to access information and how it further relates to the adoption of management practices.

Farmers' decision to adopt certain practices is essentially information-seeking and information-processing activity that clarifies the advantages and disadvantages of adopting practices (Kumela et al., 2018). In Ghana, information on the identification and management of FAW was the main message disseminated to farmers through all the information sources identified. Yet access to information is differentiated based on gender in many developing countries, including Ghana (Ankrah et al., 2020), with women farmers being in a disadvantaged position in most cases due to unfavourable gender roles in such countries. Mudege et al. (2017) and Ragasa et al. (2013) found gender norms, damaging stereotyping and more as barriers that hinder female farmers from accessing agricultural resources (including information), agricultural extension and agricultural training in Malawi and Ethiopia. Also, many other researchers (Ankrah et al., 2020; Chatterjee et al., 2020) have argued that typical traditional customs do not support the use of ICTs especially among women because of time, poverty and challenging economic opportunities. This hindrance further influenced female farmers' ability to adopt new practices. It is, however, interesting to note that other gender studies in some parts of Ghana found that women farmers have higher access to agricultural resources such as credit than their male counterparts (Anaglo et al., 2014). In terms of adoption, Muriithi et al. (2018) iterated that there exists no difference in the adoption of agricultural practices based on gender, while Gebre et al. (2019) found gender as a significant factor that affects the adoption of farming practices in Ethiopia. In the context of an emergency like a pest outbreak, knowledge of these dynamics in gender roles is entirely essential in devising policies

and strategies.

There are many sources of information for farmers. Okwu and Umoru (2009) classified three significant sources of information available to farmers: extension agents, mass media, and fellow farmers.

Agricultural extension is widely known as one of the significant sources of information available to farmers, especially in developing countries. Several studies on the adoption of farming practices have cited the information source to have influenced farmers' decision to adopt certain farming and management practices (Anang, 2018; Kotu et al., 2017; Mittal and Mehar, 2015; Onyeneke, 2017). In Ghana, the established system practice is that Agricultural Extension Agents (AEAs) are allocated to different geographical areas to assist farmers with information delivery. As a result, the government relied on this system for information delivery to fight the FAW. However, the AEAs usually cannot reach all their allocated farmers with information because they are understaffed, with the farmer to AEAs ratio being 1:1200 instead of the FAO standard of 1:400. Whereas there are no data on the disgregation of AEAs among men and women, many researchers have argued that in southern Ghana there are very few women working as AEAs. (Anaglo et al., 2020; Anaglo1 et al., 2014; Ankrah et al., 2020)

Many researchers and policymakers have also suggested the mass media as an efficient and effective way to deliver timely information to farmers, particularly in developing countries where farmers live in widely dispersed and diverse communities. The mass media includes radio, television, mobile platforms, text messaging, and many more (Larochelle et al., 2019). In the case of FAW infestation, government campaigns were mainly sharing posters with pictures of the crop pest and how to manage it and through radio station information sharing. This strategy was expected to be effective because most farm households in the selected area of study have access to radio sets, which are treated almost as a part of farm household culture. Therefore, information through radio stations was expected to reach most farmers regardless of their economic class or educational level. These media campaigns were considered gender-neutral, and that both men and women would have access to the information being shared. However, how effective this strategy has been regarding the information reaching farmers (and if both men and women could access this) is yet to be known since FAW infestation is still a problem for farmers.

On the other hand, the peer farmer has the advantage of the social multiplier effect. It is an effective information source for educating and informing farmers about farming practices (Anaglo et al., 2020). Whereas farmer-to-farmer information sharing can be a formally organised program where farmers are trained to share certain information, the peer farmer information sources identified in this study entirely occurred by word of mouth and based on farmers' interest to share information among themselves.

METHODOLOGY

Research design

Three regions in which maize production occurs were selected. This includes the Central Region, the Bono Region, and the Ashanti Region of Ghana. Apart from the fact that the Bono Ahafo and Ashanti regions are among Ghana's major maize production areas, these three regions were selected based on a report from the agricultural extension directorate on FAW infestation in these three regions. Two districts from each region were randomly selected using a simple random sampling technique. With the Awutu Senya West and Awutu Senya East districts from the Central region, The Tein and Wenchi municipal from the Bono region, and the West Akim and the Afram Plain South districts from the Eastern region. Out of each district, the list of registered maize farmers was obtained from the district assemblies. Farmers were randomly selected using simple random sampling but with a constant sampling fraction (5%). The sample size was calculated based on the number of registered maize farmers in each selected district. This ensured that every farmer in the population had an equal chance of being selected. In the central region, 135 Farmers were sampled from the two districts, while 95 and 110 farmers were sampled from the Bono and Eastern regions, respectively. (See Table 1 for sample distribution).

This study was conducted using the survey method. A survey is used mainly for explanatory and descriptive research; it is used in research with individuals or groups as a unit of analysis. In this case, it was used to assess and describe the management practices employed by farmers in fighting FAW, sources of information available to farmers on FAW and FAW control measures, and the relationship that exists between their accessibility to information and their decision to adopt management practices, with maize farmers being the unit of analysis. A questionnaire was administered to farmers by researcher enumerators in the local language of the farmers. Researchers trained the enumerators before they administered the questionnaire" Farmers' consent was sought before they participated in this research. The purpose and use of this study were explained to the farmers before they participated. The questionnaire administration lasted one month, from the second week of January 2020 to the Second week of February 2020.

Information on the demographics of farmers was collected. Also, farmers were asked if they knew of FAW and had

experienced infestation on their farms to determine their awareness of the FAW infestation. To assess their adoption of management practices, they were asked if they had previously applied FAW management practices on their farms. Farmers who indicated they had adopted control measures were further questioned on which type of control measure they adopted with a list of control measures (Use of pesticides, Handpicking eggs and caterpillars, Frequent weeding, Traditional method; the use of sand, highly concentrated salt) identified through the pre-testing of the questionnaire and being supported in the reviewed literature being provided for them to select from.(Asare-Nuamah, 2020; Kansiime et al., 2019). Adoption in this study means a farmer applied FAW management practice on the farm to control or manage the pest.

To determine information access and information sources available to farmers, they were asked if they could access information on FAW infestation and management practices. Farmers who indicated access to information about the pest were asked to select from a list of information sources (peer farmers, AEAs, and the Media) which once again was provided to them through sources identified from the literature review and pre-testing of the questionnaire (Kotu et al., 2017).

Table 1. Study regions and districts with sample distribution

Region	District/ Municipal	N
Bono	Wenchi Municipal	57
	Tain District	38
Eastern	Afram Plains South	52
	West Akim	58
Central	Awutu Senya East	72
	Awutu Senya West	63
	Total	340

Source: Field survey, 2020.

Data were analyzed using the statistical package for social sciences (version 22). Descriptive statistics such as percentages, cross tabulation, frequencies, and more were used to present findings in the data. It was expected that farmers affected by FAW infestation would have a high probability of adopting management practices if they had access to relevant information on FAW and FAW management practices. The hypothesis, therefore, was that increasing access to information increased the probability of adopting management practices.

To determine if there exists a relationship between accessing information from AEAs and the adoption of FAW management practices, a chi-square analysis was conducted between 'yes access information from AEAs', no do not access information from AEAs, and yes adopt management practice and no do not access management practices. Also, between gender, access to information and adoption of management practices

RESULTS

Farmer Characteristics

Table 2, presents the demographic characteristics of respondents. The average age of the respondents was 47 years. Most farmers were males, with the percentage of males being 73% and females being 27%. It was also observed that over 69% of the farmers were above forty years old. The mean household size of respondents was few (5%) of the respondents had tertiary education; however, most farmers (62%) had primary education. This means people with primary education dominate the farmers. The farmers had an average maize farm size of 5.3 acres and a total farm size average of 7.8 acres. The average farming experience of the respondents is 22 years. Over 90% of the farmers had more than five years of experience in maize farming.

Gender and farmer demographics

Results reviewed that the gender of farmers had a significant relationship with farmer demographics in general. Men farmers were more educated than women with only 6.5% of women farmers having high school and tertiary education, 24% of male respondents attending high school and tertiary. The same pattern was identified for farming experience and maize farm size. 51% of male respondents had farm size above 20 hectares whereas the women respondents with 20 hectares and above were only 31%. Table 3 below presents the results on intersection of Gender and farmer demographics.

Table 2. Demographic characteristics

Variables		Frequencies	Percentages (%)	Mean	SD
Gender	Male	247	72.6		
	Female	93	27.4		
Age (years)	21-40	110	32.4		
	41-60	197	57.1	46.76	11.25
	Above 60	36	10.6		
Educational Level	No formal Education	61	17.9	1.07	0.723
	Basic	212	62.4		
	Secondary	50	14.7		
	Tertiary	17	5.0		
Maize farming Experience	Less than 5 years	32	9.4	22.47	12.552
	More than 5 years	308	90.6		
Maize farm size	Less than 5 Ha	186	54.7		
	5 – 20 Ha	77	22.6	5.279	6.768
	Above 20 Ha	77	22.6		

Table 3. Intersection of Gender and farmer Demographic

		Gender	
Variables		Male	Female
Maize Farming Experience	Less than 5years	43	8
	More than 5years	203	84
	Test; Chi=4.033 P=0.045 df=1		
Educational level	No formal education	33	28
	Basic	153	59
	Secondary	45	5
	Tertiary	16	1
	Test; Chi=22.107 P=0.000 df=3		
Maize farm size	Less than 5 Ha	74	33
	5 – 20 Ha	48	31
	Above 20 Ha	125	29
	Test; Chi=14.548 P=0.002 df=3		
Age	21-40	83	21
	41-60	133	63
	Above 60	28	9
	Test; Chi=8.298 P=0.040 df=3		

FAW Management Practices Adopted by Farmers

All the farmers interviewed knew of FAW, and most farmers had experienced FAW infestation on their farms. However, only a few (2.4%) of these farmers said they had not experienced infestation on their farms. Farmers adopted different management practices to fight FAW on their farms. Most farmers (74.7%) interviewed adopted one or more management practices. Out of the farmers who adopted management practices, over 69% used pesticides. Most farmers who used pesticides reported that it was effective.

Handpicking was the second management practice adopted most by farmers, with 39.2% of farmers who adopted management practices using it. More than half of the adopters of handpicking also reported its effectiveness. Frequent weeding and traditional methods (use of neem tree extract, use of ash) were the management practices, with few farmers adopting them. While frequent weeding had only 11.4% of farmers adopting it, the traditional method had about 0.6% of farmers adopting it.

Table 4. Maize Farmers' knowledge of FAW and their management practices

Maize Farmers FAW management practices	Freq. (Yes)	%
Knowledge on FAW	340	100
Experienced in farm	332	97.6
Controlled FAW	255	74.7
FAW Management Practice		
Use of pesticide	236	69.4
Handpicking eggs and caterpillars	100	29.4
Frequent weeding	39	11.4
Traditional method	2	0.6

Source: Survey data, 2020.

Information Sources Available to Farmers

Table 5 presents a summary of information sources available to farmers. The study found that most farmers (89.7%) interviewed had access to information on FAW. This information was from one or more of the sources below. Most farmers had multiple sources of information. Out of the total farmers interviewed, 76.5% indicated peer farmers as their source of information, making fellow farmers the major source of information available to farmers on FAW and FAW management practices. AEAs followed fellow farmers as an information source, with 60 % of farmers with access to information indicating AEAs as a source of information to them. This contradicts the researchers' expectations and the many reports that AEAs cannot reach farmers due to their more minor number. The media, which had 29.4% of farmers with information access indicating it as a source of information, was the source with the most minor percentage.

Table 5. Information sources

Information Sources	Frequency	Percent (%)
Access to information on FAW	305	89.7
Access to information from peer farmers	260	76.5
Access to information from AEAs	204	60.0
Access to information from the Media	118	34.7

Source: Survey data, 2020.

Access to information from AEAs and Adoption of Management practices

While the chi-square test (in table 6) showed a signification relationship between access to information from AEAs and the use of pesticides, the use of handpicking, and frequent weeding, the results revealed that there exists no relationship between access to information from AEAs and the use of the traditional method.

While the probability that a farmer will use pesticides increases with increased access to information from AEAs, the probability of farmers hand-picking eggs and larvae and using frequent weeding seems to decrease with increased access to information from AEAs. This is because the government's initial attempt to fight FAW infestation was mainly through disseminating pesticides to farmers through AEAs. AEAs, therefore mainly distributed and disseminated the use of pesticides to farmers. AEAs also reported that, due to other disadvantages of hand picking and frequent weeding, which is labour-intensive and difficult to use, they intentionally do not recommend it to farmers.

Access to information from peer farmers and the adoption of FAW management practices

A chi-square (in table 6) test showed a significant relationship between access to information from peer farmers and the adoption of management practices (use of pesticides and handpicking). The results showed that farmers had a higher probability of adopting management practices if they had access to information from peer farmers. Over 87% and 91% of the farmers who adopted pesticide use and handpicking, respectively, had access to information from peer farmers.

Access to information from the media and adoption of FAW management practices

It was expected that most farmers would have indicated the media as their source of information. Considering the government's campaign through the media, it was expected to influence farmers' decision to adopt management practices. However, the results showed otherwise; it was the source of information with the least number of farmers indicating that they sourced information. Also, the cross-tabulation showed that access to information from the

media had a significant relationship only with hand-picking, showing no significant association with the adoption of other management practices. It is pertinent to note that the result shows an inverse relationship between access to information from the media and the use of handpicking to control FAW infestation.

Table 6. Relationship between Sources of Information and Adoption of FAW Management Practices

	Use of Pesticides			**Use of Handpicking**		**Use of Frequent Weeding**		**Use of Traditional method**	
	NO		YES	NO	YES	NO	YES	NO	YES
Access to FAW information from AEAs	NO	65	71	125	11	129	7	136	0
	YES	39	165	115	89	172	32	202	2
	Test	Chi=31.605 P=0.000 df=1		Chi=49.642 P=0.000 df=1		Chi=8.926 P=0.000 df=1		Fisher's Exact Test=0.530	
		NO	YES	NO	YES	NO	YES	NO	YES
Access to FAW information from fellow farmers	NO	39	41	67	13	67	13	80	0
	YES	65	195	173	87	234	26	258	2
	Test	Chi=16.253 P=0.000 df=1		Chi=8.729 P=0.003 df =1		Fisher's Exact Test=0 .548		Fisher's Exact Test=1.00	
		NO	YES	NO	YES	NO	YES	NO	YES
Access to FAW information from the Media	NO	75	147	176	46	197	25	221	1
	YES	29	89	64	54	104	14	117	1
	Test	Chi=3.076 P=0.079 df=1		Chi=23.272 P=0.00 df=1		Fisher's exact test= 0.868		Fisher's exact test= 1.00	

Source: Survey data, 2020

Gender and Access to Information

It was found that male farmers had a higher probability of getting access to information than female farmers. There was a significant relationship between the gender of farmers and their ability to access information from all information sources. In all these, male farmers had higher access to information than female farmers. It is shown in Table 7 that 94% of male farmers had access to information from one or more of the information sources, and only 78% of female farmers had access to information from the information sources.

Table 7. Relationship between Gender and Access to Information

	Access to info		**Access to info from AEAs**		**Access to info from peer farmers**		**Access to info. From the media**	
	NO	**YES**	**NO**	**YES**	**NO**	**YES**	**NO**	**YES**
Female	20	73	46	46	33	61	69	24
Male	14	233	90	158	47	199	153	94
	Chi=18.829 P=0.000 df=1		Chi=5.133 P=0.023 df=1		Chi=8.418 P=0.015 df=1		Chi=4.232 P=0.040 df=1	

Source: Survey data, 2020

Gender and adoption of FAW management practices

Results showed that the gender of farmers had a significant relationship with the adoption of management practices in general. The same results were found for using pesticides (chemicals), handpicking, and frequent weeding. In all these, the probability that male farmers will adopt the management practice(s) was much higher than that of female farmers. Table 8 shows that only 56% of female farmers adopted management practices, while 82% of male farmers adopted management practices.

Table 8. Relationship between Gender and adoption of FAW management practices.

	Adopted management practices		Use of pesticides		Use of handpicking		Frequent weeding		Traditional	
	NO	YES	NO	YES	NO	YES	NO	YES	NO	YES
Female	40	53	47	46	74	19	80	13	92	1
Male	45	202	57	190	166	81	221	26	246	1
	Chi=22.148 P=0.000 df=1		Chi=23.996 P=0.000 df=1		Chi=4.974 P=0.026 df=1		Chi=0.793 P=0.373 df=1		Chi=0.519 P=0.471 df=1	

Source: Survey data, 2020

DISCUSSION

Most farmers interviewed were above forty years of age, and the mean respondent age was forty-seven. Meaning most of the farmers are middle-aged, energetic, and vibrant. Older farmers are more experienced and risk-loving, hence likely to adopt management practices (Baffoe-Asare et al., 2013). This partially explains why most farmers adopted management practices, but it also reveals that the younger generations are not engaged in farming.

Males dominate maize farming in the area. A probable explanation for this finding could be that more men are engaged in maize farming than women. This confirms what Asare-Nuamah, (2020) found that farming in Ghana is male-dominated. Sixty-two percent of the farmers had only primary education. Farmers in the area had low formal education. However, the male farmers had higher education than women. There was a difference of 2.5 acres between the mean total farm size owned by farmers and the mean maize farm size. This means respondents had extra land to cultivate other crops and diversify their sources of income as an essential strategy to increase revenue and diversify food sources. This may cushion farmers against the devastating hardship effect of FAW as they may have other crops to fall on. The average farming experience of the respondents was twenty-two years, with most farmers having ten years of experience and over. Experienced farmers are expected to be more skillful, hence should be able to adjust to unfavourable situations. This result affirms the findings of Anaglo et al. (2020), which indicated that most farmers in Ghana have over ten years of farming experience.

Farmers were well informed of the presence of FAW in their area and had experienced it on their farms. This can be explained by the fact that infestation in the area was intense, and FAW had spread to almost all farms, and this, therefore, caused most farmers to seek information on FAW. It was, therefore, not surprising that most farmers adopted management practices to curb the situation. It was expected that farmers would adopt management practices if they experienced the infestation and had access to the right information. This is consistent with Kumela et al. (2018), who confirm that farmers adopted management practices to fight FAW infestation when they experienced it on their farms in Ethiopia. The effectiveness and less-labour-intensive nature of pesticides, the fact that the government gave out pesticides to farmers for free and at a subsidised price as an intervention, and lastly AEAs recommended pesticides as the management practices desirable to be adopted by farmers explains why most farmers adopted it. This is consistent with earlier studies that reported chemical application as the main management practice adopted by farmers in other African countries such as Ethiopia and Kenya (Kansiime et al., 2019; Kumela et al., 2018).

Kumela et al. (2018) argue that access to information by farmers is an integral part of the clamour for adopting management practices. The acquisition of information about agricultural practices demystifies it and makes it more available to farmers. This explains why a significant relationship was found between access to information and the adoption of FAW management practices. Information received by farmers on FAW and its management practices may have helped farmers to understand the probable effect of the infestation and evaluate the practices and adopt them. Whereas Kansiime et al. (2019) and (Mittal and Mehar, 2015) found AEAs as a primary source of information for farmers on FAW and farming practices, Larochelle et al. (2019) argue that the Mass media is the most prominent source of information to farmers on FAW management in other African countries. Contrary to these findings, our study found peer farmers as the most common source of information available to farmers. However, our result is consistent with Anaglo et al. (2020), who found that peer farmers are the most prominent source of information available to farmers in the Eastern region of Ghana. It must, however be noted that the transfer of information between and among farmers (peer farmer source) was entirely by word of mouth and that no formally organised farmer-to-farmer training was identified. But formalised farmer-to-farmer extension helps to overcome the information access problem and enables widespread adoption of agricultural practices. Based on this we recommend that for the government to harness the full potential of peer farmer information sharing, they should adopt more formal farmer-to-farmer extension programs where farmers are formally trained to transfer information.

AEAs as an information source had a significant relationship with adopting three different management practices (use of chemicals, handpicking, and frequent weeding). This implies that the informational campaign by the AEAs on FAW management practices appears well received by the farmers. Extensive education and increased AEAs numbers are further required to improve adoption of management practices. This confirms Kotu et al. (2017) and Onyeneke (2017) found that contact with AEAs on agricultural management practices influences farmers' adoption decisions about the management practices. Also, peer farmers being the most accessed source of information by farmers, is significantly related to the adoption of two management practices (use of chemicals and handpicking). A probable explanation could be that after farmers received information from AEAs and the media, they assessed the recommended practices based on experience and their implementation, got well informed and communicated what they thought was effective (chemicals and handpicking) to their colleagues. This is supported by the findings as further probing from participants ascertains that information from peer farmers was helpful and valuable that they relied on their peers.

The study revealed that women have less access to information compared to men. A probable explanation for this could be that there exists a wide gender gap and inequalities which lead to differential access to information among men and women. For instance, it is factual that attending meetings with AEAs is seen as a designated duty of the male head of a family; hence they receive the information. Also, many other researchers (Ankrah et al., 2020; Chatterjee et al., 2020) have argued that typical traditional customs do not support the use of ICTs especially among women because of time, poverty and challenging economic opportunities. It is therefore possible that men did have owned more phones, television and other digital means through which information were being disseminated hence they had better chances of getting the information . Ankrah et al. (2020) and Mudege et al. (2017) argue that gendered access to agricultural resources though stifles growth, still exists in developing countries. This is consistent with Mudege et al. (2017) and Ragasa et al. (2013), who found that gender norms and other factors hinder women farmers from accessing agricultural resources, including information. They further argued that this affects women farmers negatively on their adoption abilities. Therefore, it was not surprising to realise that women had less probability of adopting management practices. This is also consistent with earlier studies that found gender influences adoption decisions (Anaglo et al., 2014; Gebre et al., 2019).

CONCLUSION AND RECOMMENDATIONS

Many studies have been conducted on the role of information sources in adopting farming practices and how this relates to gender. Yet very little is known about these issues in the context of emergencies. Concerning FAW management practices in Ghana, very little is known about how information access, gender, and adoption of management practices. This study further bridges the gap on how this plays out in emergencies like pest outbreaks. It further puts into perspective the role of gender in adoption and access to information in the context of pest outbreaks. However, the limitation of this study is that it is a relational study and hence cannot prove causality, though the researchers engaged in further probing to ascertain findings. Experimental studies would be needed in the future. Nonetheless based on the findings we recommend that the government consider gender roles in devising further policies and interventions. We also recommend consideration of organized peer-to-peer information sharing.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The author read and approved the final manuscript. The author verify that the text, figures, and tables are original and that they have not been published before.

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Data availability

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Consent to participate

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Consent for publication

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RESEARCH ARTICLE

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Isolation and characterization of plant growth promoting rhizobacteria (PGPR) from rhizosphere of *Helianthus annuus* L.

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Abstract

Plant growth-promoting rhizobacteria (PGPR) support plant growth through direct and indirect mechanisms. To investigate PGPR strains that support plant growth, 21 bacterial isolates, mostly *Bacillus* ssp. and *Pseudomonas* ssp., were isolated from different rhizospheric soils of sunflowers in Kırşehir districts in 2020. All isolates were characterized morphologically, biochemically by screening under in vitro conditions for plant growth-promoting properties such as nitrogen fixation, IAA (indoleacetic acid) production, siderophore production, HCN (hydrogen cyanide) production, inorganic phosphate solubility. It was also screened for extracellular enzyme production and antifungal activity against *Fusarium oxysporum*. Among the 21 isolates, 3 isolates (MH-35-4, MH-49-4, MH-64-3) fixed nitrogen, 2 isolates (MH-59-6, MH-64-3), produced siderophores, 8 isolates (MH-35-4, MH-35-6, MH-54-3, MH-54-4, MH-59-1, MH-59-2, MH-59-4, MH-59-8) produced HCN, 6 isolates (MH-35-6, MH-54-4, MH-59-1, MH-59-2, MH-59-4, MH-59-8) produced IAA, and 7 isolates (MH-35-4, MH-35-6, MH-59-1, MH-59-2, MH-59-4, MH-59-8, MH-64-3) solubilized inorganic phosphate. Additionally, only 2 isolates (MH-54-3, MH-54-4) were positive amylase tests, 8 isolates (MH-35-6, MH-54-4, MH-59-1, MH-59-2, MH-59-4, MH-59-6, MH-59-7, MH-59-8) were positive citrate tests, 8 isolates (MH-35-1, MH-35-4, MH-35-7, MH-49-4, MH-54-4, MH-59-6, MH-59-7, MH-64-3) were positive protease tests, and 6 isolates (MH-35-1, MH-35-3, MH-35-7, MH-54-3, MH-54-4, MH-59-7) were positive gelatin hydrolysis tests. Among 21 isolates, 38% were determined as hydrogen cyanide producers, 10% as siderophore producers, 29% IAA producers, 33% as phosphate solubilizers and 14% as nitrogen fixers. Isolate MH-35-6 showed the highest antifungal activity against *Fusarium oxysporum* with an inhibition rate of 53.57%. This was followed by isolates MH-54-1 (51.19%), MH-54-3 (47.61%) and MH-59-2 (38.09%), respectively. Therefore, our study reveals that bacteria that promote plant growth in sunflowers can be used to increase crop yield and as a biocontrol agent.

Keywords: *Helianthus annuus* L., Plant growth-promoting rhizobacteria (PGPR), *Fusarium oxysporum*, MALDI-TOF MS

INTRODUCTION

The need for food has gradually increased due to the growing global population, which has highlighted the significance of agricultural productivity and prompted the development of sustainable agricultural practices. The cornerstone of ecological agriculture and the growth of green production policies is the use of biofertilisers, which are fertilisers that do not damage the environment or the natural world, as opposed to chemical fertilisers used in traditional agriculture (Yadav, 2020).

Food security has become a major problem worldwide due to the increasing global population, decreasing arable land resources, and climate change. Therefore, limited arable land resources need to be used more efficiently to produce more food. It is known that chemical fertilizers contributed to the continuous increase in agricultural food production in previous years. Even though agricultural production has increased at the desired rate, it has led to the destruction of nature and the environment in terms of its results, increasing environmental pollution and causing the natural balance to deteriorate. The development and use of alternative products against the use of chemical fertilizers is of great importance for sustainable agriculture and environmental protection. Therefore, in recent years, research on the development of new methods that are beneficial to the environment and human health in agriculture has gained momentum. One of these studies is the use of environmentally friendly plant growth-promoting formulations (inoculants), which have a significant effect on increasing crop yield (Jiang et al. 2021).

The soil we live on is home not only to visible creatures but also to millions of microorganisms. The rhizosphere is a habitat in which the soil is rich in nutrients, intense biological and chemical activities occur, the plant root system is surrounded, and millions of microorganisms live (Tabassum et al. 2017). Lorenz Hiltner was the first to define the term "rhizosphere" in 1904 (Shrivastava et al. 2015). Plant roots synthesise, accumulate, and secrete various compounds in addition to providing mechanical support and facilitating water and nutrient uptake (Walker et al. 2011). These heterogeneous compounds produced by plant roots act as chemical attractants for actively used soil microbial communities. Chemicals called root exudates are substances that roots release into the soil. Microorganisms found in plant roots that have many benefits for the development and growth of the plant are called Plant Growth Promoting Rhizobacteria (PGPR). Although the term PGPR was first used by Kloepper et al. (1980) for fluorescent Pseudomonas, which are used for biocontrol purposes against pathogens and contribute to the growth of the plant, its current meaning was used by Kapulnik et al. (1981) for rhizobacteria that have the feature of promoting plant growth. Today, PGPR is expressed for all bacteria in the rhizosphere that ensure the growth and development of the plant through one or more mechanisms (Haghighi et al. 2011). Some of the most important known properties of PGPRs are: phosphate dissolving, producing IAA hormone and siderophore, and nitrogen fixation.

Numerous bacteria from the genera *Pseudomonas*, *Bacillus*, *Azospirillum*, *Agrobacterium*, *Azotobacter*, *Rhizobium*, *Enterobacter*, and *Phyllobacterium* have been identified as PGPR. The most extensively documented PGPRs among these are *Pseudomonas*, *Azospirillum*, and *Bacillus*. They significantly increase the growth and yield of agronomic crops (Bashan et al. 2010). Sunflower (*Helianthus annuus* L.) is an important oilseed plant with high adaptability and is cultivated in large areas around the world (Mahapatra et al. 2021). A crucial raw material for the edible oil, chemical, cosmetic, paint, motor oil, biodiesel, hydraulic oil, soap, polish, and plastic industries is the sunflower. Sunflower, whose homeland is known as North America, has the largest cultivation area and production in Türkiye. Today, more than half of the vegetable oils produced in Türkiye are obtained from sunflowers. Sunflowers cultivation areas must be expanded, and the yield obtained per unit area must be increased to close the vegetable oil deficit in our country (Abdullah et al. 2023).

The soil physico-chemical structure, climatic conditions, and microbial population distribution of each region vary. Therefore, in order to prepare effective biofertilizer formulations, the biotic and abiotic conditions of the soil in that region must be well known. Particularly local bacterial species that are widespread in their own region are of great importance in the preparation of these formulations and the creation of local culture collections, and studies are concentrated on this subject. The isolation and identification of PGPR bacteria from the soil where sunflower cultivation is carried out in Kırşehir province and its districts and their PGP (Plant Growth Promoting) properties were investigated in this study. It was aimed at obtaining the microbial fertiliser inventory in the sunflower rhizosphere cultivated in our province in this context. This study will lead to the preparation of effective microbial fertilizer with local bacterial species prevalent in the region for future studies.

MATERIALS AND METHODS

Sample Collection and Isolation of Rhizobacteria

The soil samples collected in 2020 from the rhizosphere region of the sunflower (*Helianthus annuus* L.) plant in Kırşehir (Table 1) and its districts were thoroughly mixed and homogenised, and serial dilutions of 10^{-1}-10^{-6} were prepared from each of them to isolate the bacterial samples for use in the study (Figure 1).

In petri dishes with nutrient agar media, planting was done using the spreading technique. To obtain a pure culture, petri dishes were incubated for 2-4 days at 28±2°C. All the isolates obtained at the end of the incubation were transferred to a nutrient broth medium. Each purified colony was kept in a 20% glycerol solution. Bacterial isolates were deposited in the Culture Collection at the Microbiology Laboratory of Kırşehir Ahi Evran University in Türkiye.

Identification of Bacterial Strains

MALDI-TOF mass spectrometry was used to identify the resulting rhizospheric bacteria. The MALDI Biotyper CA System is a powerful tool for rapid and accurate identification of microorganisms and uses unique molecular fingerprints.

Morphological and Biochemical Characterization

The strains were identified using Bergey's Manual of Determinative Bacteriology and characterized by Gram stain, colony color, motility test, and biochemical tests including catalase activity, oxidase activity, and KOH (3%) (Krieg &Holt, 1984).

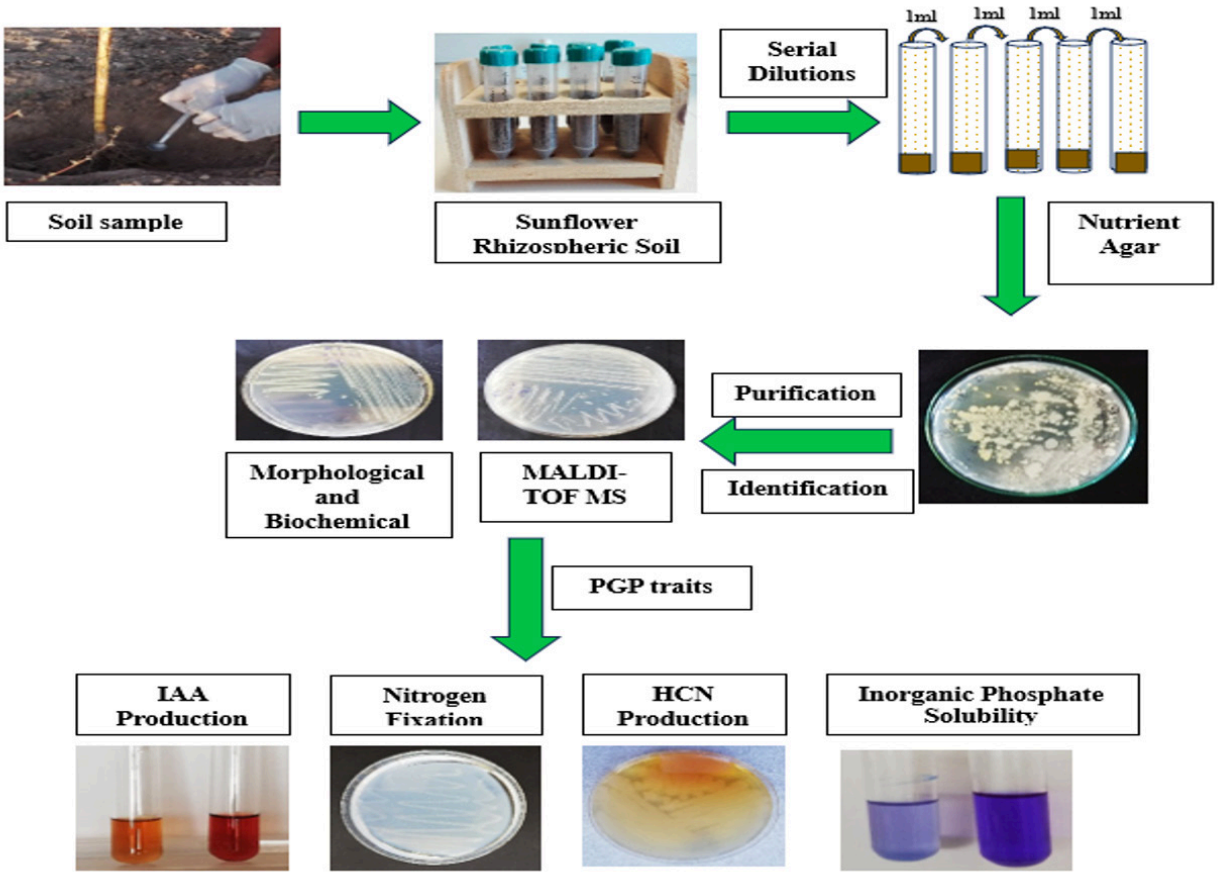

Figure 1. Flowchart Representation of the Process Used to Detect the Characteristics of Isolates From Sunflower Rhizospheric Soil

Assessment of Extracellular Enzyme Production

Screening for amylase production was done as per the methodology Smibert & Kreig (1994). Starch agar medium (yeast extract 5g, NaCl 10g, tryptone 10g, starch 5g, agar 15g, pure water 1000 ml) was prepared to determine amylase activity. After planting on starch agar medium, it was placed in an oven set at 30ºC and incubated for 1 day. At the end of the incubation, iodine solution (I_2 1g, KI 2g, pure water 300 ml) was dropped onto the petri dishes and waited for 5 minutes to examine the zone formation. Zone formation around the colony indicates the presence of amylase. The protease production of the isolates was determined according to the protocol described by Smibert & Kreig (1994). Agar (skimmilk agar) medium containing milk was prepared for the protease production test, bacteria were cultivated using the streak seeding method and incubated at 37°C for three days. Zones around the colony formed after incubation showed the presence of protease. For the citrate test, 10ml of slanted Simmans agar medium was prepared and the pH was adjusted to 6.9. Isolates were inoculated into 10ml tubes with a loop and incubated at 28˚C for 7 days. At the end of incubation, the color change from green to blue in the tube was evaluated as positive (Marakana et al. 2018). After preparing the required amount of gelatin agar medium, isolates taken from the 24-hour fresh culture were inoculated into 5ml tubes and then incubated at 28ºC for 7-14 days. After incubation, it was placed in the refrigerator at +4ºC. The tubes were kept in the refrigerator for 2-3 hours. Gelatin production was considered

positive if the tubes remained in liquid form when removed from the refrigerator (Nathan et al. 2011). Extracellular enzyme activity assays were conducted in triplicate.

Table 1. Locations, Altitudes, Longitude and Latitude of Sunflower Rhizosphere Samples

Isolates	Location	Altıtude	Latitude	Longitude
Bacillus pseudomycoides MH-35-1 *Bacillus megaterium* MH-35-3 *Chryseobacterium elymi* MH-35-4 *Pseudomonas koreensis* MH-35-6 *Bacillus weihenstephanensis* MH-35-7 *Bacillus simplex* MH-35-8, *Bacillus oligofermantans* MH-35-9 *Bacillus cereus* MH-49-2 *Pseudarthrobacter polychromogenes* MH-49-4 *Bacillus simplex* MH-49-8	Kaman/İsahocalı	1280	39°25'00.6"	33°53'58.8"
Bacillus megaterium MH-54-1 *Bacillus mojavensis* MH-54-3 *Stenotrophomonas sp* MH-54-4	Akçakent/Mahsenli	1268	39°34'10.6"	34°10'44.5"
Pseudomonas koreensis MH-59-1 *Pseudomonas koreensis* MH-59-2 *Pseudomonas koreensis* MH-59-4 *Acinetobacter calcoaceticus* MH-59-6 *Stenotrophomonas rhizophila* MH-59-7 *Pseudomonas koreensis* MH-59-8	Boztepe/Külhüyük	1150	39°20'15.1"	34°15'47.0"
Bacillus simplex MH-64-2 *Aromatoleum evansii* MH-64-3	Çiçekdağı/İbikli	1140	39°31'50.0"	34°20'36.0"

Evaluation of Plant Growth Promotion

Determination of Nitrogen Fixing Capacity

The isolates were tested using the nitrogen fixation protocol described by Wilson & Knight (1952). Firstly, the isolates were streaked on nutrient agar medium and incubated for 24h at 28±2ºC. Using the streaking method, each of the newly isolated cultures that had emerged from incubation was injected into petri dishes that contained solid Burk's N-free medium (Wilson & Knight, 1952; Park et al. 2005). They were incubated in this medium at 28±2°C for four days, and the plates were checked hourly and graded according to their development. Three-time intervals were determined for nitrogen fixation activity (+++: development after 6h, ++: development after 12h, +: development after 24h).

Evaluation of Siderophores-Producing Isolates

Schwyn & Neilands (1987) used Chrome Azurol S agar in this method to determine whether the isolates produced siderophores. The isolates were seeded onto the medium using the spot-seeding method and incubated at 28°C for 4 days. At the end of the incubation, the formation of yellow-orange colour around the bacteria was considered positive, and the formed zone diameters (mm) were measured (Öğütcü & Avsar, 2020). For siderophore activity, three-time intervals were determined (+++: color change after 1h, ++: color change after 6h, +: color change after 24h).

Assessment of Isolate Inorganic Phosphate Dissolving Capacity

The isolates' inorganic phosphate-dissolving capacities were determined using the protocol described by Mehta & Nautiyal (2001). Pure bacterial cultures grown on nutrient agar medium were inoculated into tubes containing 5 ml of NBRIP-BPB Medium (National Botanical Research Institute's Phosphate), and the control tubes were not inoculated. For three days, all tubes were incubated at 30±2°C and 180 rpm. Although there was no color change (blue-purple) in the control group tubes after incubation, color expansion was observed in some of the inoculated tubes.

HCN-Producing Isolates

The isolates' HCN production was determined using the method proposed by Bakker & Schippers (1987). Bacteria were inoculated on a nutrient agar medium containing 0.44% glycine, and filter papers (1.5 cm in diameter) impregnated

with picric acid (0.5% picric acid, 2% sodium carbonate) were placed on the Petri plate's edge without touching the medium.

The petri dish mouths were tightly sealed with paraffin and incubated at 28±2°C for 4 days. Picric acid-impregnated papers turned from yellow to brown at the end of the incubation period, which was considered a positive result (Temiz, 2010). For HCN activity, three-time intervals were determined (+++: color change after 6h, ++: color change after 12h, +: color change after 24h).

Identification of Isolates Producing Indole-3-Acetic Acid (IAA)

The isolates' IAA production abilities were determined using the protocol described by Sarwar & Kremer (1995). Pure bacterial cultures were grown for and 48h at 36±2°C. Fresh cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). The appearance of pink indicates the presence of IAA. For IAA activity, three time intervals were determined (+++: color change after 1h, ++: color change after 6h, +: color change after 24h).

Antifungal Activity

The fungal isolate (*Fusarium oxysporum*) used in the study was obtained from the culture collection unit of Ahi Evran University, Faculty of Agriculture, Department of Plant Protection. Using a potato dextrose agar (PDA) medium, all the isolates were tested for antifungal activity against *F. oxysporum*. Bacterial isolates were grown on a nutrient agar medium at 25 °C for 24h to obtain fresh cultures. A 6 mm mycelial disc of fungi, *F. oxysporum* was placed in the centre of the plates and incubated at 28°C for 7 days. Bacterial isolates were drawn on the edge of the petri dish with a swab and incubated in the dark at 25°C for one week. As a control, only the pathogenic fungus isolate was placed in the middle of the petri dish containing PDA, and the evaluation was made when the pathogen fungus isolates covered the control petri dish. The diameter of the fungus in the application petri dishes was measured in mm. The percent inhibition rate of bacteria and fungus colony development was determined by Mari et al. (1996), it was calculated using the percentage of inhibition of radial development formula. For each isolate, the experiments were conducted with three replicates.

$$\% \text{ Inhibition} = (C - T) / (C - M) \times 100$$

C: Colony diameter of the pathogen in the control application

M: Diameter of micellar disc (6 mm)

T: Colony diameter of the pathogen in bacterial application

Data Analysis

Data for antifungal activity were analyzed in three replicates for using JMP Pro 17.0 statistical software. Dependant variables with normal distribution were presented as mean ± Standart Devision (SD). Analysis of variance (ANOVA) was used for antifungal activity measurements. The Tukey test was used to determine the differences between the mean levels of factors.

RESULTS AND DISCUSSIONS

Identification of Isolates

PGPR supports plant growth directly or indirectly by colonizing plant roots and reducing the population of harmful microorganisms. The rhizosphere is the ecological niche with a rich nutrient presence between plant roots and soil microorganisms. Therefore, rhizobacteria contribute to soil fertility and sustainability. In this report, we looked at the bacterial ecology in the rhizosphere of sunflower. Soil samples were collected from 4 different locations (Kaman, Akçakent, Boztepe, *Çiçekdağı*) of Kırşehir city in T*ürkiye*. 21 isolates were obtained by serial dilution method. For bacterial identification, MALDI-TOF mass spectrometer was used. According to the MALDI-TOF MS results, 21 isolates belonging to 7 different genera were identified. Among these isolates, *Bacillus*, *Pseudomonas* and *Stenotrophomonas* were in the first three places (Table 2, Figure 2). Pramanik et al. (2018) used MALDI-TOF MS and FAME analysis to identify the bacteria they isolated from heavy metal-contaminated rice rhizospheres in India and tested them for plant growth-promoting properties. Similarly, Çevik & Ogutcu (2020) identified 51 isolates from non-agricultural soils using the MALDI TOF MS method. They determined that some bacteria (*Bacillus* sp., *Pseudomonas* sp., *Enterobacter* sp., and *Paenibacillus* sp.) possessed plant growth-promoting properties.

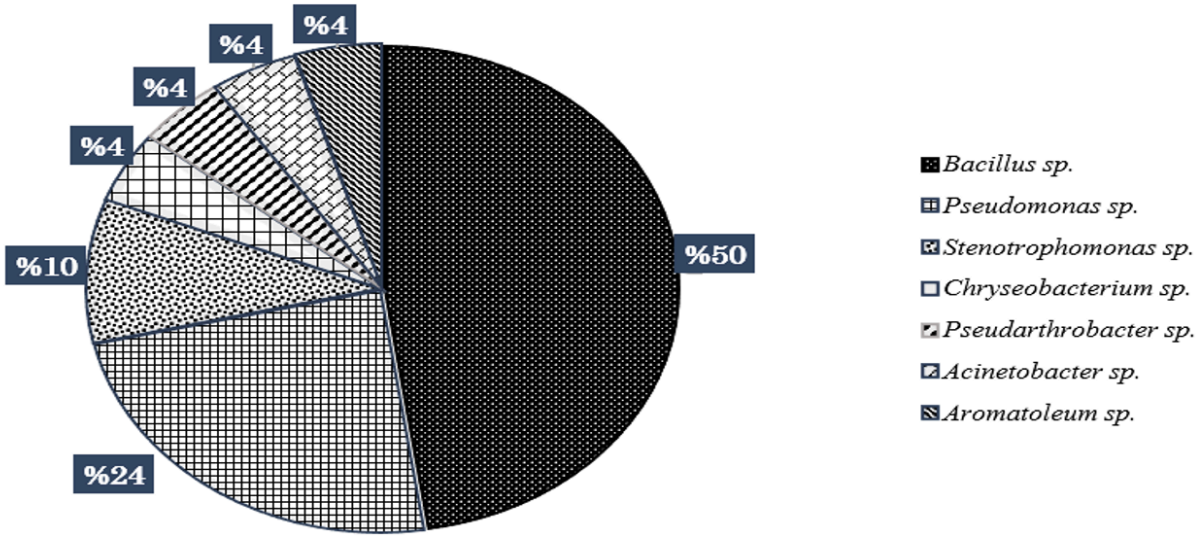

Figure 2. Percentages of Bacteria Isolated from Sunflower Rhizosphere

Biochemical and Morphological Characterization of Isolates

The biochemical tests such as catalase, and oxidase test were carried out for phenotypic identification of isolates (Krieg & Holt, 1984). It has been observed that bacterial isolates obtained from sunflowers have different biochemical and morphological characters. The majority of the isolated bacteria were Gram-positive, with a lower proportion being Gram-negative. In the current study, Among all isolates, 11 were Gram (+) and 10 were Gram (-); Except for two isolates (MH-49-4, MH-64-3), the others were catalase (+); Except for 7 isolates (MH-35-4, MH-35-6, MH-54-3, MH-59-1, MH-59-2, MH-59-4, MH-59-8), the others were oxidase (-). Details are shown in Table 2.

Production of Extracellular Enzymes

Among 21 isolates, all except for two isolates (*Bacillus mojavensis* MH-54-3, *Stenotrophomonas sp*. MH-54-4) were negative amylase test, 8 isolates were positive citrate and protease test, and 6 isolates (*Bacillus pseudomycoides* MH-35-1, *Bacillus megaterium* MH-35-3, *Bacillus weihenstephanensis* MH-35-7, *Bacillus mojavensis* MH-54-3, *Stenotrophomonas sp*. MH-54-4, *Stenotrophomonas rhizophila* MH-59-7) were positive gelatin hydrolysis test in the present study (Figure 3, Table 3). Biocontrol agents are antagonists that reduce disease intensity by acting against pathogens in plants. Antagonists show their effect against pathogens by producing hydrolytic enzymes such as protease, amylase and chitinase that damage the fungal cell wall. Similarly, PGPs in the rhizosphere produce hydrolytic enzymes such as proteases, cellulases, amylases, and so on to combat phytopathogens, which disrupt the pathogens' cell walls and cause cell death (Khalil et al. 2022). According to Petrović et al. (2024), enzymes such as protease, pectinase, and xylanase help bacteria colonise plant tissues and form symbiotic relationships with host plants. Bashir et al. (2021) reported that *Exiguobacterium auranticum*, *Paenibacillus* sp, and *Priestia koreensis* isolated from sunflower leaves produced amylase, protease, and chitinase. Fatima et al. (2022) reported that *Pseudomonas aeruginosa* IR-57, *Bacillus subtilis* IR-27, and *Serratia* sp. (IS-1) from chickpea rhizosphere produced protease, amylase, and HCN, and this resulted in an increase in the antifungal potential of the isolates. Among our isolates, some isolates that produced protease enzymes and were positive for HCN production (*Chryseobacterium elymi* MH-35-4, and *Stenotrophomonas sp*. MH-54-4) exhibited relatively high antifungal activity against the pathogen. Similarly, the protease-producing *Bacillus weihenstephanensis* MH-35-7 isolate exhibited antifungal activity (27.38%) in present study (Table 3). Our results for enzymatic activity are consistent with other researchers (Moustaine et al., 2017; Bashir et al., 2023).

Table 2. Morphological and Biochemical Characterization of Isolates

MALDI-TOF MS results	Morphological Characterization			Biochemical Characterization		
	Gram staining	Colony color	Motility test	KOH %3	Catalase activity	Oxidase activity
Bacillus pseudomycoides MH-35-1	+*	cream	-	-	+	-
Bacillus megaterium MH-35-3	+	white	+	-	+	-
Chryseobacterium elymi MH-35-4	-	pale yellow	-	+	+	+
Pseudomonas koreensis MH-35-6	-	white	+	-	+	+
Bacillus weihenstephanensis MH-35-7	+	white	+	-	+	-
Bacillus simplex MH-35-8,	+	cream	+	-	+	-
Bacillus oligofermantans MH-35-9	+	white	-	-	+	-
Bacillus cereus MH-49-2	+	whitish	+	-	+	-
Pseudarthrobacter polychromogenes MH-49-4	+	white	-	-	-	-
Bacillus simplex MH-49-8	+	cream	+	-	+	-
Bacillus megaterium MH-54-1	+	pale yellow	+	-	+	-
Bacillus mojavensis MH-54-3	+	white	+	-	+	+
Stenotrophomonas sp. MH-54-4	-	yellow	+	+	+	-
Pseudomonas koreensis MH-59-1	-	white	+	+	+	+
Pseudomonas koreensis MH-59-2	-	white	+	+	+	+
Pseudomonas koreensis MH-59-4	-	white	+	+	+	+
Acinetobacter calcoaceticus MH-59-6	-	white	-	+	+	-
Stenotrophomonas rhizophila MH-59-7	-	yellow	+	+	+	-
Pseudomonas koreensis MH-59-8	-	white	+	+	+	+
Bacillus simplex MH-64-2	+	cream	+	-	+	-
Aromatoleum evansii MH-64-3	-	white	+	+	-	-

Note: * +, positive; –, negative

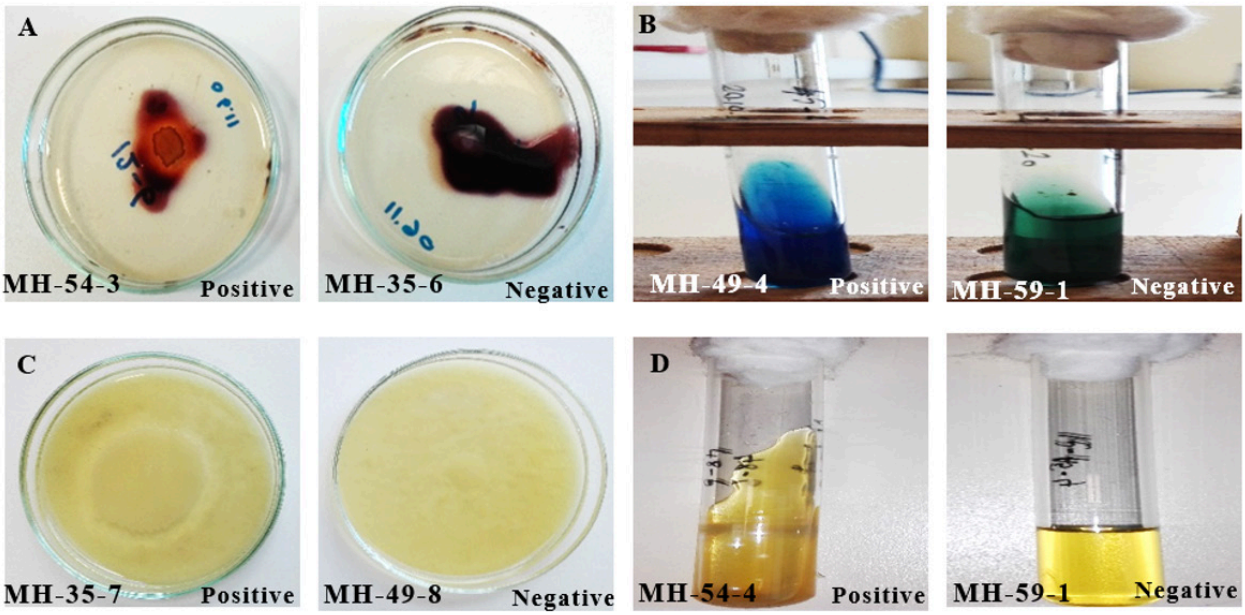

Figure 3. Extracellular Enzyme Production of Isolates (A. Amylase test B. Citrate test C. Protease test D. Gelatin hydrolysis)

Table 3. Extracellular Enzyme Production Results of Isolates

Isolates	Extracellular Enzymes			
	Amylase Test	Citrate Test	Protease Test	Gelatin Hydrolysis
Bacillus pseudomycoides MH-35-1	-	-	+	+
Bacillus megaterium MH-35-3	-	-	-	+
Chryseobacterium elymi MH-35-4	-	-	+	-
Pseudomonas koreensis MH-35-6	-	+	-	-
Bacillus weihenstephanensis MH-35-7	-	-	+	+
Bacillus simplex MH-35-8,	-	-	-	-
Bacillus oligofermantans MH-35-9	-	-	-	-
Bacillus cereus MH-49-2	-	-	-	-
Pseudarthrobacter polychromogenes MH-49-4	-	-	+	-
Bacillus simplex MH-49-8	-	-	-	-
Bacillus megaterium MH-54-1	-	-	-	-
Bacillus mojavensis MH-54-3	+	-	-	+
Stenotrophomonas sp MH-54-4	+	+	+	+
Pseudomonas koreensis MH-59-1	-	+	-	-
Pseudomonas koreensis MH-59-2	-	+	-	-
Pseudomonas koreensis MH-59-4	-	+	-	-
Acinetobacter calcoaceticus MH-59-6	-	+	+	-
Stenotrophomonas rhizophila MH-59-7	-	+	+	+
Pseudomonas koreensis MH-59-8	-	+	-	-
Bacillus simplex MH-64-2	-	-	-	-
Aromatoleum evansii MH-64-3	-	-	+	-

Note: * +, positive; –, negative

PGP Attributes of Isolates

Of the 21 isolates, only 7 (MH-35-4, MH-35-6, MH-59-1, MH-59-2, MH-59-4, MH-59-8, MH-64-3) were found to dissolve inorganic phosphate (Figure 4). This showed that it had a share of 33% among all isolates. In addition, it was defined that 3 (MH-35-4, MH-49-4, MH-64-3) of 21 isolates do nitrogen fixation (14%), 2 (MH-59-6, MH-64-3) produce siderophores (10%), 8 (MH-35-4, MH-35-6, MH-54-3, MH-54-4, MH-59-1, MH-59-2, MH-59-4, MH-59-8) produce HCN (38%) and 6 (MH-35-6, MH-54-4, MH-59-1, MH-59-2, MH-59-4, MH-59-8) produce IAA (29%) (Table 4).

PGPR promotes plant growth via a variety of mechanisms, including IAA production, nitrogen fixation, siderophore production, and phosphate solubilization. According to Beattie (2006), approximately 30% of the bacteria isolated from the sunflower rhizosphere consist of the genera *Stenotrophomonas*, *Agrobacterium*, *Pseudomonas* and *Rhizobium*. Recent studies are showing that *Stenotrophomonas* sp., a member of the PGPR family, supports plant growth (Ghosh et al. 2020; Singh et al. 2020; Mushtaq et al. 2021). In the current study, it was determined that *Stenotrophomonas* sp. MH-54-4 isolate produced HCN and IAA. *Stenotrophomonas* strains have been found as endophytes in rice (Sun et al. 2008), sugarcane (Morgado et al. 2015), wheat (Majeed et al. 2015) and maize (Ercole et al. 2023). Pseudomonas are among the foremost rhizospheric bacteria because they colonise aggressively and use a variety of carbon sources (Dorjey et al. 2017). Pandey et al. (2013) reported that *Pseudomonas* sp. isolate RP1 showed plant growth-promoting including phosphate solubilization, IAA production, and HCN production.

An essential macronutrient, phosphorus is required for many important plant metabolic functions, including signal transduction, membrane integrity, synthesis of energy, cell division, and photosynthesis. The bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aereobacter*, *Flavobacterium*, and *Erwinia* are known to be capable of phosphorus solubilization. According to reports, *Rhizobium*, *Bacillus*, and *Pseudomonas* sp. have the highest P solubilization activity (Ahmad et al. 2008; Chaiharn & Lumyong, 2011). Beneduzi et al. (2008) studied the phosphate solubility of bacteria isolated from wheat rhizosphere and found only 9 phosphate-solubilizing bacteria. Hameeda et al. (2008) isolated 207 bacteria from the maize rhizosphere and found that only 5 dissolved phosphate. Ambrosini et al. (2012) determined that among 299 isolates from the sunflower rhizosphere, 59 were phosphate-soluble (including *Burkholderia* sp. and *Achromobacter* sp.), and especially *Azospirillum* sp.Vi 22 isolate had a high rate of nitrogen fixation.

According to reports, rhizosphere soil typically contains higher concentrations of phosphate-solubilizing bacteria than non-rhizospheric soil (Verma & Shahi, 2015; Rawat et al. 2021). The eports available on *Pseudomonas* sp. isolated from different sources showed phosphate solubilization (Rosas et al. 2006; Khan et al. 2014; Paul & Sinha, 2017). Phosphate-solubilizing bacteria play a crucial role in the growth of plants by converting insoluble phosphorus into soluble phosphates that plants can use. Therefore, the use of plant growth-promoting phosphate-solubilizing bacteria in agriculture not only preserves soil fertility but also reduces costs. Liang et al. (2023) determined that among 31 isolates obtained from the rhizosphere of *Festuca arundinacea*, *Acinetobacter calcoaceticus*, *Buttiauxella* sp, and *Erwinia pyriflorinigrans* solubilized inorganic phosphate between 203.96 and 412.22 µg/mL. Soares et al. (2023), who investigated the phosphate solubilization abilities of isolates in different pH environments, determined that *P. aeruginosa* UFT01 and *B. cereus* UFT42 dissolved phosphate in all pH ranges. In present study, *Pseudomonas koreensis* MH-59-1, *Pseudomonas koreensis* MH-59-2, *Pseudomonas koreensis* MH-59-4 showed phosphate solubilization (Figure 4E).

IAA (indole-3-acetic acid), produced by bacteria, takes part in physiological events such as increasing root development, cell elongation, and cell division differentiation in plants. (Glickmann & Dessaux, 1995). IAA is the most common and best-characterized phytohormone. It is estimated that 80% of bacteria isolated from the rhizosphere can produce IAA (Sokolova et al. 2011). Several PGPR genera, including *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Pantoea*, *Pseudomonas*, and *Serratia*, have been found to produce IAA. Sivasakthivelan and Stella (2012) reported that the *Azospirillum lipoferum* SA-17 strain isolated from the sunflower rhizosphere produced IAA in high amounts (89.9 µg 25 ml^{-1}), and that *Pseudomonas fluorescens* SP-10 and *Bacillus megaterium* SB-17 strains fixed nitrogen. Raval and Desai (2012) measured the IAA production of bacteria isolated from sunflower rhizosphere on a spectrophotometer (535 nm) and determined that the highest IAA production was in *Pseudomonas* M6S3 and *Bacillus* M7S3 isolates. Similarly, Pandey et al. (2013) determined that some of the bacteria they isolated from the sunflower rhizosphere produced IAA. They also determined spectrophotometrically that the highest IAA producer belonged to the *Pseudomonas stutzeri* (78 µg/ml). Adeleke et al. (2022) reported that 20 of 50 endophytic bacteria obtained from sunflower had plant growth-promoting properties among them, *S. maltophilia* JVB5 (23.36 µg/ml), *B. cereus* T4S (20.72 µg/ml) and *S. indicatrix* Bovıs40 (46.43 µg/ml) isolates produced IAA.

The findings of this study revealed that *Pseudomonas* (24%) and *Bacillus* (50%) were the most common bacterial genera in sunflower rhizospheres. Further, all five isolates identified as *Pseudomonas koreensis* (MH-35-6, MH-59-1, MH-59-2, MH-59-4, MH-59-8) were positive for IAA production and solubilizing inorganic phosphate when evaluated for PGP properties (Figure 4A, Figure 4E). *Bacillus* species, which are the most common in soil, have a high potential to become microbial fertilizers, especially due to their ability to form spores, being easy to grow and store, and having protective properties against plant pathogens (Forchetti et al. 2007). *Bacillus* species used as biofertilizers promote plant growth by synthesizing plant growth hormones, fixing nitrogen, solubilizing phosphates and producing siderophores (Borriss, 2011; Riaz et al. 2021; Mushtaq et al. 2021). PGP features were not determined in *Bacillus* isolates, except for isolate *Bacillus mojavensis* MH-54-3 in current study (Table 4).

Nitrogen, which is necessary for all living things, participates in the structure of many substances such as amino acids, vitamins and nucleotides. Although nitrogen is 78% concentrated in the atmosphere, it cannot be used directly by plants. The conversion of atmospheric nitrogen into ammonium is known as the process of biological nitrogen fixation or diazotrophy. Biological nitrogen fixation, which occurs thanks to the nitrogenase enzyme complex, occurs in two ways: symbiotic and nonsymbiotic (Deka et al. 2015). The genera *Rhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Allorhizobium*, *Mesorhizobium*, and *Frankia* contain symbiotic nitrogen-fixing bacteria (Hayat et al. 2012). Non-symbiotic bacterial genera are; *Azotobacter*, *Azospirillum*, *Clostridium*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *Pseudomonas* are bacteria. Goes et al. (2012) showed that 3 *Bacillus* species among 57 bacteria obtained from different sunflower tissues (roots, stems, florets, and rhizosphere) fixed nitrogen. Chai et al. (2023) determined that *Acinetobacter calcoaceticus* HMAJ1, *Pseudomonas piscium* HMAJ5, and *Pseudarthrobacter psychrotolerans* NCZ1 have the nitrogenase activity necessary for nitrogen fixation. In the current study, isolates of *Chryseobacterium elymi* MH-35-4, *Pseudarthrobacter polychromogenes* MH-49-4 and *Aromatoleum evansii* MH-64-3 fixed nitrogen (Figure 4B). In previous studies, the genus *Chryseobacterium* and *Pseudarthrobacter* isolated from different plant rhizospheres was reported to fix nitrogen (Lucas Garcia et al. 2004; Nishioka et al. 2016; Dhole et al. 2017; Gopalakrishnan et al. 2017; Bushra et al. 2023). Moreover, *Chryseobacterium* can produce siderophore, protease, cellulase, amylase, xylanase, and exhibit antifungal properties (Pathma & Sakthivel, 2013).

One of the many strategies for preventing pathogenic microorganisms from growing in the rhizosphere is the production of HCN (Pandey et al. 2013). It inhibits electron transport chains and energy sources in cells, leading to pathogen death. Studies have shown that bacterial species like *Bacillus* sp., *Pseudomonas* sp., *Stenotrophomonas* sp., and *Alcaligenes* sp. may prevent the pathogen attach in plants by stimulating the HCN production mechanism (Miljaković et al. 2020; Hamid et al. 2021; Ferioun et al. 2023). HCN production by *Bacillus* sp. and *Pseudomonas*

aeruginosa has been previously reported (Kumar et al. 2012; Sebastian et al. 2021; Devi et al. 2023). Manasa et al. (2017) reported that the *Rhizobium* RR-1 strain obtained from sunflower rhizosphere produced HCN. Singh et al. (2019) determined that *Bacillus thuringiensis* SF 23, *Pseudomonas aeruginosa* SF 44, *B. subtilis* SF 48, and *Bacillus subtilis* SF 90 isolate produced HCN. Similarly, Bashir et al. (2021) determined that *Exiguobacterium auranticum* SV10 and *Priestia koreensis* LV19 isolates, which are endophyte bacteria obtained from sunflower leaves, produced HCN. Isolates *Chryseobacterium elymi* MH-35-4, *Pseudomonas koreensis* MH-35-6, *Bacillus mojavensis* MH-54-3, *Stenotrophomonas sp.* MH-54-4, *Pseudomonas koreensis* MH-59-1, *Pseudomonas koreensis* MH-59-2, *Pseudomonas koreensis* MH-59-4 and *Pseudomonas koreensis* MH-59-8 produced HCN in present study (Figure 4C).

The amount of iron available in the rhizosphere for microbial assimilation is very limited. In this case, organisms secrete iron-binding ligands known as siderophores, which bind to the ferric ion and make it available to the host organisms so that they can survive (Gupta & Gopal, 2008). Rhizospheric bacteria produce siderophores, which enhance rhizosphere colonisation and are crucial for iron mineralization and plant supplementation. Fluorescent pseudomonads are known to produce siderophores such as pyoverdines. Khare et al. (2011) reported that *P. aeruginosa* could produce siderophores and pyoverdines at different NaCl concentrations (0-500 mM).

Ambrosini et al. (2012) reported that the bacteria they obtained from the sunflower rhizosphere mostly belonged to the *Enterobacter*, *Burkholderia* and *Klebsiella* genera, and 27 of them produced siderophores. Similarly, Sivasakthivelan and Stella (2012) reported that high amounts of siderophores were produced by strains of *Pseudomonas fluorescens* (8.26 µg ml^{-1}) and *Bacillus megaterium* (7.80 µg ml^{-1}) among the various isolates obtained from the sunflower rhizosphere. Further studies revealed that bacterial strains are siderophore producers (Zou et al. 2020; Fiodor et al. 2023). Koçak and Boyraz (2024) reported that among 5 isolates obtained from sunflower rhizosphere (*Bacillus cereus*, *Bacillus simplex*, *Brevibacterium frigoritolerans*, *Bacillus toyonensis*, *Bacillus toyonensis*), the most effective siderophore producer was *Brevibacterium frigoritolerans*. Huang et al. (2023) reported that *Acinetobacter calcoaceticus* DP25 and *Acinetobacter calcoaceticus* DP27, obtained from the rhizosphere of *Lespedeza davurica*, produced 53.13% and 86.67% of siderophores, respectively. Interestingly, In the current study, 2 isolates (*Acinetobacter calcoaceticus* MH-59-6 and *Aromatoleum evansii* MH-64-3) produced siderophores (Figure 4D).

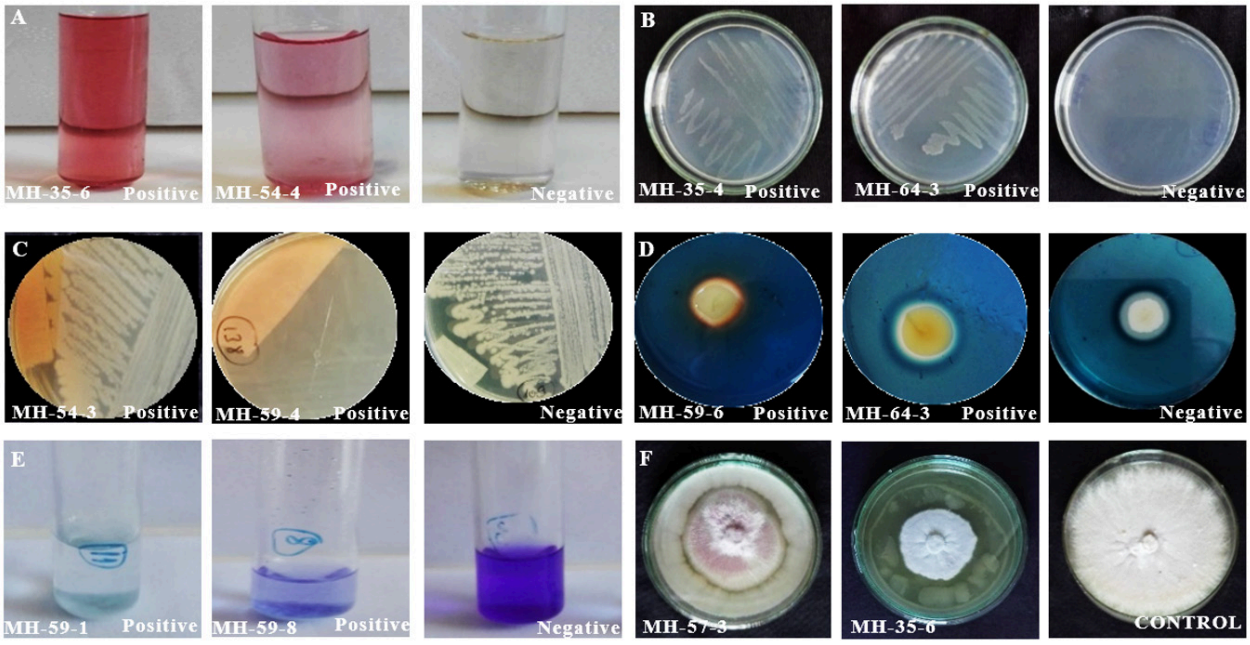

Figure 4. PGPR Test Results of Isolates (A. IAA production B. Nitrogen fixation; C. HCN production D. Siderophore production E. Inorganic phosphate solubization) and antifungal activity against *Fusarium oxysporum* (F)

Antifungal Activity

Sunflower wilt is caused by *Fusarium oxysporum*, the most frequent soil-borne pathogen. *F. oxysporum* invades the host's root system and blocks the water-conducting tissues, causing root rot and eventual plant death. Synthetic fungicides used against *Fusarium* sp. are both ineffective and harmful to environmental health (Gulya, 2016; Bashir et al. 2021). Therefore, the use of biofungicides as an alternative to chemical pesticides is gaining importance day by day. Forchetti et al. (2007) determined that endophyte bacteria (*Bacillus pumilus*, *Achromobacter xiloxidans*) in sunflowers grown under drought conditions showed antifungal activity at different rates against pathogenic fungi (*Fusarium* sp., *Sclerotinia sclerotiorum*, *Alternaria* sp.).

In this study, Antifungal activity of isolates obtained from sunflower rhizosphere was tested against *F. oxysporum* using PDA (Potato dextrose agar) medium and the inhibition percentages varied between 16.66% and 53.57%. Among the isolates, *Pseudomonas koreensis* MH-35-6 isolate showed the strongest antagonism against the pathogen with a high percentage inhibition value (53.57%), followed by *Bacillus megaterium* MH-54-1 isolate (51.19%). The *Stenotrophomonas rhizophila* MH-59-7 isolate (15.47%) showed the weakest effect against the pathogen (Table 4, Figure 5). Previous studies have shown that PGPRs improve plant growth and fungal diseases in tomatoes, wheat and sunflowers (Shittu et al. 2009; Moussa et al. 2013; Waqas et al. 2015).

Bacillus spp. is considered an effective microorganism with remarkable abilities for synthesising a wide range of beneficial substances. Production of antifungal metabolites by PGPR such as *Bacillus* is a well-documented biocontrol agent against phytopathogens (Majeed et al. 2018). Recently, several Bacillus species have been accepted as biocontrol agents against phytopathogens because of their ability to produce biosurfactant lipopeptides with antimicrobial activity. According to Koumoutsi et al. (2004), *B. amyloliquefaciens* FZB42 secretes fengycin and bacillomycin D, which have antagonistic activity against *Fusarium oxysporum*. Shobha and Kumudini, (2012) reported that seven Bacillus isolates revealed significant inhibitory effects on mycelial radial growth against *F. oxysporum* in vitro. Singh et al. (2017) determined that fifteen *B. subtilis* strains reduced *F. oxysporum* pathogen growth by varying rates ranging from 47% to 85.5%. Singh et al. (2019) determined the inhibition rates of *Bacillus* strains (*Bacillus thuringiensis* Rhizo SF23 and *Bacillus subtilis* Rhizo SF48) isolated from the sunflower rhizosphere against *Fusarium* sp. as 43.54% and 47.85%, respectively. Similarly, Mishra et al. (2023) determined that *Pseudomonas guariconensis* IIPRMKCP-9, *Bacillus amyloliquefaciens* IIPRAJCP-6, *Bacillus haynesii* IIPRMKCP-10, *Bacillus cereus* IIPRAMCP-5, *Bacillus subtilis* IIPRSHEP-6, and *Seratia macrescens* IIPRMKCP-3 isolates inhibited *F. oxysporum* mycelial growth by more than 80%. Bacillus isolates revealed varying degrees of antifungal activity against the fungal pathogen *F. oxysporum* in the present study. Among the Bacillus ssp., *Bacillus megaterium* MH-54-1 showed the maximum inhibition rate of 51.19%, followed by *Bacillus mojavensis* MH-54-3 (47.61%) and *Bacillus pseudomycoides* MH-35-1 (35.71%) in current study (Table 4). Our findings are consistent with the other works.

PGPR's antagonistic nature against plant pathogens is associated with the production of secondary metabolites that impede pathogen growth and progression. *Pseudomonas* spp. produces a number of antifungal compounds: chitinases, glucanases, proteases, siderophores, butylbenzenesulfonamide, hydroxymethyl, hydroxyphenyl, which prevent various pathogen diseases. Parveen et al. (2020) determined that *Pseudomonas aeruginosa* PGPR-11 showed antifungal activity against sunflower root pathogenic fungi (*Rhizoctonia solani*, *F. solani*, *Macrophomina phaseolina* and *F. oxysporum*). Bashir et al. (2021) examined the antifungal activities of endophytic bacteria (*Exiguobacterium auranticum* SV7, *Paenibacillus* sp. SV10 and *Priestia koreensis* LV19) obtained from sunflower against *Fusarium* sp. They determined that among the three endophytic bacteria, the most effective isolate against *Fusarium* sp. belonged to the *Priestia koreensis* LV19 isolate with an inhibition rate of 53%, and the least effective isolate belonged to the *Paenibacillus* sp. SV10 isolate with an inhibition rate of 19.2%. Thakker et al. (2023) reported that *Pseudomonas aeruginosa* OG101 inhibited *F. oxysporum* mycelial growth by 24.4%. Similarly, Chaurasiya et al. (2023) reported that *Pseudomonas* sp. PGP 18 isolate inhibited the *F. oxysporium* that causes lentil wilt disease by 67.41%. Likewise, Among the Pseudomonas ssp., *Pseudomonas koreensis* MH-35-6 isolate showed the highest antifungal activity (53.57 %), followed by *Pseudomonas koreensis* MH-59-2 (39.09%) and *Pseudomonas koreensis* MH-59-8 (36.90%) in present study (Table 4, Figure 4F). Our result coincides with Majeed et al. (2018) who showed *Pseudomonas* sp. AF-54 antifungal activity against *F. oxysporum* in Arabidopsis.

Table 4. Plant Growth Promoting Test Results and Antifungal Activity Values Against *Fusarium oxysporum* of the Isolates

Isolates	PGP Traits					Antifungal Activity	
	Inorganic phosphate solubility	Nitrogen fixation	Siderophores production	HCN production	IAA production	Colony diameter of F. oxysporum (mm) Mean ± SD	Inhibition percentage (%)
Bacillus pseudomycoides MH-35-1	-	-	-	-	-	60±1.52 ^{ij}	35.71
Bacillus megaterium MH-35-3	-	-	-	-	-	68±0.57 ^{fgh}	26.19
Chryseobacterium elymi MH-35-4	+	++*	-	+***	-	65±1.52 ^{h}	29.76
Pseudomonas koreensis MH-35-6	+	-	-	+	+++	45±1.15 ^{l}	53.57
Bacillus weihenstephanensis MH-35-7	-	-	-	-	-	67±0.57 ^{gh}	27.38
Bacillus simplex MH-35-8	-	-	-	-	-	70±1.52 ^{cde}	23.80
Bacillus oligofermantans MH-35-9	-	-	-	-	-	74±1.52 ^{bcd}	19.04
Bacillus cereus MH-49-2	-	-	-	-	-	75±1.0 ^{ab}	17.85
Pseudarthrobacter polychromogenes MH-49-4	-	+	-	-	-	68±0.57 ^{gh}	26.19
Bacillus simplex MH-49-8	-	-	-	-	-	76±0.57 ^{ab}	16.66
Bacillus megaterium MH-54-1	-	-	-	-	-	47±1.52 ^{kl}	51.19
Bacillus mojavensis MH-54-3	-	-	-	+	-	50±0.33 ^{k}	47.61
Stenotrophomonas sp. MH-54-4	-	-	-	+	+	70±1.33 ^{efg}	23.80
Pseudomonas koreensis MH-59-1	+	-	-	+	+++	60±1.0 ^{ij}	35.71
Pseudomonas koreensis MH-59-2	+	-	-	+	+++	58±0.33 ^{j}	38.09
Pseudomonas koreensis MH-59-4	+	-	-	+	+++	63±1.52 ^{i}	32.14
Acinetobacter calcoaceticus MH-59-6	-	-	+	-	-	75±0.57 ^{abc}	17.85
Stenotrophomonas rhizophila MH-59-7	-	-	-	-	-	77±1.0 ^{a}	15.47
Pseudomonas koreensis MH-59-8	+	-	-	+	+++	59±0.57 ^{ij}	36.90
Bacillus simplex MH-64-2	-	-	-	-	-	71±0.33 ^{def}	22.61
Aromatoleum evansii MH-64-3	+	++	+	-	-	76±1.0 ^{a}	16.66

*For nitrogen fixation activity (+++: development after 6 hours, ++: development after 12 hours, +: development after 24 hours). **For siderophore and IAA activity activity: (+++: color change after 1h, ++: color change after 6h., +: color change after 24h). ***For HCN activity: (+++: color change after 6h, ++: color change after 12h, +: color change after 24h)

For antifungal activity: p<0,01; statistically significant level. a-l: The difference between the means shown by different letters in the same column is statistically significant. (Mean ± SD: Mean±Standard Deviation)

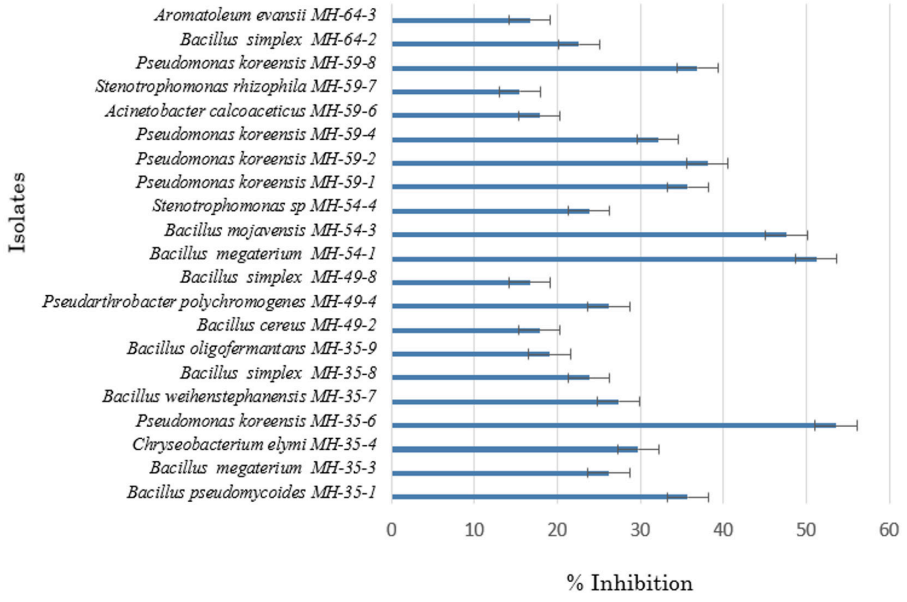

Figure 5. Percent Inhibition Rates of Isolates Against *Fusarium oxysporum*

CONCLUSION

This study reported the isolation, characterization and identification of PGPR from the rhizosphere of sunflowers grown in Kırşehir province of Türkiye. Among the PGPB properties of the obtained isolates, biological nitrogen fixation, phosphorus solubilization, siderophores production, HCN production, IAA production and antifungal activity against the fungal pathogen *Fusarium oxysporum* are the most interesting features. Numerous studies conducted in the past few decades have documented the advantages of using *Bacillus* species as biocontrol and biofertilizers, such as *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus cereus* and *Bacillus pumilus*. The predominance of bacillus species among our isolates can be considered as an advantage in terms of developing inoculant-based formulations from bacillus species. *Bacillus* ssp. and *Pseudomonas* ssp. in sunflower acts as an antipathogen because it inhibits the growth of specific pathogens. In future, PGPR is expected to eventually replace artificial growth regulators, chemical fertilizers, and pesticides, which have many harmful effects on the environment and human health.

The isolates obtained in this study are suitable candidates for the development of biotechnological tools aimed at increasing the yield of sunflower plants in environmentally friendly ways and contributing to the protection against the *Fusarium oxysporum* that causes disease in this plant. Moreover, Further study, including efficiency tests in greenhouses and fields, is needed to determine the role of PGPR-based microbial fertilizers as biofertilizers.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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Data availability

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RESEARCH ARTICLE

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Morphological and agronomical characterization of beef type tomato hybrids

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Abstract

Tomato (*Solanum lycopersicum L.*) is one of the most important vegetable crops and agro-morphological characterization has a key role in the development of new varieties. In this study, 228 samples of the tomato hybrid type "Beef" (*Solanum lycopersicum L.*) were characterized by comparing with 11 standard varieties based on 24 quantitative traits and 2 qualitative traits to reveal the phenotypic diversity by using conventional descriptors proposed by IPGRI (1996) and UPOV (2011). A significant level of variability was found in most of the traits studied among the genotypes in two locations. A high level of broad-sense heritability (H^2) was detected for many traits such as the number of fruits, firmness, immature fruit color, stem length up to the first inflorescence, total height, and number of days to the first flowering in both locations. There was a highly significant positive correlation among the color values (L*, a*, b*, c*, h*) but no positive correlation between a* and h*. Number of locule had a positive correlation with fruit width and fruit weight, and a positive correlation was determined between fruit length and pericarp thickness in both locations. While fruit weight had a highly significant negative correlation with the number of fruits and number of flowers, there was a highly significant negative correlation between the number of locules and the fruit length-to-width ratio in both locations. Results of PCA showed that PC1 and PC2 accounted for around 15.6% and 13.7% of total variation and 13.8% and 11.8% of total variation for Location 1 and Location 2, respectively. The first five principal components accounted for around 54.2% of the total variation for Location 1 and 48.2% of the total variation for Location 2. Cluster analysis grouped the 239 genotypes under six cluster groups for Location 1 and seven cluster groups for Location 2. Results of the cluster analysis revealed that Cluster 3 for Location 1 and Cluster 2 for Location 2 had prominent genotypes for some of the agronomically important traits like yield. The results showed that present phenotypic diversity could be useful in the selection of best-performing genotypes, which would be important candidates for the beef red tomato market in the spring season.

Keywords: Agro-morphological characterization, beef type tomato, *Solanum lycopersicum*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most popular and consumed vegetable crops and belongs to the Solanaceae (nightshade) family, including many important agronomic crops such as eggplant, pepper, and potato (Jenkins, 1948; Peralta et al., 2008). Tomato is one of the most produced vegetable crops in the world and total production of tomatoes is around 180 million tons in a cultivation area of 5 million hectares (FAOSTAT, 2021). China, India, Turkey, USA,

and Italy are the top countries in tomato production in the world. Turkey is one of the countries that has an important share in total production of tomato in the world and its tomato production is around 13 million tons in a cultivation area of 180 thousand hectares (FAOSTAT, 2021). Tomato has a high nutritional value, is a great source for human nutrition, and is used for fresh and processed consumption like sauces, paste, ketchup, and juices (Gosselin and Trudel, 1984). Ripe tomato includes mainly 95% of water and 5% of other components (sugar, polyphenols, vitamins, etc.) and lycopene of 20-50 mg per 100 g ripe fruit (Davies and Hobson, 1981). Tomato variety "Beefsteak", also known as "Beef", can have determinate or indeterminate growth habits. Beef tomatoes have generally flattened or round shape, and more than three locules with green shoulder. There are some essential characteristics for a variety that is used for fresh consumption, these are mainly; high yield, external and internal fruit quality, earliness, long shelf-life and resistance to biotic and abiotic stresses (Prohens-Tomás, and Nuez, (Eds.), 2007). In a breeding program, it is crucial to measure and analyze important morphologic and agronomic traits properly and to comprehend and benefit more from phenotypic variation. Phenotypic characterization is generally implemented with conventional morphological and agronomical descriptors that are mainly seedling, plant, inflorescence, flower, fruit, and agronomic traits (IPGR, 1996). Phenotypic characterization also provides good estimation about parental lines that are used to make new hybrids.

MATERIALS AND METHODS

Plant Materials

11 samples of standard red tomato type "beef" as control and 228 samples of the red tomato hybrid type "beef" which were newly developed by tomato breeders at Enza Zaden were studied by using morphological and agronomical descriptors proposed by IPGRI (1996) and UPOV (2011). Control varieties were named as A, B, C, D, E, F, H, I, J, K, and M and newly developed hybrids were named as between G1 and G228.

Growth Conditions and Experimental Design

The trials were conducted in two locations and coded Location 1 (L1), namely Enza Zaden R&D Turkey station, and Location 2 (L2), namely Kurşunlu region, Antalya, Turkey between February and June 2021. The experimental plots were arranged as double rows: 1.4 m between each double row, 0.5 m between rows within a double row, and 0.4 m between plants. Transplantation of tomato seedlings was carried out at the end of January 2021 in Location 2 and around mid-February 2021 in Location 1. All the plants were tied up with rope to support them as they all had indeterminate growth habits. The apex of all the plants was cut when control varieties had a seventh inflorescence. General agronomic practices such as drip irrigation, weeding control, and fertilizing were carried out.

All the genotypes were transplanted in two different trials into non-heated greenhouses with an Augmented randomized complete block (ARCBD) experimental design due to the limited amount of seeds and limited space, and the number of blocks was determined according to the following formula: $b \geq [(10/r-1)]+1$

Where, r is the number of control varieties used in this study and b is the number of blocks (Federer and Raghavarao, 1975). As 11 standard variety samples were used as control varieties, two blocks were decided as sufficient according to the formula. Therefore, control varieties were used in two blocks and each block included newly tested genotypes.

Phenotypic Analysis

The descriptors were the number of days to the first flowering (FD), number of days to the first maturity (MD), plant height (TH), stem length between 1st and 2nd truss (L1.2), stem length up to the first inflorescence (L1trs), leaf attitude (LA), leaf length (LL), leaf width (LW), number of truss on main stem (NT), number of flowers (NF), number of fruit (NFr), the ratio of fruit set (FS), immature fruit color (IMC), fruit external color (L, a, b, c, h), fruit length (FL), fruit width (FW), the fruit length-to-width ratio (FL.FW), pericarp thickness (PT), number of locules (NL), fruit weight (Fwe), fruit firmness (F), and average yield (Y). A total of 26 morphological and agronomical traits were characterized as 2 qualitative traits and 24 quantitative traits.

Data Collection

All the data were collected from randomly selected 4 individual plants from 10 plants within each genotype. The number of days elapsed from the planting date to the first flowering was determined in 50% of all plants within hybrid when they had the first fully open flower. Similarly, the number of days elapsed until the first maturity was also determined in 50% of all plants within hybrid when they had the first mature fruit. Total plant height, stem length between 1st and 2nd trusses, and stem length up to first inflorescence were measured on 4 plants per hybrid by 2 meters, and leaf length and leaf width were measured on 4 plants per hybrid by using a 30-cm ruler, respectively. Leaf attitude and immature fruit color were scored for each genotype according to the morphological descriptors used in this study. Fruit weight was measured on collected 4 marketable representative fruits (1 fruit per plant) by using a

weighing scale and recorded for each plant within the genotype. The average yield per hybrid sample was calculated by multiplying an average number of fruits per hybrid sample with the mean weight of 4 marketable representative fruits per hybrid sample. Fruit external color was measured on 3 parts of a marketable, ripe representative fruit per plant within each hybrid by using a Colorimeter PCE CSM device and L*, a*, b*, c*, and h* values were obtained. These values indicate lightness, red or green coordinates, yellow or blue coordinates, color scale (red, yellow, blue, and green) and saturation, respectively. Fruit firmness was also measured on 3 parts of fruit per plant by using a Force Gauge device and firmness values were obtained as Newton (N) unit. Pericarp thickness and number of locules were recorded by cutting fruit cross-sectionally. Pericarp thicknesses were measured by using a slide gauge and number of locules was counted.

Statistical Analysis

As there are too many genotypes to be characterized with a limited number of seeds and a limited experimental field area, an augmented randomized complete block design was used, and data were analyzed by using R statistical software (R 4.1.0 version). Analysis of variance (ANOVA) was run by using the 'augmented RCBD' package in R program (Aravind *et al*., 2019). Descriptive statistics, genetic variability and frequency distribution were also performed by using augmented RCBD package in R program. Phenotypic, genotypic, and environmental variances (σ^2p, σ^2g, σ^2e) were calculated by using a mean square from ANOVA result (Federer and Searle, 1976) according to the formula as:

σ^2p = Mean sum of squares of newly tested genotypes, σ^2e = Mean sum of squares of residual

$\sigma^2g = \sigma^2p - \sigma^2e$

Phenotypic and genotypic coefficients of variation (PCV and GCV) were also calculated according to Burton (1951, 1952). The broad-sense heritability was obtained based on Lush (1940) method as in the formula: $H^2 = \sigma^2g / \sigma^2p$

And estimation was categorized according to Johnson *et al*., (1955) as:

H^2	Category
x < 30	Low
30 ≤ x <60	Medium
≥ 60	High

Pearson's correlation analysis was performed by using the function 'cor()' and plots were obtained with the 'corrplot' and 'Performance Analytics' R packages. Principal component analysis (PCA) was performed by using 'corrplot', 'factoextra' and FactoMiner' R packages. Cluster analysis was applied as hierarchical two-way clustering through Ward method by using SAS JMP 16.0 version and was obtained for both locations.

RESULTS

Descriptive statistics was carried out to interpret 228 beef-type tomato hybrids in terms of 26 morphological and agronomical traits in two locations, (L1) and (L2) (Table 1). Analysis of variance (ANOVA) revealed significant differences between blocks for firmness, days to the first flowering, fruit length, fruit length-to-width ratio, fruit set, fruit weight, hue value, stem length up to the first inflorescence, leaf attitude, number of flowers, number of fruits, number of truss and yield in Location 1 (treatment adjusted) (Table 2). Block effects were also significant for most of the traits except for a*, fruit length, fruit set, fruit weight, hue value, stem length between 1st and 2nd trusses, stem length up to the first truss, leaf width, and pericarp thickness in Location 2 (treatment adjusted). Block effects were significant for stem length up to the first truss (17.28*) in Location 1 and there were significant differences between blocks for firmness, fruit length, number of days until the first maturity, and total height in Location 2 (block adjusted). All the genotypes including control varieties in Location 1 showed significant differences in firmness, days to the first flowering, fruit set, immature fruit color, stem length up to the first truss, leaf attitude, number of flowers, pericarp thickness and total height (treatment adjusted). Significant differences were also found among genotypes for days to the first flowering, fruit weight, immature fruit color, length between 1st and 2nd trusses, stem length up to the first truss, leaf length, number of fruits, number of truss and total height in Location 2 (treatment adjusted).

Genetic variability analysis was applied based on the ANOVA results. The broad-sense heritability was calculated as the highest for the number of fruit (92.09%), rate of fruit set (88.91 %), stem length up to the first truss (88.11%), and number of flowers (87.24%) and the lowest heritability was found for fruit width (4.15%), fruit weight (4.61%), and hue value (10.05%) in Location 1. The heritability was recorded as the highest for plant total height (94.77%), fruit weight (90.27%), and stem length between 1st and 2nd trusses (85.02%), and the lowest heritability was estimated for hue value (1.51%), fruit width (11.52%) and L* value (22.7%) in Location 2. The broad-sense heritability could not be calculated mostly for color values, as well as a fruit length-to-width ratio and pericarp thickness, because

environmental variance (EV) was higher than phenotypic variance (PV) (Table 3).

Correlation analysis for agro-morphologic traits was done separately for Location 1 and Location 2 (Figure 1). The Pearson correlation coefficient showed highly significant positive correlations between color values (L*, a*, b*, c*, and h*) in both locations. Fruit width had highly significant and positive correlations with fruit length, number of locule, and leaf length and fruit length had highly significant and positive correlations with pericarp thickness, leaf length and leaf width, number of days to the first flowering and stem length up to the first truss in both locations. Highly significant and negative correlations were also found between the number of days to the first flowering and number of flowers, number of fruits, total height; and between the number of days to the first maturity and number of fruits, number of fruit and fruit weight in both locations (Figure 1).

Table 1. Descriptive statistics of 239 beef-type genotypes for 26 agro-morphological traits

Location 1								Location 2						
Trait	**Mean**	**SE**	**SD**	**Min**	**Max**	**Skewness**	**Kurtosis**	**Mean**	**SE**	**SD**	**Min**	**Max**	**Skewness**	**Kurtosis**
L	35.77	0.08	1.3	32.71	39.99	0.35 *	3.2 ns	34.53	0.08	1.2	31.88	38.62	0.55 **	3.67 ns
a	32.94	0.12	1.82	26.97	37.92	-0.11 ns	3.42 ns	31.65	0.14	2.18	24.04	40.13	-0.08 ns	4.78 **
b	30.01	0.15	2.32	23.62	36.9	0.26 ns	2.94 ns	28.85	0.14	2.14	22.59	36.48	0.49 **	4.09 **
c	44.58	0.17	2.64	36.56	52.36	0.13 ns	3.37 ns	42.74	0.15	2.38	34.97	48.11	-0.42 **	3.56 ns
h	42.23	0.1	1.54	38.76	47.23	0.53 **	3.16 ns	42.3	0.13	2.04	38.01	50.3	0.64 **	3.71 *
NF	40.71	0.51	7.84	21.66	61.34	0.2 ns	2.77 ns	41.31	0.47	7.23	25.42	62.09	0.11 ns	2.53 ns
NFr	27.82	0.38	5.9	12.77	45.48	0.32 *	3.19 ns	32.75	0.37	5.79	19.89	49.44	0.27 ns	2.63 ns
FS	68.93	0.52	8.06	48.02	93.14	0.23 ns	3.03 ns	79.43	0.5	7.74	55.96	96.49	-0.45 **	3.19 ns
FL	57.2	0.23	3.6	48.2	66.05	0.16 ns	2.64 ns	63.24	0.27	4.13	53.16	77.79	0.29 ns	3.25 ns
FW	72.11	0.24	3.65	61.97	81.47	-0.11 ns	2.81 ns	79.03	0.31	4.85	64.94	91.77	-0.07 ns	3.08 ns
FL.FW	0.79	0.0033	0.05	0.67	0.97	0.48 **	3.53 ns	0.8	0.0033	0.05	0.66	0.94	0.19 ns	2.87 ns
NT	6.71	0.04	0.6	4.98	8.52	0.24 ns	3.28 ns	6.84	0.04	0.58	5.59	8.41	0.15 ns	2.47 *
F	27.79	0.16	2.43	21.41	33.26	-0.16 ns	2.52 ns	22.06	0.19	2.9	15.06	30.03	0.12 ns	2.69 ns
NL	4.63	0.06	0.99	2.62	7.63	0.48 **	2.83 ns	4.49	0.05	0.84	2.26	7.51	0.12 ns	3.52 ns
PT	6.3	0.05	0.76	4.46	8.31	0.06 ns	2.61 ns	9.41	0.06	0.87	4.9	11.98	-0.54 **	5.62 **
Fwe	186.39	1.52	23.47	125.48	252.48	0.12 ns	2.74 ns	220.31	1.85	28.67	150.59	296.29	0.34 *	2.91 ns
Y	5167.83	61.96	957.91	3108.76	7850.24	0.28 ns	2.78 ns	7133.84	72.39	1119.12	4483.75	9996.67	0.15 ns	2.7 ns
LL	40.28	0.22	3.34	34.14	50.36	0.51 **	2.91 ns	35.81	0.21	3.25	28.17	43.33	0.08 ns	2.51 ns
LW	46.84	0.31	4.72	35.14	57.64	0.22 ns	2.4 *	39.61	0.33	5.1	27.53	53.16	0.17 ns	2.77 ns
LA	7.46	0.11	1.77	1	9	-0.88 **	2.99 ns	6.89	0.09	1.37	3	9	-0.25 ns	2.94 ns
IMC	4.92	0.09	1.36	1	9	-0.25 ns	4.42 **	4.28	0.11	1.66	1	9	0.42 **	3.95 *
L1trs	27.67	0.32	4.89	18.11	43.39	0.43 **	3.03 ns	31.33	0.25	3.8	20.48	42.02	-0.01 ns	2.96 ns
L1.2	22.62	0.32	4.89	9.48	36.02	0.04 ns	2.94 ns	24.99	0.28	4.36	14.3	35.3	0.0033 ns	2.45 *
TH	165.6	0.96	14.88	133.68	203.32	0.42 **	2.69 ns	174.19	0.86	13.36	136.02	219.98	0.05 ns	3.92 *
FD	22.31	0.14	2.14	16.91	29.09	-0.1 ns	2.52 ns	33.26	0.14	2.16	28.55	40.55	0.65 **	3.85 *
MD	82.39	0.19	2.93	75.77	91.23	0.5 **	3.37 ns	100.54	0.28	4.28	94.59	113.41	0.91 **	3.62 ns

ns P > 0.05; * P <= 0.05; ** P <= 0.01 SE : Standard Error, SD : Standard deviation, Min : Minimum, Max : Maximum; L, a, b, c, h : Color values, NF : Number of Flower, NFr : Number of fruits, FS : Fruit set (%), FL : Fruit length, FW : Fruit width, FL.FW : Fruit length-to-width ratio, NT : Number of truss, F : Firmness, NL : Number of locule, PT : Pericarp thickness, Fwe : Fruit weight, Y : Yield, LL : Leaf length, LW : Leaf width, LA : Leaf attitude, IMC : Immature fruit color, L1trs : Stem length up to the first truss, L1.2 : Stem length between 1st and 2nd truss, TH : Total Height, FD : Days to the first flowering, MD : Days to the first maturity.

Table 2. Mean squares from the ANOVA made on the evaluated traits for 228 genotypes and 11 control varieties.

	Source	Df	a	b	c	F	FD	FL	FL.FW	FS	FW	Fwe	h	IMC
L1 (Treatment Adjusted)	Block (ignoring treatments)	1	0.34 ns	12.8 ns	0.16 ns	45.57 **	14.88 **	122.26 **	0.01 *	2814.97 **	35.14 ns	4008 *	15.25 *	0.14 ns
	Treatment (eliminating blocks)	238	3.17 ns	5.44 ns	6.88 ns	5.65 *	4.74 **	11.67 ns	0.0026 ns	60.45 **	13.73 ns	564.11 ns	2.39 ns	1.93 *
	Control	10	3.18 ns	7.53 ns	5.26 ns	3.42 ns	2 ns	15.3 ns	0.0034 ns	44.02 **	21.5 ns	706.31 ns	1.39 ns	3.71 **
	Test and Test vs. Control	228	3.17 ns	5.34 ns	6.95 *	5.75 *	4.86 **	11.51 ns	0.0026 ns	61.17 **	13.38 ns	557.87 ns	2.43 ns	1.85 *
	Residuals	10	2.48	6.28	2.71	1.58	0.78	7.33	0.0014	8.09	13.02	549.21	2.25	0.58
L1 (Block Adjusted)	Treatment (ignoring blocks)	238	3.16 ns	5.46 ns	6.86 ns	5.84 *	4.8 **	12.16 ns	0.0027 ns	72.18 **	13.87 ns	580.38 ns	2.45 ns	1.93 *
	Control	10	3.18 ns	7.53 ns	5.26 ns	3.42 ns	2 ns	15.3 ns	0.0034 ns	44.02 **	21.5 ns	706.31 ns	1.39 ns	3.71 **
	Test vs. Control	1	4.76 ns	10.15 ns	10.39 ns	8.96 *	63.31 **	7.8 ns	0.01 *	177.89 **	1.23 ns	367.9 ns	0.94 ns	6.84 **
	Test	227	3.15 ns	5.35 ns	6.92 ns	5.93 *	4.67 **	12.04 ns	0.0026 ns	72.96 **	13.59 ns	575.77 ns	2.5 ns	1.83 *
	Block (eliminating treatments)	1	3.78 ns	7.69 ns	4.61 ns	0.59 ns	0.18 ns	6.71 ns	0.00023 ns	22.77 ns	1.08 ns	135.01 ns	0.61 ns	0.18 ns
	Residuals	10	2.48	6.28	2.71	1.58	0.78	7.33	0.0014	8.09	13.02	549.21	2.25	0.58
L2 (Treatment Adjusted)	Block (ignoring treatments)	1	11.33 ns	37.78 *	74.42 *	528.09 **	8.84 *	2.4 ns	0.09 **	5.89 ns	562.71 **	19.49 ns	8.06 ns	10 **
	Treatment (eliminating blocks)	238	4.86 ns	4.71 ns	5.75 ns	8.55 ns	4.77 *	15.71 ns	0.0022 ns	55.59 ns	24.2 ns	831.82 **	4.3 ns	2.88 **
	Control	10	4.94 ns	5.11 ns	7.12 ns	10.73 ns	7.91 **	34.05 *	0.0038 ns	80.47 ns	36.04 ns	994.3 **	5.05 ns	5.24 **
	Test and Test vs. Control	228	4.86 ns	4.69 ns	5.69 ns	8.45 ns	4.63 *	14.91 ns	0.0022 ns	54.5 ns	23.69 ns	824.69 **	4.27 ns	2.77 **
	Residuals	10	6.36	6.77	10.7	4.03	1.35	7.6	0.0026	35.01	22.71	80.1	4.22	0.44
L2 (Block Adjusted)	Treatment (ignoring Blocks)	238	4.91 ns	4.86 ns	6.05 ns	10.49 *	4.79 *	15.55 ns	0.0026 ns	55.1 ns	26.48 ns	830.57 **	4.33 ns	2.91 **
	Control	10	4.94 ns	5.11 ns	7.12 ns	10.73 ns	7.91 **	34.05 *	0.0038 ns	80.47 ns	36.04 ns	994.3 **	5.05 ns	5.24 **
	Test vs. Control	1	0.39 ns	4.84 ns	2.8 ns	0.03 ns	3.13 ns	43.14 *	0.0015 ns	354.39 **	113.7 *	838.23 **	7.49 ns	4.32 *
	Test	227	4.93 ns	4.85 ns	6.02 ns	10.52 *	4.66 *	14.62 ns	0.0026 ns	52.66 ns	25.67 ns	823.32 **	4.29 ns	2.8 **
	Block (eliminating Treatments)	1	0.18 ns	2.45 ns	1.98 ns	66.16 **	4.55 ns	39.49 *	7.1e-05 ns	124.04 ns	22.35 ns	315.97 ns	0.75 ns	1.64 ns
	Residuals	10	6.36	6.77	10.7	4.03	1.35	7.6	0.0026	35.01	22.71	80.1	4.22	0.44

Table 2. Mean squares from the ANOVA made on the evaluated traits for 228 genotypes and 11 control varieties (continued).

	Source	Df	L	L1.2	L1trs	LA	LL	LW	MD	NF	NFr	NL	NT	PT	TH	Y
L1 (Treatment Adjusted)	Block (ignoring Treatments)	1	4.87 ns	53.82 ns	22.8 *	21.9 **	2.12 ns	0.84 ns	13 ns	522.73 **	1395.94 **	0.29 ns	1.59 **	0.05 ns	65.03 ns	20005273.6 **
	Treatment (eliminating Blocks)	238	1.7 ns	23.4 ns	23.23 **	3.01 **	11.52 ns	23.21 ns	8.76 ns	60.66 **	31.03 **	1.01 ns	0.35 ns	0.58 *	231.02 *	854123.15 ns
	Control	10	1.16 ns	25.98 ns	31.27 **	3.78 **	19.89 ns	48.03 ns	7.54 ns	85.14 **	30.17 **	1.83 ns	0.26 ns	0.33 ns	333.56 *	579008.68 ns
	Test and Test vs. Control	228	1.73 ns	23.29 ns	22.88 **	2.97 **	11.15 ns	22.12 ns	8.81 ns	59.59 **	31.07 **	0.97 ns	0.35 ns	0.6 **	226.52 *	866189.58 ns
	Residuals	10	1.16	16.89	2.68	0.73	8.36	16.21	5.34	7.7	2.93	0.69	0.16	0.15	72.7	685359.7
L1 (Block Adjusted)	Treatment (ignoring Blocks)	238	1.72 ns	23.6 ns	23.25 **	3.09 **	11.52 ns	23.2 ns	8.81 ns	62.85 **	36.89 **	1.01 ns	0.36 ns	0.59 *	231.23 *	938178.46 ns
	Control	10	1.16 ns	25.98 ns	31.27 **	3.78 **	19.89 ns	48.03 ns	7.54 ns	85.14 **	30.17 **	1.83 ns	0.26 ns	0.33 ns	333.56 *	579008.68 ns
	Test vs. Control	1	8.16 *	1.09 ns	95.34 **	8.77 **	0.07 ns	51.13 ns	15.41 ns	405.77 **	67.21 **	8.72 **	0.004 ns	1.47 *	1366.6 **	4871824.18 *
	Test	227	1.72 ns	23.6 ns	22.58 **	3.04 **	11.2 ns	21.99 ns	8.84 ns	60.36 **	37.05 **	0.93 ns	0.36 ns	0.59 *	221.72 *	936672.08 ns
	Block (eliminating Treatments)	1	0.03 ns	6.01 ns	17.28 *	0.73 ns	0.41 ns	2.91 ns	1.14 ns	2.56 ns	1.64 ns	0.34 ns	0.01 ns	0.0012 ns	14.73 ns	110.81 ns
	Residuals	10	1.16	16.89	2.68	0.73	8.36	16.21	5.34	7.7	2.93	0.69	0.16	0.15	72.7	685359.7
L2 (Treatment Adjusted)	Block (ignoring Treatments)	1	7.34 *	0.04 ns	0.13 ns	18.5 **	63.59 **	14.22 ns	52.9 *	224.06 *	200.41 **	12.21 **	3.36 **	3.96 ns	1557.5 **	7218137.88 **
	Treatment (eliminating Blocks)	238	1.45 ns	19.69 **	15.08 **	1.85 ns	11.07 **	27.21 ns	17.73 ns	53.05 ns	32.78 **	0.76 ns	0.31 *	0.79 ns	157.24 **	1271629.69 ns
	Control	10	2.19 ns	35.6 **	26.93 **	1.35 ns	20.3 **	52.99 *	16.55 ns	52.95 ns	52.37 **	2 **	0.46 **	1.64 ns	225.06 **	1606527.52 ns
	Test and Test vs. Control	228	1.41 ns	18.99 **	14.56 **	1.87 ns	10.66 **	26.08 ns	17.79 ns	53.05 ns	31.92 **	0.71 ns	0.3 *	0.75 ns	154.26 **	1256941.19 ns
	Residuals	10	1.1	2.85	2.84	1.2	2.68	16.34	8.18	23.13	7.29	0.37	0.09	0.82	8.41	685694.18
L2 (Block Adjusted)	Treatment (ignoring Blocks)	238	1.46 ns	19.68 **	15.08 **	1.93 ns	11.32 **	27.27 ns	17.77 ns	53.7 ns	33.6 **	0.81 ns	0.32 *	0.81 ns	163.21 **	1301918.85 ns
	Control	10	2.19 ns	35.6 **	26.93 **	1.35 ns	20.3 **	52.99 *	16.55 ns	52.95 ns	52.37 **	2 **	0.46 **	1.64 ns	225.06 **	1606527.52 ns
	Test vs. Control	1	4.37 ns	14.21 *	30.24 **	9.44 *	40.12 **	129.02 *	5.59 ns	178.02 *	21.58 ns	2.51 *	3.01 **	0.97 ns	51.77 *	4431593.2 *
	Test	227	1.42 ns	19 **	14.49 **	1.92 ns	10.8 **	25.69 ns	17.88 ns	53.19 ns	32.83 **	0.75 ns	0.31 *	0.77 ns	160.98 **	1274712.85 ns
	Block (eliminating Treatments)	1	3.38 ns	1.92 ns	0.01 ns	4.4e-31 ns	3.96 ns	0.56 ns	43.68 *	67.98 ns	4.25 ns	1.25 ns	0.13 ns	0.53 ns	135.01 **	9319.1 ns
	Residuals	10	1.1	2.85	2.84	1.2	2.68	16.34	8.18	23.13	7.29	0.37	0.09	0.82	8.41	685694.18

ns P > 0.05; * P <= 0.05; ** P <= 0.01, L1 : Location 1, L2 : Location 2, L, a, b, c, h : Color values, NF : Number of Flower, NFr : Number of fruit, FS : Fruit set (%), FL : Fruit length, FW = Fruit width, FL.FW : Fruit length-to-width ratio, NT : Number of trusses, F : Firmness, NL : Number of locule, PT : Pericarp thickness, Fwe : Fruit weight, Y : Yield, LL : Leaf length, LW : Leaf width, LA : Leaf attitude, IMC : Immature fruit color, L1trs : Stem length up to the first truss, L1.2 : Stem length between 1st and 2nd trusses, TH : Total Height, FD : Days to the first flowering, MD : Days to the first maturity.

Table 3. Genetic variability estimates from the ANOVA results.

	Location 1								Location 2							
Trait	**PV**	**GV**	**EV**	**GCV**	**PCV**	**ECV**	**HBS**	**Category**	**PV**	**GV**	**EV**	**GCV**	**PCV**	**ECV**	**HBS**	**Category**
L	1.72	0.56	1.16	2.09	3.67	3.01	32.57	Medium	1.42	0.32	1.1	1.64	3.45	3.03	22.7	Low
a	3.15	0.67	2.48	2.49	5.39	4.78	21.42	Low	4.93	-	6.36	-	7.01	7.97	-	-
b	5.35	-	6.28	-	7.7	8.35	-	-	4.85	-	6.77	-	7.63	9.02	-	-
c	6.92	4.2	2.71	4.6	5.9	3.69	60.8	High	6.02	-	10.7	-	5.74	7.65	-	-
h	2.5	0.25	2.25	1.19	3.74	3.55	10.05	Low	4.29	0.06	4.22	0.6	4.89	4.86	1.51	Low
NF	60.36	52.66	7.7	17.82	19.08	6.82	87.24	High	53.19	30.05	23.13	13.27	17.65	11.64	56.5	Medium
NFr	37.05	34.12	2.93	21	21.88	6.15	92.09	High	32.83	25.53	7.29	15.43	17.49	8.24	77.79	High
FS	72.96	64.86	8.09	11.68	12.39	4.13	88.91	High	52.66	17.65	35.01	5.29	9.14	7.45	33.51	Medium
FL	12.04	4.7	7.33	3.79	6.07	4.73	39.08	Medium	14.62	7.02	7.6	4.19	6.05	4.36	48	Medium
FW	13.59	0.56	13.02	1.04	5.11	5	4.15	Low	25.67	2.96	22.71	2.18	6.41	6.03	11.52	Low
FL.FW	0.0026	0.0012	0.0014	4.42	6.45	4.7	46.92	Medium	0.0026	-	0.0026	-	6.34	6.38	-	
NT	0.36	0.21	0.16	6.8	8.98	5.87	57.31	Medium	0.31	0.21	0.09	6.74	8.08	4.46	69.57	High
F	5.93	4.35	1.58	7.5	8.76	4.53	73.33	High	10.52	6.49	4.03	11.55	14.7	9.1	61.67	High
NL	0.93	0.25	0.69	10.73	20.87	17.9	26.45	Low	0.75	0.37	0.37	13.62	19.23	13.57	50.2	Medium
PT	0.59	0.44	0.15	10.56	12.22	6.15	74.7	High	0.77	-	0.82	-	9.31	9.64	-	
Fwe	575.77	26.56	549.21	2.76	12.87	12.57	4.61	Low	823.32	743.22	80.1	12.37	13.02	4.06	90.27	High
Y	936672.08	251312.38	685359.7	9.7	18.73	16.02	26.83	Low	1274712.85	589018.67	685694.18	10.76	15.83	11.61	46.21	Medium
LL	11.2	2.85	8.36	4.19	8.31	7.18	25.4	Low	10.8	8.11	2.68	7.96	9.18	4.57	75.16	High
LW	21.99	5.78	16.21	5.13	10.01	8.6	26.28	Low	25.69	9.35	16.34	7.72	12.8	10.21	36.38	Medium
LA	3.04	2.31	0.73	20.38	23.37	11.43	76.07	High	1.92	0.72	1.2	12.3	20.1	15.9	37.45	Medium
IMC	1.83	1.25	0.58	22.7	27.49	15.5	68.2	High	2.8	2.37	0.44	35.9	39.07	15.42	84.43	High
L1trs	22.58	19.9	2.68	16.12	17.17	5.92	88.11	High	14.49	11.66	2.84	10.9	12.15	5.38	80.43	High
L1.2	23.6	6.71	16.89	11.45	21.48	18.17	28.43	Low	19	16.16	2.85	16.08	17.44	6.75	85.02	High
TH	221.72	149.02	72.7	7.37	8.99	5.15	67.21	High	160.98	152.57	8.41	7.09	7.28	1.66	94.77	High
FD	4.67	3.89	0.78	8.84	9.69	3.96	83.25	High	4.66	3.31	1.35	5.47	6.49	3.49	71.13	High
MD	8.84	3.5	5.34	2.27	3.61	2.8	39.61	Medium	17.88	9.7	8.18	3.1	4.21	2.84	54.24	Medium

PV : Phenotypic Variance, GV : Genotypic Variance, EV : Environmental Variance, PCV : Phenotypic coefficient of variation, GCV : Genotypic coefficient of variation, ECV : Environmental coefficient of variation, HBS : Broad-sense Heritability, L1 : Location 1, L2 : Location 2, L, a, b, c, h : Color values, NF : Number of Flowers, NFr : Number of fruits, FS : Fruit set (%), FL : Fruit length, FW = Fruit width, FL.FW : Fruit length-to-width ratio, NT : Number of trusses, F : Firmness, NL : Number of locules, PT : Pericarp thickness, Fwe : Fruit weight, Y : Yield, LL : Leaf length, LW : Leaf width, LA : Leaf attitude, IMC : Immature fruit color, L1trs : Stem length up to the first truss, L1.2 : Stem length between 1st and 2nd trusses, TH : Total Height, FD : Days to the first flowering, MD : Days to the first maturity.

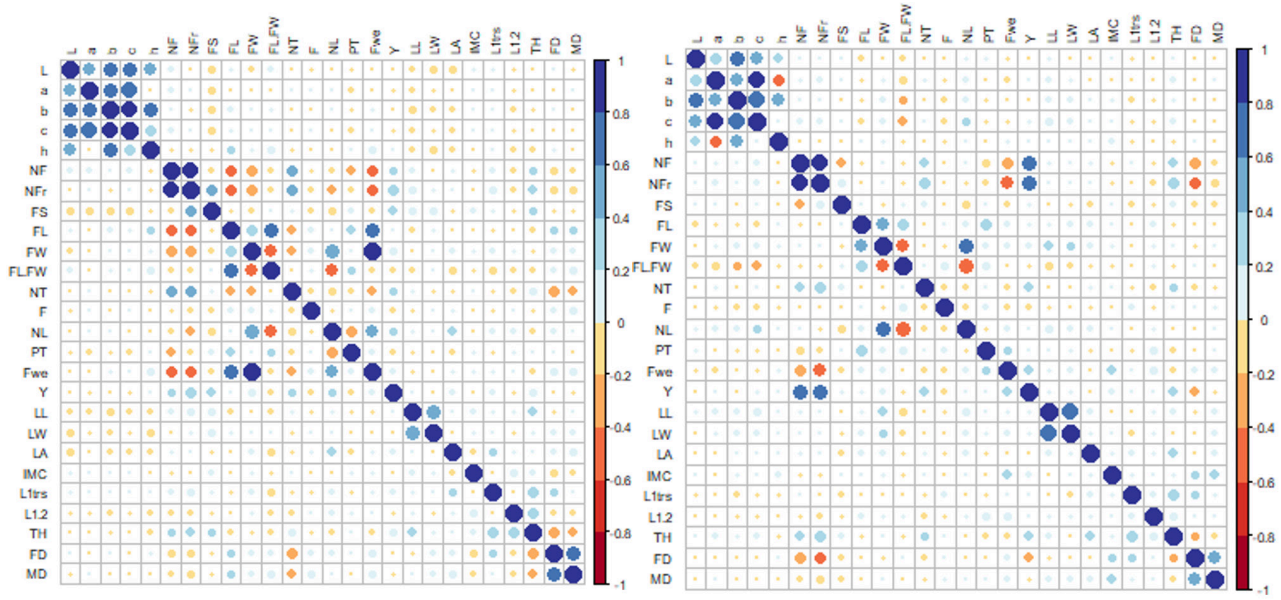


Figure 1. Correlation matrix for the traits in Location 1 (left) and Location 2 (right).

The first five principal components (PCs) accounted for 54.2343% of total variation for Location 1 and 48.1802% of total variation for Location 2 (Table 4). The first two components PC1 (15.5936%) and PC2 (13.7491%) for Location 1, and PC1 (13.7779%) and PC2 (11.8345%) for Location 2 made contribution to higher variation and they were used for biplots. Principal component analysis (PCA) indicated that PC1 for Location 1 accounted for 15.59 (%) of total variation by showing positive correlation with number of flowers, number of fruits, ratio of fruit set, number of trusses, yield, leaf length, leaf width, and total height; whereas, PC1 in Location 2 accounted for 13.7779 (%) of total variation and showed a positive correlation with fruit length, firmness, pericarp thickness, fruit weight, number of days to the first flowering and number of days to the first maturity. PC2 in Location 1 accounted for 13.7491 (%) of total variation by having positive correlation with number of flower, number of fruit, fruit length-to-width ratio, number of trusses, firmness, and total height; whereas, PC2 in Location 2 accounted for 11.8345 (%) of total variation and correlated positively with fruit length, fruit width, firmness, number of locule, pericarp thickness, fruit weight, leaf length, leaf width, leaf attitude, immature fruit color, stem length between 1st and 2nd truss, total height, days to the first flowering, and days to the first maturity. Biplots belonging to the both locations showed variability of genotypes studied for 26 agro-morphologic traits (Figure 2). Genotypes were scattered in four groups according to x and y axis. The genotypes present in positive axis were mostly correlated with pericarp thickness, number of locule, fruit width, fruit weight, days to the first flowering and maturity for Location 1 and with number of trusses, number of flowers and fruits, yield and total height for Location 2. The genotypes present in negative axis were correlated with these traits, negatively.

Table 4. Eigenvalue, variance (%), and cumulative variance (%) of the first five principal components

	Location 1					Location 2				
Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
L	0.4342	-0.3227	0.4889	-0.1317	0.0503	0.1610	-0.7778	0.0844	-0.0431	0.1225
a	0.5724	-0.2877	0.3329	0.0543	0.3975	0.1302	-0.7041	0.2469	0.0461	-0.0497
b	0.5916	-0.4872	0.4167	-0.1783	-0.0064	0.3259	-0.8610	0.1809	-0.0092	0.1733
c	0.6972	-0.4202	0.4021	-0.0349	0.2079	0.2265	-0.8299	0.2112	0.0177	0.0780
h	0.0949	-0.2275	0.1004	-0.2447	-0.3512	0.3258	-0.5143	-0.0014	-0.0595	0.2658
NF	0.6205	0.5136	-0.2063	0.1492	-0.0465	-0.6507	-0.3056	0.2033	0.3280	-0.0516
NFr	0.6599	0.6368	-0.1296	0.0063	0.0199	-0.7834	-0.1587	0.1608	0.2543	0.2693
FS	0.0085	0.1794	0.1741	-0.2920	0.0859	-0.3464	0.2103	-0.0585	-0.0542	0.5480
FL	-0.1289	-0.0812	-0.4589	-0.4772	0.2016	0.6589	0.0607	-0.2789	-0.0899	0.4346
FW	0.2847	-0.4317	-0.6213	-0.0918	-0.2453	0.6282	0.3244	0.5683	-0.1496	0.0989
FL.FW	-0.4308	0.3878	0.1801	-0.3385	0.4743	0.1280	-0.1995	-0.7703	0.0505	0.3227
NT	0.28477	0.3803	-0.0440	-0.0394	0.0726	-0.5797	-0.1730	0.0202	-0.0328	-0.0010
F	-0.2050	0.1088	0.0820	0.0389	0.2214	0.2308	0.0621	-0.1580	0.0992	-0.1017
NL	0.3255	-0.5015	-0.4287	0.1176	-0.3842	0.3634	0.2299	0.7107	0.0859	-0.1640
PT	-0.2031	-0.1021	-0.1748	-0.5097	0.3200	0.1402	0.1294	-0.4211	-0.3612	0.2271
Fwe	-0.2591	-0.3917	-0.3426	-0.0879	0.0112	0.7400	0.2538	0.3436	-0.1453	0.2341
Y	0.4850	0.3899	-0.4034	-0.0451	0.0307	-0.1896	0.0879	0.3953	0.1342	0.3969
LL	0.3078	-0.3265	-0.3556	-0.1434	0.3613	-0.2677	0.2595	0.1594	0.0991	0.4104
LW	0.1981	-0.3112	-0.3687	-0.2381	0.3505	-0.0665	0.2821	-0.0073	0.2326	0.4769
LA	0.1244	-0.0706	-0.2878	0.2526	0.1543	0.0429	0.2685	0.2898	0.3745	-0.1368
IMC	-0.0272	-0.2084	-0.1217	0.2793	0.0823	-0.0456	0.0301	0.1260	-0.4387	0.1984
L1trs	-0.0558	0.0313	-0.1175	0.5602	0.2827	-0.0679	0.0126	0.3939	0.2421	0.3058
L1.2	-0.0724	-0.1188	-0.2698	0.0154	0.2983	-0.0126	0.1893	0.1613	-0.4674	-0.0757
TH	0.4217	0.2956	-0.1613	0.3127	0.3197	-0.4319	0.0596	0.2812	-0.3646	0.3695
FD	-0.3988	-0.4293	0.1312	0.4159	0.1777	0.4449	0.1131	-0.1235	0.6372	0.1335
MD	-0.2200	-0.3412	-0.0806	0.3903	0.2888	0.3881	0.1386	-0.2018	0.6335	0.0839
Eigen Value	4.0543	3.5747	2.6344	2.0186	1.8187	3.5822	3.0769	2.4140	1.8146	1.6388
Variance (%)	15.5936	13.7491	10.1325	7.7638	6.9951	13.7779	11.8345	9.2847	6.9795	6.3033
Cumulative Variance (%)	15.5936	29.3427	39.4753	47.2392	54.2343	13.7779	25.6125	34.8973	41.8768	48.1802

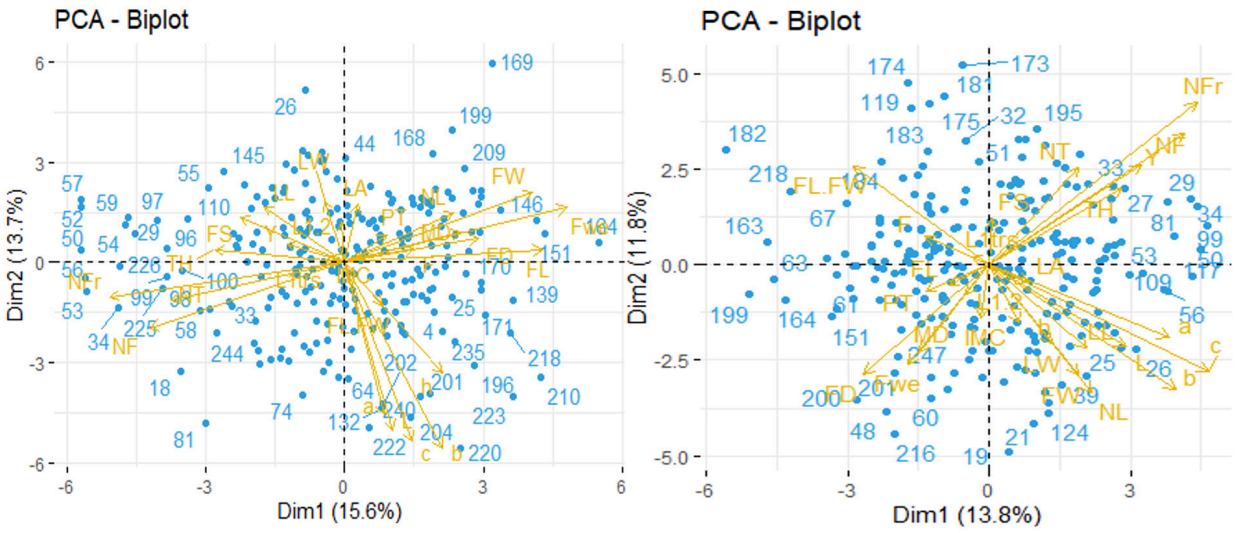
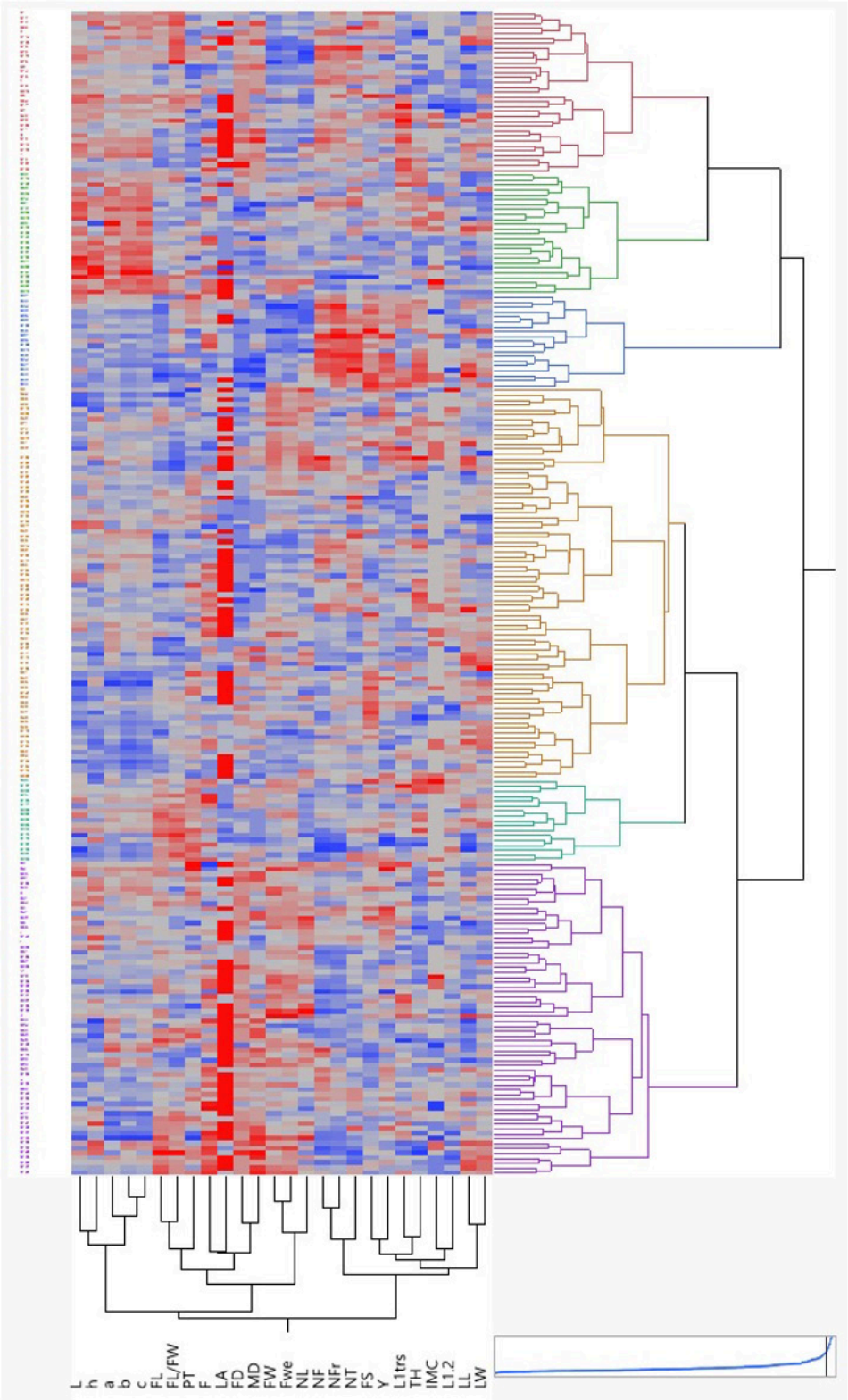


Figure 2. Biplots showing the correlation of 26 agro-morphological traits with 239 genotypes for Location 1 (left) and Location 2 (right).

The cluster analysis grouped the 239 genotypes into six cluster groups for Location 1 (Figure 3) and seven cluster groups in Location 2 (Figure 4). The number of genotypes was the highest in Cluster 4 (80), followed by Cluster 6 (64), Cluster 1 (33), Cluster 2 (25), Cluster 3 (19) and Cluster 5 (18) for Location 1 as shown in Figure 3 and Table 5. Genotypes belonging to Cluster 2 had higher color values (L*, a*, b*, c*, h*) in other word they are highly light and saturated, and lesser in ratio of fruit set, yield, leaf length and leaf width for Location 1. The genotypes were agglomerated mostly into Cluster 6 (60) and Cluster 3 (50), followed by Cluster 1 (38), Cluster 7 (31), Cluster 2 (29), Cluster 4 (20) and Cluster 5 (11) for Location 2. Cluster 5 for Location 2 was characterized by high lightness and saturation, high firmness, high pericarp thickness, moderate yielding, high stem length between 1st and 2nd trusses and low total height and late flowering and maturity. Cluster 1 for Location 2 was characterized by more flowers, moderate fruit set, the lowest fruit length, the widest fruit, low firmness, high yielding, mostly drooping leaf attitude, the tallest plant height, and moderately early mature (Table 6).



L, a, b, c, h : Color values, NF : Number of Flowers, NFr : Number of fruits, FS : Fruit set (%), FL : Fruit length, FW = Fruit width, FL.FW : Fruit length-to-width ratio, NT : Number of trusses, F : Firmness, NL : Number of locules, PT : Pericarp thickness, Fwe : Fruit weight, Y : Yield, LL : Leaf length, LW : Leaf width, LA : Leaf attitude, IMC : Immature fruit color, L1trs : Stem length up to the first truss, L1.2 : Stem length between 1st and 2nd truss, TH : Total Height, FD : Days to the first flowering, MD : Days to the first maturity.

Figure 3. Two-way hierarchical clustering analysis for Location 1.

Table 5. Mean values of agro-morphological traits in different clusters of genotypes of beef tomato type in Location 1.

Cluster	1	2	3	4	5	6
L	36.5	37.7	35.3	35.3	35.4	35.5
a	33.7	35.4	32.0	32.5	32.7	32.6
b	31.3	34.2	28.5	29.0	29.5	29.7
c	46.1	49.3	42.9	43.6	44.1	44.2
h	42.8	43.9	41.6	41.7	41.9	42.3
NF	46.2	39.2	50.9	40.5	33.1	38.1
NFr	32.2	24.5	39.8	27.7	23.2	25.1
FS	70.3	62.6	79.0	69.4	70.5	66.6
FL	57.3	57.5	53.1	55.6	61.2	59.4
FW	68.9	71.9	68.3	72.6	71.7	74.7
FL/FW	0.8	0.8	0.8	0.8	0.9	0.8
NT	6.8	6.6	7.4	6.7	6.6	6.5
F	27.3	28.0	27.2	27.3	28.5	28.5
NL	4.1	4.5	4.0	4.8	4.0	5.3
PT	5.9	6.1	6.2	6.3	7.2	6.5
Fwe	171.7	185.8	157.1	183.8	195.0	205.0
Y	5304.0	4716.5	6118.4	4926.7	4791.2	5314.2
LL	39.9	38.6	42.0	41.0	39.8	39.8
LW	47.4	44.0	47.5	47.1	47.6	47.0
LA	7.6	6.2	7.0	7.7	5.2	8.4
IMC	4.5	5.2	5.1	5.2	5.6	4.4
L1trs	29.8	27.9	29.2	26.7	26.9	27.3
L1.2	20.3	22.9	19.3	25.2	26.3	21.6
TH	162.5	169.1	180.3	167.4	174.4	157.7
FD	23.9	21.3	20.0	21.3	21.9	24.0
MD	84.1	80.9	79.6	81.5	81.4	84.3
Count	33.0	25.0	19.0	80.0	18.0	64.0

L, a, b, c, h : Color values, NF : Number of Flower, NFr : Number of fruit, FS : Fruits set (%), FL : Fruit length, FW = Fruit width, FL.FW : Fruit length-to-width ratio, NT : Number of truss, F : Firmness, NL : Number of locule, PT : Pericarp thickness, Fwe : Fruit weight, Y : Yield, LL : Leaf length, LW : Leaf width, LA : Leaf attitude, IMC : Immature fruit color, L1trs : Stem length up to the first truss, L1.2 : Stem length between 1st and 2nd trusses, TH : Total Height, FD : Days to the first flowering, MD : Days to the first maturity.

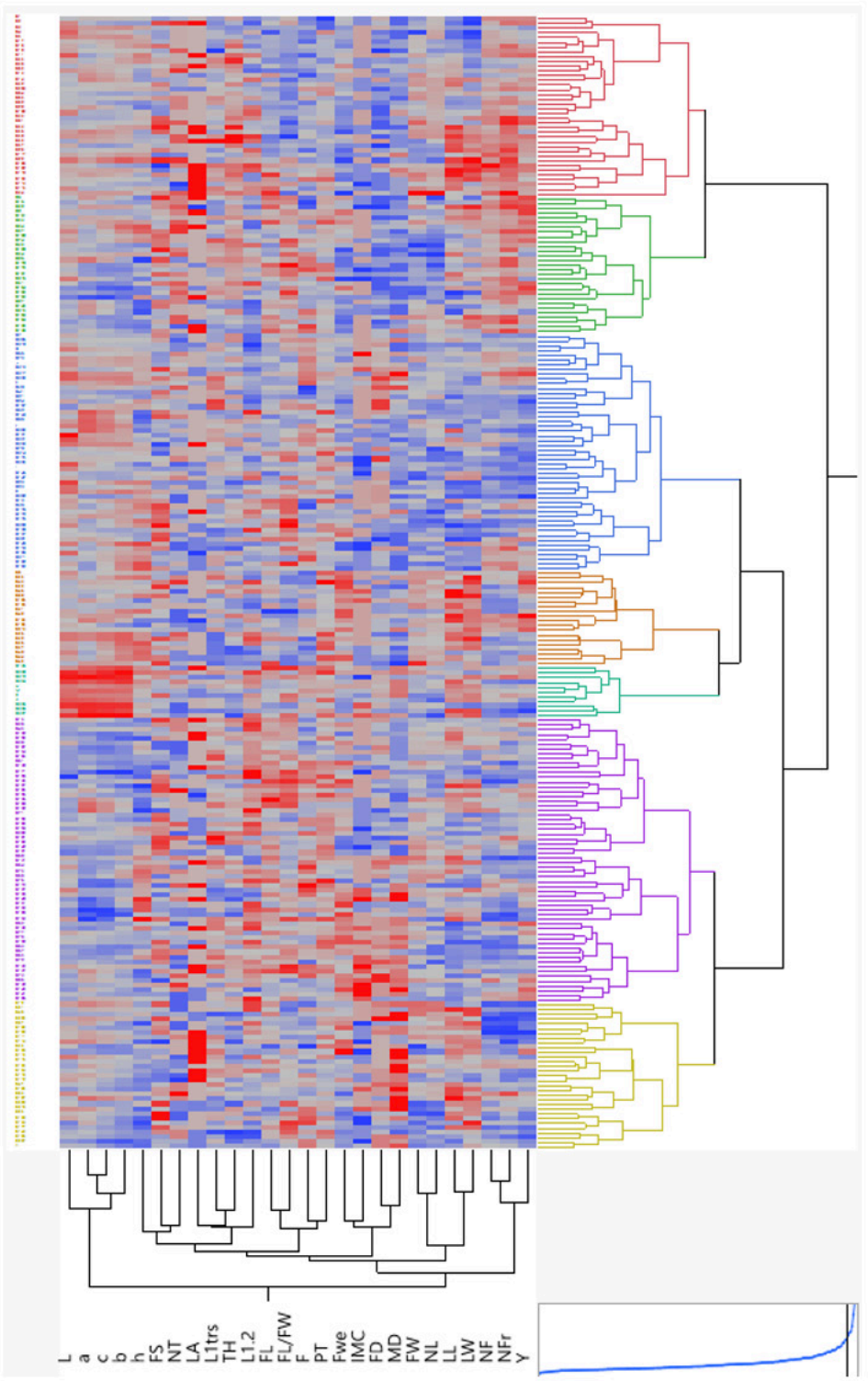


Figure 4. Two-way hierarchical clustering analysis for Location 2.

Table 6. Mean values of agro-morphological traits in different clusters of genotypes of beef tomato type in Location 2

Cluster	1	2	3	4	5	6	7
L	35.0	34.3	35.3	34.9	36.9	33.6	34.3
a	32.2	31.3	32.2	32.2	37.2	30.3	32.1
b	29.5	27.9	29.9	30.6	35.1	27.5	27.7
c	43.7	42.0	44.0	44.5	51.2	41.0	42.4
h	42.4	41.7	42.8	43.4	43.3	42.2	40.8
NF	48.9	47.3	39.5	39.0	39.6	38.6	36.5
NFr	38.2	38.7	31.2	31.3	31.3	30.2	28.6
FS	78.6	82.0	79.4	80.8	78.9	79.0	79.1
FL	61.1	64.1	61.7	62.2	63.1	65.2	61.9
FW	81.4	77.3	76.0	83.2	77.5	80.2	77.9
FL/FW	0.8	0.8	0.8	0.7	0.8	0.8	0.8
NT	7.1	7.2	6.7	6.7	6.6	6.8	6.7
F	20.5	22.5	22.6	20.3	23.6	23.3	22.2
NL	5.2	3.9	4.1	5.1	4.0	4.4	4.7
PT	8.5	9.4	9.3	9.8	10.0	9.7	9.3
Fwe	203.1	208.4	210.9	252.5	214.7	234.5	218.6
Y	7696.3	8044.7	6554.3	7914.6	6713.5	7033.1	6176.4
LL	36.8	35.4	33.6	38.4	37.3	35.0	37.2
LW	39.0	39.9	36.4	45.0	42.3	38.4	42.2
LA	7.6	7.0	6.3	6.3	6.4	6.8	7.1
IMC	3.9	4.0	4.0	4.7	4.6	4.9	3.6
L1trs	31.4	31.0	31.5	29.4	30.5	32.9	30.7
L1.2	25.0	23.0	23.3	24.1	26.5	27.4	24.1
TH	184.8	179.4	171.5	168.8	166.1	174.2	166.1
FD	31.8	31.2	34.0	32.7	33.8	33.9	34.7
MD	99.0	98.0	99.4	99.0	102.6	101.3	105.7
Count	38.0	29.0	50.0	20.0	11.0	60.0	31.0

L, a, b, c, h : Color values, NF : Number of Flowers, NFr : Number of fruits, FS : Fruit set (%), FL : Fruit length, FW = Fruit width, FL.FW : Fruit length-to-width ratio, NT : Number of trusses, F : Firmness, NL : Number of locules, PT : Pericarp thickness, Fwe : Fruit weight, Y : Yield, LL : Leaf length, LW : Leaf width, LA : Leaf attitude, IMC : Immature fruit color, L1trs : Stem length up to the first truss, L1.2 : Stem length between 1st and 2nd trusses, TH : Total Height, FD : Days to the first flowering, MD : Days to the first maturity.

DISCUSSION

The desired genotypes should be compact, open plant, early, firm, and fast ripening, have a low light tolerance, a good fruit quality, deep red color, and a long shelf-life and fruit weight should be at least around 200-220 grams. The analysis of agro-morphological traits enables to describe the variability between different genotypes (Figas et al., 2015). The results of descriptive statistics showed that there was an important level of diversity among genotypes evaluated. Variability was high especially for number of flowers, ratio of fruit set, fruit weight, yield and height in both locations. Even though number of flowers was similar in both locations, the ratio of fruit set was higher in Location 2, so yield was higher in this location compared to Location 1. The lower yield in Location 1 may be associated with the fact that there were water and/or nutrient deficiencies such as phosphorus, zinc and boron during fruit set as also revealed by Wang et al., (2017). As well as the fruit set in the upper trusses was less in both locations; this was probably

because of high daily mean temperature (Sato et al., 2006). Optimum temperature for fruit set is between 20-24 °C, (Charles and Harris, 1972; De Koning, 1994), a temperature higher than 35 °C was observed in both locations. The highest number of fruits was found in G34 genotype for both locations and this genotype had the lowest fruit weight in both locations. Genotypes like G34 may be more stable for yield across two regions of Antalya. Transplantation of tomato seedlings was done one month earlier in Location 2 than Location 1. As climatic conditions were better during the time of transplantation in Location 1 than Location 2, number of days to the first flowering and maturity occurred in a shorter time in Location 1 even though transplantation was done almost a month ago in Location 2. The colder climatic condition below 10 °C in Location 2 may affect also fruit set (Picken, 1984) and cause slightly damages like catface on a few fruits belonging to trusses in the middle part of the plants and these fruits remain smaller. Although the cold affected fruit set in Location 2 at a particular time, it had still a higher ratio of fruit set in this location than Location 1. The use of augmented randomized complete block design (ARCBD) in this study enabled to make comparisons between newly tested genotypes and control varieties. The result of ANOVA showed the genotypic variability and a high level of heritability for many traits for both locations (Table 2) indicated that this diversity could be maintained in different environmental conditions. This is useful in the selection of the well-adapted and best performing genotypes. However, the broad-sense heritability for yield for each location was not high because of environmental effect. Avdikos et al., (2011) and El-Gabry et al., (2014) also reported the same result showing that yield was influenced more by environment as it is a very complex trait and therefore it did not have a high heritability. Combinations of high temperature with other factors like high humidity due to climate change can also affect the fruit set (Hanson et al., 2002) and increase the need of irrigation and clearly affects the yield changes. While fruit weight showed a high heritability in Location 2, it showed a low heritability in Location 1. This indicated that Location 2 had more stable greenhouse conditions in terms of fruit weight, and this may be due to differences in application of fertilizer and excessive application of fertilizer in case of sufficient nutrients and due to the fact that it caused the reduction in production as also indicated by Sainju et al., (2003). A low level of heritability was found for fruit width and hue value in both locations, thus demonstrating that these traits were not useful for the selection. Ortiz and Izquierdo (1994) also found stability changes in different traits. The small changes in agronomic practices may also differ the estimation of environmental effect. Principal component analysis (PCA) demonstrated that PC1 and PC2 accounted for around 15.6% and 13.7% of total variation and 13.8% and 11.8% of total variation in Locations 1 and 2, respectively. According to the PCA, the variability for Location 1 was obtained by number of flowers, number of fruits, yield and total height; and fruit length, fruit width, number of locule, fruit weight accounted for the variability in Location 2. Number of fruits, and b* and c* values made the highest contribution to help the variation for both locations. The first five principal components for all traits accounted for 54.23% and 48.18% of total variation. This result was lower in comparison with similar studies (Cortés-Olmos et al., 2015; Renna et al., 2019). Cortés-Olmos et al., (2015) found that the first two principal components accounted for 71% of the total variation in the characterization of 166 traditional tomato varieties and Renna et al., (2019) also determined that the first three principal components accounted for 79% of the total variation in the evaluation of three local tomato varieties. As all the tomato genotypes were the same variety, namely beef and specific to spring growing season and market, variability shown by multivariate principal component analysis was lower in contrast to the other studies done with core collections, landraces, or local varieties probably due to locations used, different genotypes, and number of genotypes. Cluster analysis revealed that all studied traits enabled to divide clusters into groups and traits' mean values in different clusters showed what genotypes became prominent with which traits. Yield has always been considered as an important trait and one of the main interests for growers and breeders, too. Two main traits determining average yield per plant are number of fruits and fruit weight which were grouped in Cluster 3 and Cluster 6 as a superior trait for these clusters for Location 1, respectively. As yield is one of the important selection criteria, common genotypes present in these clusters for both locations would probably be the potential genotypes for further evaluations. Fruit size and fruit quality traits were the more effective discrimination criteria as they affect the marketability of variety.

CONCLUSION

The present study showed that commonly used conventional agro-morphological descriptors provided detailed information of the tomato hybrid type "Beef" in two locations. This feature of the descriptors proved their importance for the characterization and evaluation of diversity. Even though there was no clear separation for some of the traits between the genotypes, some of the agronomically important traits like number of fruits, fruit weight, yield and fruit quality provided variability between genotypes. Phenotypic and statistical evaluation of genotypes revealed that some of the genotypes showing high adaptability demonstrated acceptable performances in terms of yield and fruit quality in both locations. The selected genotypes could be evaluated in multi-environmental conditions to figure out whether they are a good candidate to be released as a new variety. The two main concerns of today are climate change and population increase. Tomato (*Solanum lycopersicum*) is one of the most consumed vegetable crops in the world; therefore, grower needs high-yielding varieties with a good adaptation to different environmental

conditions for the compensation of global market needs. The present study also provided an estimation to plan for future breeding strategies by showing the positive and negative sides of developed hybrids, and gave opportunity to make better combinations of parents.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

This article is derived from Ali ÜNAL's Master thesis. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Occupational health and safety in flour Mills: A research and risk assessment

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Abstract

Flour mills are one of the workplaces with occupational health hazards due to irregular workplaces and environments where insufficient attention is paid to workers' safety. In this context, considering the extent of difficulties faced by workers in flour mills, hazards and risks that could affect the health and occupational safety of workers were investigated in a sample of flour mills in Iğdır province. The data obtained as a result of the research was ranked using the matrix method. The study identified 68 specific risks, of which 38% were identified as unacceptable risks requiring immediate action and 33% as risks requiring medium-term action. The results show that among the factors negatively affecting the health of workers, the respiratory hazard level of wheat dust with RS:15 (l:3, s:5) and the explosion hazard with RS:20 (l:4, s:5) occupy significant positions. Despite the emphasis on the importance of hygiene standards, serious deficiencies were identified, such as RS:20, which is considered high risk. In addition, the presence of many machines, such as conveyors, silos, compressors and rollers, was found to increase the risks on the system in the range of RS:20-25, which could lead to serious injury or death. Bacteria and microorganisms, such as RS:16-18, proliferate due to the low availability of personal hygiene facilities in the factory. For each identified risk, the necessary control measures are proposed and it is emphasized that protective measures should also be taken even after the implementation of these measures. Among the factors that negatively affect the health of workers in flour mills are the lack of use of personal protective equipment, working in dirty conditions, the presence of elements that can cause respiratory problems, as well as injuries related to carrying heavy loads on slippery floors and using unprotected machinery.

Keywords: Flour mill, Occupational health and safety, Hazard, Flour dust, Hazards

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INTRODUCTION

The history of milling probably dates back to prehistoric times. Modern flour milling systems, as we know them today, have a history of about 250-300 years. Flour, defined as the ground particles of grains and legumes, is considered a hazardous substance by the Health Safety Executive (HSE). The American Conference of Governmental Industrial Hygienists (ACGIH) defines flour as a complex organic dust and recommends that it be kept below a threshold limit value (TLV) of 0.5 mg/m3 in the workplace (Kakooei and Marioryad, 2005).

Flour dust, like many other organic substance dusts, is a harmful and potentially hazardous material (El Karim et al., 1986). It is known to cause occupational asthma and can sensitize the respiratory tract, leading to reactions such as allergic rhinitis (Smith and Lumley, 1996). It can trigger asthma attacks in people

with asthma and can lead to chronic bronchitis. It also has irritating properties and can cause short-term respiratory, nasal, and ocular symptoms (Ajeel and Al-Yasin, 2007). In order to mitigate these harmful effects, occupational exposure limits have been accepted in work environments ranging from 0.5-10 mg/m3 for an 8-hour shift (Kakooei and Marioryad, 2005). The maximum exposure limits are set at 10 mg/m3 in the United Kingdom and the United States, while Italy sets it at 0.5 mg/m3 (REF). In our country, according to the dust control regulations published in the appendices, the threshold limit value (TWA) for grain dust is set at 10 mg/m3 (Babel & Rajvanshi, 2013).

In the last five years, 17 people have lost their lives in our country due to work accidents in the production of ground cereals and vegetable products. The average number of work accidents in this sector between 2018 and 2022 was 734, while in 2021, three people were diagnosed with occupational diseases (SGK, 2023). Occupational disease is a temporary or permanent illness, physical or mental disability that occurs in the course of the employee's work or due to a recurring cause arising out of the nature of the work or working conditions (Horozoğlu, 2017).

The flour manufacturing process, classified as "Hazardous" in the "Regulation on Workplace Hazard Classes Related to Occupational Health and Safety", generally consists of stages such as raw material supply, storage, cleaning, washing, tempering, grinding, sieving, and storage. Throughout this process, areas where grinding occurs, called rollers, transfer screws for product transfer between processes, cleaning with sieves, distribution, and washing sections, are areas where vibration and noise exposure are particularly intense (Ali & Mohamed, 2023). However, the presence of many machines operating mechanically and electrically, along with personnel movement in the workplace, brings along a series of risks. During this process, especially physical hazards arising from machine movements and electrical-related risks are significant. Additionally, there is a risk of workers being exposed to health issues such as dust particles, skin irritation, and respiratory tract infections (Tiikkainen et al., 1996). Companies should identify potential hazards, take appropriate precautions, and train and inform workers. Furthermore, regular maintenance and inspections are crucial.

In the crushing, grinding, sieving, and washing sections where flour mill production takes place, workers may experience problems such as hearing loss due to excessive noise. Measurements taken at these points have shown that the noise level is well above the legal limits (Yağmur, 2016). Similarly, vibration exposure in these areas exceeds action levels and will adversely affect workers. Those working in enclosed spaces are exposed to these effects throughout their working hours. However, workers outside the production area are distant from this effect.

The manufacturing sector, due to its high workload, requires more attention to occupational health and safety (Tekin & Rizvan, 2016). Research shows that there are more workplace accidents in the manufacturing sector than in the service sector (Çalış & Büyükakıncı, 2021). These bitter experiences better illustrate the importance of sensitivity in the manufacturing sector. The accidents that can occur on a production line can cause larger and more serious problems than in other work environments, such as offices; however, the criteria for risk assessment may also change, taking into account the frequency and likelihood of accidents.

The occurrence of work accidents and occupational diseases in workplaces has significant destructive effects both morally and economically. Due to factors such as the unsuitable nature of the ambient air and the variety of machinery and equipment used, flour mills are considered hazardous workplaces where workers face a high probability of occupational accidents. With this aim, to evaluate the risks arising during the work conducted in the sampled factory, whether the measures taken to ensure the health and safety of mill workers are adequate and meet legal requirements is examined. Are workers exposed to particles, gases, fumes, and vapors in the air? How do workers perceive occupational health and safety measures? Are there any risks associated with the tools, machinery, or equipment used, and what measures are taken against these risks? To what extent are workers exposed to thermal comfort conditions? Are workers exposed to excessive noise or vibration in the workplace? Are there any chemicals in the workplace that could cause illness or health problems for workers? These questions have been investigated.

The prioritization and treatment of risks was linked to the implementation of identified control measures and the impact of these measures was discussed. Implementing control measures includes improving work practices, enhancing communication, supporting education and training, and ensuring supervision and maintenance. Improving work methods assists in making workplace activities safer, while communication aims to promote clear and effective communication among employees. Education and training activities ensure that employees acquire the necessary knowledge and skills to recognize risks and apply control measures correctly (Şerifoğlu & Sungur, 2007). Supervision involves monitoring the effectiveness of control measures in the workplace and taking corrective actions when necessary. Maintenance contributes significantly to occupational safety and worker health by ensuring regular maintenance of equipment and tools used.

MATERIALS AND METHODS

Quantitative or qualitative methods traditionally used in risk assessment methodologies are tending to be replaced by hybrid methods in modern times. Among the many methods, which can be expressed in hundreds of ways, risk assessment decision matrices are the most popular because of their broad and easy-to-understand application areas (Gul & Ak, 2018). The method, which aims to rank risks, risk sources, or risk treatments according to risk level, was initially used in military systems and later became widespread, being preferred primarily in many sectors (Bakx & Nyce, 2017).

The Severity-Likelihood matrix is a method that combines qualitative or semi-quantitative results with probability rankings to determine risk levels or risk assessments. These matrices allow us to analyze the relationship between two or more variables through visual diagrams. In addition, they help identify factors that influence the problematic events and understand the relationship between those factors and the events, as well as aid in problem solving. The format and definitions of the matrix vary depending on the context of use, and selecting an appropriate design is critical (Korshunov et al., 2020). The risk posed by workplace accidents is defined as the combination of the probability of occurrence in the risk matrix and the severity of the consequences. The severity-likelihood matrix is used to rank risks or assess risk sources and treatments according to risk levels. When various risks are identified, it serves as a common screening tool to determine which risks require more detailed analysis, which risks should be prioritized, or which risks should be reported to senior management (Oliveira et al., 2018). Toward these objectives, two different risk assessment decision matrix methods are used: X-type and L-type. In this study, the L-type matrix was preferred because it contributes to understanding complex issues and supports multidimensional thinking. This method also aids in identifying factors contributing to or affecting the problem or event and determining their relationship. Its prominent advantage is graphically representing the degree of relationship between each variable. For the effective application of this method in risk analysis processes in complex work environments, the competence of a single person is not sufficient. Conducting studies requires disciplined teamwork under the leadership of an experienced team leader. The research has adopted a comprehensive approach to ensure the effective implementation of the risk management process, which begins with the identification of potential hazards. The process of identifying hazards and analyzing them is a crucial step in determining potential risks (Lyon & Hollcroft, 2012). The potential impact of each hazard and the size of the risk have been meticulously evaluated (Figure 1). Subsequently, measures and strategies necessary to minimize or eliminate these risks have been identified.

After potential hazards are identified in the hazard list recorded in the work log, the detailed identification of the risks they pose begins (Wijeratne et al., 2014). Each risk should be subjected to evaluation using the following methods:

Likelihood of the risk occurring: The likelihood of the risk occurring is assessed and assigned to one of five different probability categories.

Level of damage caused by the event: The level of the risk is assessed and assigned to one of five different impact categories.

Risk Level: The level of risk is calculated by multiplying the probability of the risk occurring by the impact of the risk.

As a result of this calculation, it is determined whether the risk is low, medium, or high. The risk level is equal to the product of the probability of the event occurring and the severity of the event (RS = I x S, RS: Risk, I: Likelihood, S: Severity).

While the matrix technique can be used for risk assessment, other techniques can also be used. The matrix technique visualizes potential hazard levels, making them easier to comprehend. However, it's important that the method used for risk assessment aligns with the characteristics and needs of the workplace. Expert opinions and necessary teamwork contribute significantly to assessing the hazards identified, converting them into risks, and determining the probability and magnitude of the resulting harm. The obtained values are transferred to the risk assessment matrix. Based on the scoring in the matrix, a risk map is obtained, ranging from highest to lowest priority. Risks that are high and require immediate attention for treatment are marked in red, risks that are planned to be addressed in the near investment periods, considering the medium-term or financial situations of the business, are considered notable risks and marked in yellow, while risks that do not require urgent action are marked in green (Lindholm et al., 2022).

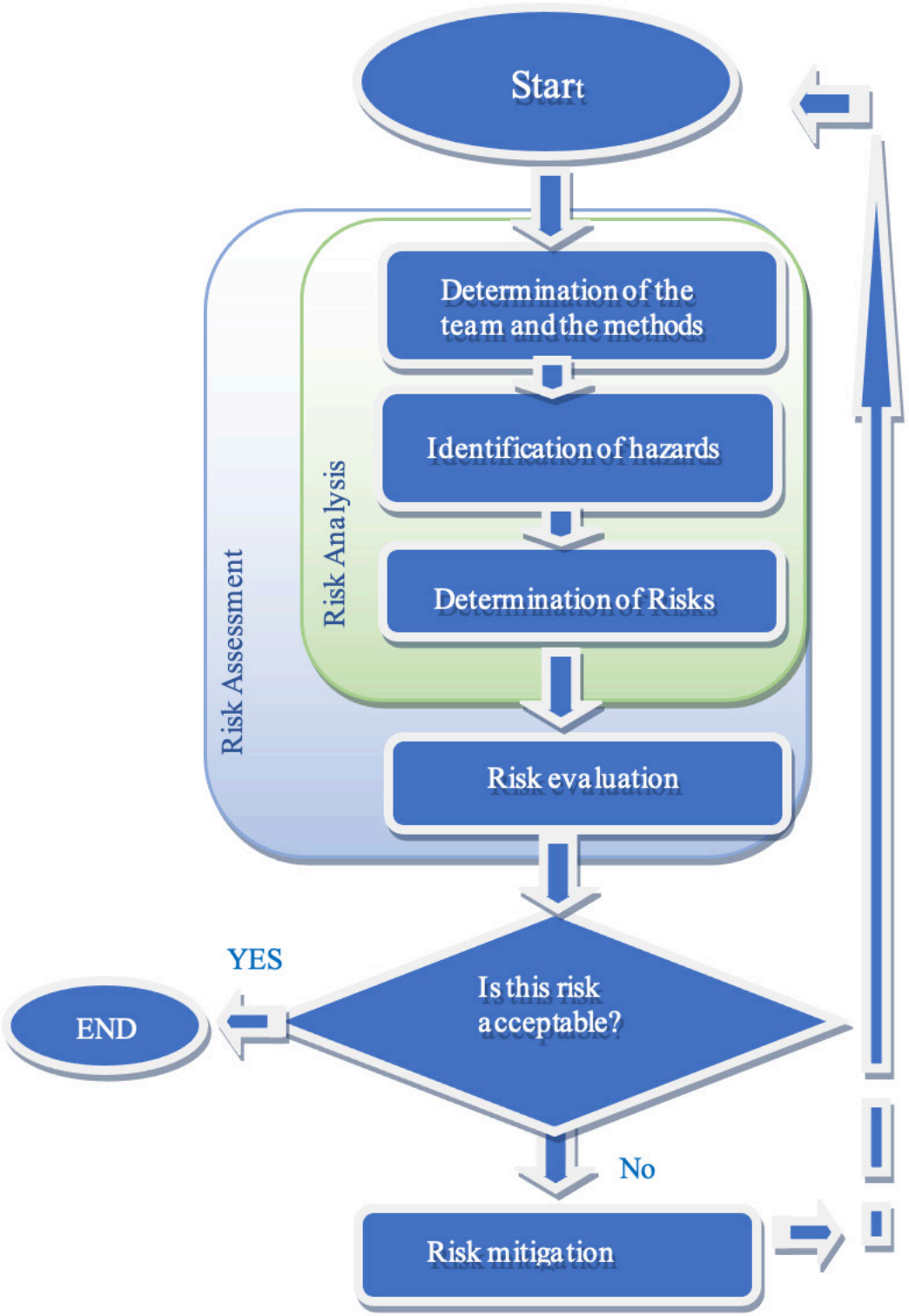

Figure 1. Risk assessment stages

Table 1. Severity and likelihood matrix

Severity			Likelihood			Likelihood	Severity				
Severity		Rating	Likelihood	Rating			1	2	3	4	5
1	Considerable	No Loss of Work Hours, No First Aid Required	1	Very low	Once a year	1	1 Very Low Risk	2 Low Risk	3 Low Risk	4 Low Risk	5 Low Risk
2	Important	No Loss of Working Days, No First Aid Required	2	Low	Once 3 months	2	2 Low Risk	4 Low Risk	6 Low Risk	8 Signi. Risk	10 Signi. Risk
3	Serious	Minor Injury Requires Treatment	3	Moderate	Once a month	3	3 Low Risk	6 Low Risk	9 Signi. Risk	12 Signi. Risk	15 High Risk
4	Very Serious	Death Serious Injury, Occupational Disease	4	High	Once a week	4	4 Low Risk	8 Signi. Risk	12 Signi. Risk	16 High Risk	20 High Risk
5	Catastrophe	Multiple Deaths, Permanent Disability	5	Very High	Everyday	5	5 Low Risk	10 Signi. Risk	15 High Risk	20 High Risk	25 Very High Risk

RESULTS AND DISCUSSION

The process in the flour mill where the study is conducted involves various stages from the procurement of wheat to the packaging and shipment of flour. This process involves several critical steps. First, starting with the procurement of wheat, the moisture content, temperature, and storage conditions of the product are meticulously controlled. This is essential to ensure the quality of the wheat, which is stored in low humidity, dry, and well-lit environments (+4 ºC). This stage also includes the cleaning and separation of foreign materials in the wheat, such as pieces of metal, dust, soil, and plant debris. This is followed by a stage called "blending", in which the same type of wheat is subjected to a homogeneous milling process, improving the quality and consistency of the final product. The wheat, free of foreign matter and impurities, is washed to obtain a more homogeneous product. Then, through the "clean wheat tempering" process, the husk is removed to obtain the highest quality flour. This stage is critical to improving the quality of the flour and achieving the desired product specifications. Finally, the wheat is ground. This complex process is carried out through a system integrated with rollers, sieves, and other machines. Sieves are used to separate and classify the materials ground in the rollers. The final product is packaged in a warehouse with suitable storage conditions and prepared for shipment.

Workers in flour milling are exposed to many hazards, with major risks including electricity, careless behavior, rotating machinery, and exposure to flour dust, which can lead to workplace accidents or occupational diseases. During the preliminary hazard analysis, risks were addressed in three separate sections: general factory operations, production, and other departments. The presence of three separate electrical panels in the factory and multiple splitters connected to sockets, especially, poses a high risk with a probability of occurrence (P) of 1:5 and severity (S) of 5, resulting in a high-risk score (RS) of 20 (Table 2). This poses a serious risk of death or disability for users. Therefore, in electrical work, open and un-insulated elements should be checked and corrected, proper grounding should be done in compliance with regulations, electrical panels should be regularly maintained by qualified personnel, warning signs should be placed, and insulating mats should be placed in front of them (Tosun, 2022). Lightning protection systems should be installed according to the project of the installation to eliminate the risk of RS:20 from lightning strikes and checked annually. The resistance of the lightning protection grounding system should not exceed 10 ohms (ETTY, 2001).

In emergencies such as fire, it is necessary for fire extinguishers to be of sufficient quantity and easily accessible, eliminating any barriers to intervention. The identified deficiency regarding the insufficient number of fire extinguishers has been classified as a risk class that requires immediate intervention with an RS:20 score (Table 2). Fire extinguishers and, when necessary, fire detectors and alarm systems will be present in closed and open areas of the workplace with effective and adequate fire extinguishing equipment based on the size of the workplace, the nature of the work performed, the physical and chemical properties of the substances used, and the number of employees (Şimşek & Aydoğdu, 2020). Fire extinguishing equipment will be easily usable, placed in visible and easily accessible

locations, and free from obstacles in front of them. Fire extinguishing devices will be marked in accordance with the Safety and Health Signs Regulation, placed in appropriate locations, and permanent (SGİY, 2012).

The inadequacy of signs indicating prohibition, warning, command, escape route, or firefighting equipment in emergencies results in a high-risk value (P: 3, S: 4, RS: 12) (Table 2). For this issue, signs should be made of sufficient quantity, suitable for the environment in which they are used, and made of impact and weather-resistant materials. The dimensions, colorimetric, and photometric properties of the signs will ensure that they are easily visible and understandable (SGİY, 2012).

The inadequate ventilation and hygiene conditions of shower cabins will lead to the proliferation of microbiological organisms, thus posing a risk of occupational diseases (P: 3, S: 3, RS: 9) (Table 2). These conditions will be prevented by suitable ventilation with aspiration and ventilation systems to prevent odor and dirt. Shower cabins will be adequately heated according to the season, with the temperature not falling below 25°C, and provided with hot and cold running water, clean towels, and bathrobes provided by the employer, stored in special cabinets. Used towels and robes shall not be used by others until they have been washed, dried and thoroughly cleaned. Workers' lockers will be locked, and their cleaning will be carried out according to guidelines prepared by the employer. In areas where toxic, hazardous, dusty, and dirty work is performed, employees should be provided with two separate lockers for storing work clothes and external clothing. Maintenance of dressing rooms, wardrobes, and lockers will be carried out by the employer, and employees should not be allowed to work with wet clothes (Eser, 2015).

Among the identified deficiencies in the workplace is the lack of regularity in employees' night shifts and inadequate rest periods, resulting in a risk of accidents and illnesses (L: 3, S: 5, RS: 15). The maximum duration of work in night shifts should be 7.5 hours, and employees should be scheduled to work during the day in the second work week after working at night for a maximum of one work week. During shift changes, workers should not be required to work continuously without being rested for at least eleven hours (İş Kanunu, 2003).

The absence of a sufficient number and quality of emergency exit doors that enable all employees to immediately and safely evacuate the workplace in any hazardous situation poses a risk of injury and death in emergencies (L: 4, S: 5, RS: 20) (Table 2). Emergency exits and doors should open directly and unobstructed to the outside or to a safe area, and the number, size, and location of doors should be appropriate to the nature of the work, the size of the workplace, and the number of employees. Additionally, emergency exit doors should not be locked or obstructed (BYKHY, 2007).

The neglected condition of fuel tanks poses a risk of fire or explosion (L: 3, S: 5, RS: 15). These tanks should be securely placed on solid bases and surrounded by suitable safety walls. In the event of a fire, there should be remotely controlled fire extinguishing systems and pressure valves that open and close automatically in response to pressure changes.

The failure of employees to receive occupational health and safety training is a significant risk with an RS value of 12. This training should be provided particularly before starting work, when there is a change in workplace or job, when there is a change in work equipment, or when new technology is implemented. Trainings should be updated in accordance with new and emerging risks and should be repeated at regular intervals as necessary. To reduce the risks associated with confined spaces with an RS value of 6, adequate fresh air supply should be ensured based on the nature of the work. When using forced ventilation systems, the system should always be operational. The airflow should not disturb the workers. Thermal comfort conditions should be appropriate for the nature of the work, intended use, and the energy expended by the workers; otherwise, it may lead to significant consequences, including workday losses due to the risk of work accidents with an RS value of 6.

Due to the construction style of the workplace, adequate utilization of daylight is not achieved, which may lead to work accidents and, at the same time, result in vision impairments for continuous workers. Lighting systems in work areas and passageways should be of a type that does not pose a risk of accidents for workers and should be appropriately positioned. In areas where any malfunction in the lighting system could pose a risk to workers, there should be backup lighting systems to provide emergency and adequate illumination. In workplaces, floors and surfacing are sound, dry, and as flat and non-slippery as possible, with no dangerous slopes, pits, or obstructions (YİİSGY, 2013). Floor coverings and coatings in workplaces will be made of materials suitable for cleaning to ensure proper hygiene conditions.

Arrangements will be made to ensure the safe movement of pedestrians and vehicles in both open and closed work areas. There will be adequate distance between roads open to vehicle traffic and pedestrian walkways, as well as between gates and pedestrian crossings. Considering the nature of the work being carried out in work areas, and taking into account machinery and materials, pathways for vehicle passage will be clearly marked to protect workers. Access to hazardous areas where there is a risk of falling materials or workers due to the nature of the work will be prevented using appropriate tools and equipment. Measures will be taken to protect individuals authorized to enter hazardous areas, and these areas will be clearly identified. Personnel who do not obtain a health report from the

occupational health physician cannot be employed. Those entering the workplace must undergo periodic health checks every three months, and the results must be recorded in health reports. Individuals found to be carriers during health checks must receive immediate treatment. Those who have completed treatment but have not received a clean bill of health cannot be employed. Individuals with febrile illness, skin disease, or diarrhea must be immediately sent for examination at a healthcare facility.

Table 2. Risks present throughout the factory

Hazard	Risk	Consequences	I	S	RS
Electrical Panel and Installation	Electrical leaks, electrocution	Injury, death, property damage	4	5	20
Lack of fire extinguishing systems	Failure to intervene in fire	Injury, death, property damage	4	5	20
Fire extinguishing systems not easily accessible	Failure to intervene in fire	Injury, death, property damage	4	5	20
Lack of maintenance of fire extinguishing systems	Failure to intervene in fire	Injury, death, property damage	4	5	20
Lack of lightning system (lightning rod)	Lightning	Injury, death, property damage	4	5	20
Failure to perform periodic checks of the lightning system (Lightning Rod)	Lightning strike, fire, explosion	Injury, death, property damage	4	5	20
Lack of warning and warning signs and signs	Increase in work accidents	Injury, death, property damage	3	4	12
Showers—Sinks-Toilets	Increase in occupational diseases, disease transmission from various bacteria and harmful microorganisms	Infectious diseases	3	3	9
Changing areas and wardrobes	Increase in occupational diseases, disease transmission from various bacteria and harmful microorganisms	Infectious diseases	3	3	9
Shift Work	Work accident due to insomnia, fatigue, careless work	Injury, death, property damage	3	5	15
Inappropriate Emergency exit routes and doors	Workers' inability to leave the danger zone	Injury, death	4	5	20
Fuel tank	Fire, explosion	Injury, death, property damage	3	5	15
Lack of Occupational Health and Safety training for employees	Increase in work accidents	Injury, death, property damage	3	4	12
Inadequate ventilation	Work accidents that may occur as a result of work stress, carelessness, and depression	Injury and various health problems	2	3	6
Inappropriate Ambient Temperature	Work accidents that may occur as a result of work stress, carelessness, and depression	Injury, loss of working days, material damage	2	3	6
Insufficient Lighting	Insufficient visibility, increase in work accidents	Injury, death, property damage	4	4	16
Slippery ground	Slip - fall	Injuries, fractures	2	3	6
Movement of Pedestrian and Vehicles	Impact, crush	Injury, death	3	4	12
Lack of health checks on employees	Increase in occupational diseases	Infectious diseases, respiratory diseases	3	3	9

The continuous operation of the existing electrical panel in the production area with connections left open poses significant risks (l:4, s:5, RS:20) (Table 3). Attention must be paid to ensuring that the panel's periodic maintenance, operating instructions, and necessary precautions and labeling comply with regulations. Electric motors operating with alternating voltage potentials of 230 volts or higher must be located in special motor rooms or at least 3 meters

above the ground or within an enclosure system. Motors that do not meet these conditions and specifications will be appropriately protected. Distribution panels, control equipment, and similar installations located inside workshops or accessible to workers will be placed in locked cabinets or enclosures, or their bases will be covered with non-conductive material (Mutlu & Çabuk, 2021). When isolating live equipment for maintenance and repair, the use of screens or protectors to cover these sections eliminates many risks.

According to regulations, effective and sufficient fire extinguishing equipment, as well as fire detectors and alarm systems when necessary, must be present in the workplace based on its size, the nature of the work performed, the physical and chemical properties of materials used, and the number of employees. However, the lack of sufficient fire extinguishing equipment in visible and easily accessible locations without obstacles in the existing facility poses a high risk (l:4, s:5), reaching an RS:20 level, in emergency situations (Table 3). Another crucial aspect is ensuring that fire extinguishing devices are appropriately labeled in accordance with the Safety and Health Signs Regulation, placed in suitable locations, and remain permanent.

It is possible to say that there are significant deficiencies in hygiene in the workplace. These deficiencies stem from both the educational levels of the employees and their social environments. Especially, attention is not paid to body, hand, and face hygiene throughout the day. The current situation poses a high risk of causing diseases (l:3, s:3), reaching an RS:9 level, and requires urgent action (Table 3). The importance of washing with water and soap should be emphasized in training, followed by the application of suitable antiseptics to the hands. The recommended amount of hand antiseptic (3-5 ml) should be applied to both hands and rubbed until dry. Hand sanitizing, like washing with water and soap, aims not only to reduce dirt and bacteria by mechanical action, but also to kill bacteria or prevent their reproduction by chemical action. In other words, they have bactericidal and bacteriostatic effects. For this purpose, hand-operated, elbow-operated, or sensor-operated disinfectant dispensers should be used (IHOTAHY, 2023).

The machines used in the operation are constantly at risk due to some of their feeds being exposed and not isolated (l:3, s:4, RS:12) (Table 3). Firstly, the wiring in the installation must be insulated within the channel. The body grounding of the machines must be done without fail.

The existing guards on the machines are either broken or missing, which indicates a risk that requires action (l:3, s:4, RS:12). According to our regulations, machine guards used in the workplace must be structurally sound, not create additional hazards, not be easily removable or rendered ineffective, be at a sufficient distance from the hazard zone, not obstruct the view of the equipment's operating points, only restrict access to the area where the operation is performed, and allow for the installation, removal, and maintenance of parts without their removal (MEY, 2009).

The ceiling-mounted motors in the facility create a risk of oscillatory movement (l:3, s:4, RS:12) while also generating significant noise due to being unsecured (Berberoğlu et al., 2002).

All moving parts of the drive machines along with the transmission mechanisms and all hazardous parts of the machinery and equipment shall be adequately guarded. These guards shall only be removed during inspection, adjustment, maintenance, and repair, and shall be immediately replaced upon completion of the work. In the event of a fault or deficiency in the machinery or its guard, the machine or equipment shall be immediately stopped, and the relevant personnel shall be notified. Measures preventing anyone from working on the machine or equipment with a fault or defective guard shall be taken, and the situation shall be indicated by affixing a sign on them (Ünsar, 2004).

The level of noise exposure experienced by employees, being a significant source of complaints and causing discomfort to individuals, constitutes risks that need to be taken into account (l:3, s:4, RS:12) (Table 3). Employees working in noisy environments should undergo periodic general health examinations. The noise level of the machines in the mills has SPL (sound pressure level) more than 85 dBA (permissible limit by NIOSH) in locations where they were used most of the time (Rawat & Gaikwad, 2020). Employees working in noisy environments should be selected from those who are suitable for working in such environments in terms of health. When noise exposure exceeds the lowest exposure action values, the employer should provide ear protectors readily available for the employees to use (ÇGİRKDY, 2012). When noise exposure reaches or exceeds the highest exposure action values, ear protectors should be used. The employer shall make every effort to ensure the use of ear protectors and shall be responsible for monitoring the effectiveness of the measures taken.

It is noteworthy that the compressors used in the operation are poorly maintained and old, and if precautions are not taken, there is a risk of explosion and fire (l:4, s:5, RS:20) (Table 3). In compressors, when the pressure reaches the set pressure, automatic stopping of the compressor motor should be ensured, and in case of delayed stopping of the motor, a safety device should be available to release the compressed air. The speed governor of air compressors shall be periodically inspected and maintained in good working order, and a mechanism shall be provided to monitor the flow of cooling water therein.

The ducts or pipes used in the aspiration system shall be made of non-flammable material in appropriate sections, and flexible hoses such as spiral or bendable hoses shall be used at portable suction points. Daily maintenance and cleaning of the aspiration system shall be carried out, and a general inspection and cleaning shall be performed every three months, ensuring that the installation characteristics of the system are not altered after repairs. The electric motors of aspirators shall be suitable for the working environment, and in cases where combustible and flammable substances are present in the air being drawn, the motor shall be appropriately mounted or made of a type resistant to such substances.

There should be guardrails to prevent approaching the sieves from more than a certain distance on the sieve floor. No one other than the responsible person should be allowed to enter this area while the machines are running.

Table 3. Risks identified in the production area

Hazard	Risk	Consequences	I	S	RS
System Room	Electrical leaks, electrocution	Injury, death, property damage	4	5	20
Electrical Panel and Installation	Electrical leaks, electrocution	Injury, death, property damage	4	5	20
Control panels	Electrical leaks, electrocution	Injury, death, property damage	4	5	20
Lack of fire extinguishing systems	Failure to intervene in fire	Injury, death, property damage	4	5	20
Fire extinguishing systems not easily accessible	Failure to intervene in fire	Injury, death, property damage	4	5	20
Lack of maintenance of fire extinguishing systems	Failure to intervene in fire	Injury, death, property damage	4	5	20
Lack of warning and warning signs and signs	Increase in work accidents	Injury, death, property damage	3	4	12
Inappropriate hygiene conditions	Bacteria and microorganisms occurring between hands and nails	Infective diseases	3	3	9
Electricity leaks in machines	Electrocution, shock	Injury, death, property damage	3	4	12
Insufficient Machine guards	Piece flying, hand-arm entrapment	Loss of limb, injury	3	4	12
Mill motors fixed to the ceiling	Engine falling to the ground	Injury, death, property damage	3	4	12
Roller mill machine	Hand-arm capture	Loss of limb	4	4	16
Roller mill machine	Exposure to Excessive Noise	Hearing loss	3	4	12
Compressor	Explosion	Injury, death, property damage	4	5	20
Grain Tempering machine	Hand-arm capture	Loss of limb	2	3	6
Pneumatic Fan	Fire -explosion	Injury, death, property damage	3	4	12
Sieve	Falling, Tripping	Injuries, fractures in various parts of the body	2	3	6

The lack of maintenance, cleaning, and operational procedures in the existing boiler room poses significant risks (l:4, s:5, RS:20) in the workplace (Table 4). All boilers used in the workplace must be located in a separate compartment or building resistant to fire and explosion, and no workers should be employed on the floor above the boiler room. Our regulations require boiler rooms in workplaces where explosive, flammable, or highly flammable materials are handled to have windows and doors opening to other workplaces. It is important to ensure that qualified individuals perform the inspection and testing of boilers. Before an employee enters the boiler for cleaning, maintenance, or repair, the blow-off, feedwater, steam, and hot water outlet stop valves, as well as all other valves, must be closed and warning signs placed on them.

A guide should be provided for aligning the truck properly with the wheat unloading section in the raw wheat unloading area, and the truck driver should wait in a safe area outside the vehicle during unloading. Lifting machines and vehicles should be checked before each operation, and operators should control the machine from a safe distance. No personnel should be present in this section during unloading, and the lift should be remotely controlled from outside the unloading area. The regulation states that there should be an aspiration system capable of removing wheat dust

at such points, and personnel should not enter this area until the dust is completely removed from the environment. Compressors should be remotely stopped in case of danger, and safety valves should be installed in the air tanks. Special compressor oil should be used in compressors, and the tanks should be located in a compartment resistant to explosions, or mobile compressors should be located at least 10 meters away or inside a durable compartment. When the pressure is adjusted, the compressor motor will automatically stop, and there will be a safety device to release the compressed air. The speed regulators of compressors should be periodically checked, and clean air intake should be ensured. Periodic checks should be conducted after assembly and repair to ensure the safe operation of compressors, and a pressure test should be conducted at 1.5 times the maximum pressure (BEY, 2018).

Precautions should be taken against the risk of trapping hands and arms (I:3, s:4, RS:12) in elevators used in silos, and employees should be ensured to move at a safe distance (Table 4). Work should not commence if protective parts are removed, and in case of blockage, the movement of the elevator shaft should be completely stopped to rectify the malfunction. There should be a safety mechanism that automatically stops the elevator in case of overheating, and a grounding system should be in place to prevent static electricity buildup. The cleaning of cylinders or drums at the head of belt conveyors should be done using appropriate blades or rotating brushes, and a grounding system should be installed to prevent static electricity buildup. Adequate precautions should be taken for welding and cutting operations, and conveyor mechanisms should be explosion-proof (Karadağ, 2001). Grain silos and warehouses should be equipped with dust and waterproof covers and ventilation systems. Ventilation should be provided inside the silos, and lightning rods and grounding systems should be installed to prevent static electricity or lightning risks. Before maintenance and repair, the power source of the mixing device should be cut off, and personal protective equipment attached to safety belts should be used on silo stairs. Guards should be designed to cover hazardous parts by interrupting all types of contact and should be made of castings, sheet metal, tubing or other suitable materials. Pulleys should not be used if cracked or damaged, transmission belts should be made sound, seamless, and belt joints should be securely fastened. (MEY, 2009).

Work with hazardous chemicals should be carried out in accordance with technological advancements, and appropriate processing, usage, transportation, and storage conditions should be provided based on the size of the workplace, the nature of the work performed, and the characteristics of the substances used (Table 4). Due to the potential risk of accidents, fire, and death associated with working with chemical substances (I:4, s:4, RS:16), adequate firefighting equipment and, when necessary, fire detectors and alarm systems should be provided (Table 4). In addition, appropriate conditions for personal hygiene should be provided, and the conditions for the use and storage of chemicals obtained from manufacturers should be arranged according to the information provided in the relevant forms. (BYKHY, 2007).

It is important for the personnel working in the packaging department to be trained and provided with personal protective equipment to address significant risks (I:3, s:3, RS:9). Transparent and hinged covers should be used to cover the front of the filling pipes on the sack filling machines (Table 4). Suitable work organization and mechanical systems should be used for the transportation of loads without the need for manual handling in the workplace (Özlem & Akalp, 2017). Programs should be tailored to the job and adjustable according to the operator's knowledge and experience, but they should not be changed except by the operator's intervention. Systems should be feedback-controlled to increase the efficiency of workers and provide convenience, delivering information to the operator at suitable speeds and formats. Additionally, importance should be given to the ease of perception and use of data in accordance with ergonomic principles. All these measures will eliminate the possibility of work accidents and provide a safer working environment (Figure2).

The working conditions and unsafe behaviors of employees significantly increase the risk level (I:3, s:5, RS:15) in the loading section (Table 4). Primarily, it is important to ensure that the rotating parts in the working area are enclosed to prevent entry of hands and arms, the cylinders or drums at the beginning of conveyor belts are not cleaned manually but with appropriate guards, openings on roller conveyors are covered with suitable caps, and conveyors are protected when they are in a pit or at ground level. It is necessary to install guards against belt breakage on conveyor belts and replace damaged cables (Horozoğlu, 2017). Proper placement of controls devices, keeping them away from water sources, ensuring system grounding of electrical installations, and providing appropriate personal protective equipment (such as insulating gloves) are important for occupational safety.

In office work conducted without taking occupational safety and employee health measures, especially musculoskeletal disorders can be encountered. Additionally, there are many risks (I:2, s:3, RS:6) that may lead to other occupational diseases and work accidents due to the conditions of the work process (Table 4). The work environment in executive offices presents ergonomic hazards, and symptoms of burnout have been observed among employees. For ergonomic arrangement of the workspace, the keyboard should be separate and movable, wrist support should be provided on the front, the keyboard should have a matte finish, and characters should be arranged to facilitate use.

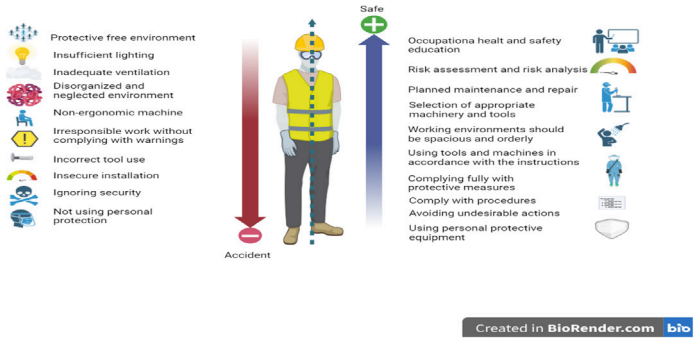

Figure 2. Causes of accidents and prevention of hazards

Table 4. Risks recorded according to fields of activity

	Hazard	Risk	Consequences	I	S	RS
Boiler Room	Inappropriate location of the Boiler Room	Fire, explosion	Injury, death, property damage	4	5	20
	Maintenance-Free Boiler	Fire, explosion	Injury, property damage	4	5	20
	Improper Working Methods	Fire, explosion	Injury, death, property damage	4	5	20
Raw (Wheat) Material Unloading	Truck	Crashing, tipping over, crushing, crushing while the truck approaches the lift	Injury, death, property damage	3	4	12
	Wheat powder	Excessive dust exposure during unloading of raw material	Lung respiratory diseases	3	4	12
	Compressor	Explosion	Injury, death, property damage	3	5	15
Raw Material Storage	Elevator	Hand and arm capture	Limb loss	3	4	12
	Transporter	Hand and arm capture	Limb loss	3	3	9
	Silos	Dust explosion	Injury, death, property damage	2	5	10
	Working at Height	Falling from high	Injury, death	3	5	15
	Blending Machine	Hand and arm capture	Limb loss	3	4	12
laboratory	Chemicals	Fire, explosion	Injury, death, property damage	4	4	16
	Lack of fire extinguishing systems	Failure to intervene in fire	Injury, death, property damage	4	5	20
Packaging	Packaging machine	Hand and arm capture	Injury, limb loss	2	4	8
	Sack Filling Benches	Exposure to excessive flour dust	Lung respiratory diseases	2	4	8
	Manual handling	Physical strains	Musculoskeletal system diseases	3	3	9
	Computer programs in Display Vehicles	Difficulty in perception and use	Various eye diseases	2	3	6
Loading	Belt Carriers	Hand and arm capture	Injury, limb loss	3	4	12
	Belt Carriers	Electric shock	Injury, death, property damage	3	5	15
Administration Building	Electrical Panel and Installation	Electrical leaks, electrocution	Injury, death, property damage	4	5	20
	Lack of fire extinguishing systems	Failure to intervene in fire	Injury, death, property damage	4	5	20
	Fire extinguishing systems not easily accessible	Failure to intervene in fire	Injury, death, property damage	4	5	20
	Vehicles with Display	Detection problems caused by the monitor	Various eye and nerve diseases	2	3	6
	Vehicles with Display	Disorders caused by keyboard use	Carpal tunnel syndrome	2	3	6
	Vehicles with Display	Unsuitable Physical strain caused by the work table and its surface	Musculoskeletal system diseases	2	3	6
	Lack of lightning system (lightning rod)	Lightning	Injury, death, property damage	4	5	20
	Lack of first aid cabinet	Failure to intervene in the injured person in the event of an accident	Injury, death, property damage	3	5	15
	Inappropriate Emergency exit routes and doors	Workers' inability to leave the danger zone	Injury, death	4	5	20

The work table or surface should be adjustable to allow the employee to work comfortably and efficiently without reflecting light. For ensuring safe exits in emergencies, emergency exit routes should directly lead outside, be unobstructed, and appropriately marked (İBEASGÖİY, 2013). Emergency exit doors should not be locked and should be prevented from being obstructed by any barriers. Backup lighting systems should be available in emergency exit routes that need to be illuminated in case of power outage.

CONCLUSION

Increasing awareness of workplace risks and taking appropriate measures to protect the safety and health of employees is crucial within the scope of occupational health and safety efforts. In this study, various hazards faced by flour mill workers and the necessary measures to reduce these hazards have been comprehensively addressed. In addition to potential risks such as electricity, careless behavior, rotating machinery, and exposure to flour dust, issues such as fire, lightning, deficiencies in signage, inadequate hygiene conditions in shower cabins, organization of night shifts, and lack of emergency exit doors have also been discussed. Measures taken to mitigate these hazards and steps that need to be taken have been outlined, emphasizing key points to be considered regarding occupational health and safety. In order to eliminate the negative effects of flour dust on workers' health, proper operation of the necessary ventilation system must be ensured. In areas where dust exposure is high, it would be advisable to use appropriate personal protective equipment such as masks. Periodic maintenance should be carried out on the electrical system and guards should always be used on machinery with rotating parts.

In this analysis, significant risks related to the maintenance and safety of electrical panels in the production area (l:4, s:5, RS:20), inadequate firefighting equipment (l:4, s:5, RS:20), compliance with hygiene standards (l:3, s:3, RS:9), lack of machine guards (l:3, s:4, RS:12), and noise levels (l:3, s:4, RS:12) have been identified, requiring urgent measures. Therefore, it is critical for employers and employees to be aware of these risks, provide the necessary training, and diligently implement the necessary measures. The lack of procedures in the boiler room exposes serious risks such as fire and explosion. Proper installation, inspected by qualified personnel, and maintained as required are essential. In the raw material unloading area, appropriate guidelines should be provided for the safe unloading of trucks and necessary precautions should be taken to ensure the safety of personnel during operations.

Identifying potential workplace hazards and taking the necessary precautions against those hazards is critical to ensuring the safety and health of employees. Employers and employees who are aware of occupational health and safety issues and take the necessary precautions will ensure that everyone has a safe working environment. Therefore, it is important to continually review workplace risks and take preventive measures.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

Author contribution

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RESEARCH ARTICLE

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Theoretical studies of phytochemicals with feline infectious peritonitis virus proteins: a search for novel antivirals

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Abstract

Feline Infectious Peritonitis Virus (FIPV) is a highly lethal pathogen affecting cats worldwide. Developing effective antiviral treatments is crucial for managing this disease. This study investigates the potential of flavonoids to act as antiviral agents and allosteric modulators against the FIPV spike protein using molecular docking simulations. Thirteen flavonoids were docked against the FIPV spike protein (PDB ID: 6JX7) in both ligand-free (cleaned) and ligand-bound (uncleaned) states to assess their binding affinities and potential allosteric effects. The docking results revealed that all tested flavonoids exhibited strong binding affinities, with docking scores ranging from -7.9 to -9.6 kcal/mol in the cleaned receptor state. Notably, Hesperidin, Morin, Hesperetin, and Quercetin maintained or even improved their binding affinities in the presence of native ligands, suggesting their potential as allosteric modulators. Comparative analysis of the binding modes in the cleaned and uncleaned receptor states further supports the allosteric modulator potential of Morin, Hesperetin, and Hesperidin. These findings highlight the promising role of flavonoids as antiviral agents and allosteric modulators targeting the FIPV spike protein. Further experimental validation and optimization of these compounds could lead to the development of effective treatments for feline infectious peritonitis. This study provides valuable insights into the application of flavonoids in the management of viral diseases and contributes to the ongoing efforts in antiviral drug discovery.

Keywords: FIPV, Flavonoid, Docking, Allosteric Modulators

Type: Research Article

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INTRODUCTION

Feline Infectious Peritonitis Virus (FIPV) is an important viral pathogen affecting cats worldwide and causes a complex and often fatal disease characterized by systemic inflammation and peritonitis. Recent advancements in understanding the molecular biology of FIPV and therapeutic interventions have provided new insights into the management and potential eradication of the disease. A recent study has highlighted the antiviral activity of an extract from the d3 Chinese herb against FIPV, suggesting a promising avenue for natural component-based therapies (Nishijıma et al., 2023). Furthermore, flavonoids such as isoginkgetin and luteolin have been found to inhibit FIPV replication and exhibit virucidal effects, providing a foundation for the development of flavonoid-based antiviral drugs (Triratapiban et al., 2023).

The strategic application of immunoinformatic approaches to develop FIPV vaccines is also gaining importance. The strategic application of immunoinformatic approaches to develop FIPV vaccines is also gaining importance. A recent study

by (Chawla et al., 2023) has used immunoinformatic techniques to predict and evaluate T-cell epitopes in the spike protein of the virus, paving the way for more targeted vaccine strategies. Another promising research direction is the identification of potential epitopes for FIPV using phylogenetic analysis, which can aid in the design of effective preventive measures (Aksono et al., 2023; Meshram & Gacche, 2015).

Antiviral drugs like GS-441524 and remdesivir have been investigated for their efficacy against FIPV, and GS-441524 has shown promising results in reducing viral replication (Barua et al., 2023). This highlights the potential for re-evaluating current antiviral agents for FIPV management. Additionally, understanding the role of specific viral proteins and genetic factors in FIPV pathogenesis can contribute to the development of targeted therapies. For instance, the ORF7a protein has been found to increase the inflammatory pathology in infected cats, pointing to a critical target for therapeutic intervention (Jiao et al., 2024).

Another study by Zeedan et al. has highlighted the antiviral effects of plant extracts on significant animal viruses, including FIPV. The study suggests that certain plant extracts demonstrate therapeutic potential by inhibiting viral replication and early stages of viral adsorption (Zeedan & Abdalhamed, 2021).

In summary, the research landscape for Feline Infectious Peritonitis Virus is rapidly evolving, with significant contributions from antiviral compounds, immunological approaches, and molecular diagnostic studies. These advancements hold the potential to not only improve the management of FIPV but also provide new insights into coronavirus biology that may be applicable to other species, including humans.

In this study, 13 flavonoids that have shown viral activity in previous studies were selected (Davies & Yáñez, 2012; Kasprzak et al., 2015; Rizk et al., 2018; Paredes et al., 2003), and their docking analyses were performed. The analyses were conducted against the FIPV virus spike protein, both with the ligands removed and in the presence of natural ligands. This approach allowed for the discussion of the competitive advantages of the flavonoid drug candidates against other ligands and the examination of their allosteric modulator potential. The results indicated that the selected flavonoids are both promising drug candidates and potential allosteric modulators.

MATERIALS AND METHODS

A total of 13 flavonoids, which have demonstrated antiviral properties in prior research, were chosen. (Davies & Yáñez, 2012; Kasprzak et al., 2015; Rizk et al., 2018; Paredes et al., 2003). Then, all ligands were obtained in 3D mol2 format from the databases provided in the reference (Pence & Williams, 2010; Groom et al., 2016; Irwin et al., 2012) . Then, using the OPENMM 7 program (Eastman et al., 2017) on Google Colab with Nvidia Tesla A100 80 GB GPUs hardware and gaff parameters (Wang et al., 2004), all ligands were optimized. As the receptor, the structure of the spike protein of feline infectious peritonitis virus strain UU4 obtained by X-ray diffraction was retrieved from the Protein Data Bank (PDB ID: 6JX7) from https://www.rcsb.org/ (Berman, 2000). Next, prepare_receptor4.py and prepare_ligan4.py scripts in MGL TOOLS (Eberhardt et al., 2021; Morris et al., 2009) were used to prepare the molecules for docking. These scripts were used to remove water molecules from the receptor, add polar hydrogens, calculate Kollman charges, and convert the files to pdbqt format. Box dimensions were calculated python script program that I developed, which uses the geometric center of the receptor as a reference. Then, virtual screening was started and docking of all ligands to the receptor was performed using vina.exe, and the results generated by vina were parsed using the vina_split.exe program. The "grep" command in Linux was used to quickly tabulate the results. Finally, the results were visualized using the Discovery Studio Visualizer program (BIOVIA, 2019).

RESULTS AND DISCUSSION

The results of the mini virtual-screening analysis are shown in the second column of Table 1. According to these results, it was concluded that all of the phytochemicals used in this study, which belong to the flavonoid class, are effective against the FIPV. All 13 compounds studied showed a docking score of -7.9 or better. This indicates that they are very potent drug candidates and is an important result of the study in terms of the binding of flavonoids to the FIPV spike protein (Xue et al., 2022).

The unexpectedly high efficacy of the ligands may necessitate a more challenging test. Typically, in the docking process, the water and ligands within the protein designated as the receptor are removed, and then water and ions are added to these "clean" receptors in molecular dynamics simulations. In most cases, the molecular dynamics results indeed support the docking results. Therefore, to address doubts about whether the ligands tested here are truly drug candidates, it would be more appropriate to investigate the allosteric effect rather than proceeding with molecular dynamics simulations using the same ligand and receptor, which are already known in the literature to mostly support

the docking process results. Consequently, in the second stage of testing, the drug candidate ligands were subjected to the docking process with the receptor whose ligands were not removed (uncleaned receptor). This way, the drug candidate ligands will compete with the naturally placed ligands inside the receptor, and the allosteric effect will also be examined. If our drug candidate ligands still show above-average efficacy in this scenario, we can confidently state that these candidates are indeed potent.

The docking results performed with the uncleaned receptor, without removing the natural ligands, are also presented in the third column of the same table (Table 1). When comparing the effects of the ligands in the clean and uncleaned receptors, it is observed that the activities of Morin, Hesperetin, Quercetin, and Hesperidin increase even in the presence of other ligands. Among these, Hesperidin exhibits the best binding score in both scenarios, leading us to conclude that it is the most promising drug candidate in this study. Luteolin and Isoquercitrin showed the same binding score despite the allosteric effect and appear unaffected by the situation, suggesting that they are more stable and predictable drug candidates. By solely examining the docking scores without conducting molecular dynamics simulations, we can also infer that these two ligands are not affected by competition with other ligands, and their drug activities within the cell will not decrease, at least in terms of competition with other ligands. It can be observed that Scutellarin, Taxifolin, Biochanin A, and Naringenin are slightly negatively affected by the allosteric effect. Catechin, Kaempferol, and Fisetin appear unable to compete with other ligands, with Fisetin showing the worst binding energy in both scenarios. However, it is still possible to say that even a -7.40 kcal/mol docking score is sufficient to keep it on the list of drug candidates.

The ability of the flavonoid drug candidates to bind to the protein even in the presence of other ligands suggested that they may act as allosteric modulators. As we will provide more detailed information in the conclusion section, allosteric modulators are highly important in drug targeting. We can determine whether a drug candidate possesses allosteric modulator properties by examining where it binds in the absence and presence of allosteric effects.

In the figures below, Figure 1 and Figure 5 show the binding modes of Morin to the cleaned and uncleaned receptors, respectively, and they are quite different from each other. In the clean receptor, the binding modes involve ASN C:3266, PHE C:3267, LYS C:3265, ASP C:3471, and GLN C:3685, whereas in the uncleaned receptor, these have changed to ASN A:776, GLN A:1195, and GLN A:979. Similarly, Figure 2 and Figure 6 for Hesperetin, and Figure 4 and Figure 8 for Hesperidin, show that they bind to very distant regions in the two different versions of the same receptor. This suggests that Morin, Hesperetin, and Hesperidin could be allosteric modulators. In the binding modes of Quercetin in Figure 3 and Figure 7, there is a situation where in Figure 3, Quercetin binds to PHE A:777, ASN A:776, LEU A:983, GLN A:979, GLN A:1195, and PHE A:1194, while in Figure 7, it binds to PHE A:777, LEU A:990, GLN A:979, and ASP A:981. Although it binds to different amino acids in both cases, its affinity for the PHE A:777 amino acid is high. However, it still has the potential to be an allosteric modulator, and this can be clarified with further studies.

Table 1. Docking Score of flavonoids with a ligand-free (clean) and uncleaned receptor (PDB ID: 6JX7)

Ligands	**Docking Score with Cleaned Receptor(S2) (kcal/mol)**	**Docking Score with Uncleaned Receptor(S1) (kcal/mol)**	**Difference (S2-S1)**
Hesperidin	-9.6	-9.7	0.1
Scutellarin	-9.2	-9.1	-0.1
Catechin	-8.5	-8	-0.5
Isoquercitrin	-8.5	-8.5	0
Kaempferol	-8.5	-8	-0.5
Taxifolin	-8.5	-8.4	-0.1
Biochanin A	-8.2	-8.1	-0.1
Morin	-8.2	-8.6	0.4
Quercetin	-8.2	-8.3	0.1
Hesperetin	-8	-8.4	0.4
Luteolin	-8	-8	0
Naringenin	-8	-7.8	-0.2
Fisetin	-7.9	-7.4	-0.5

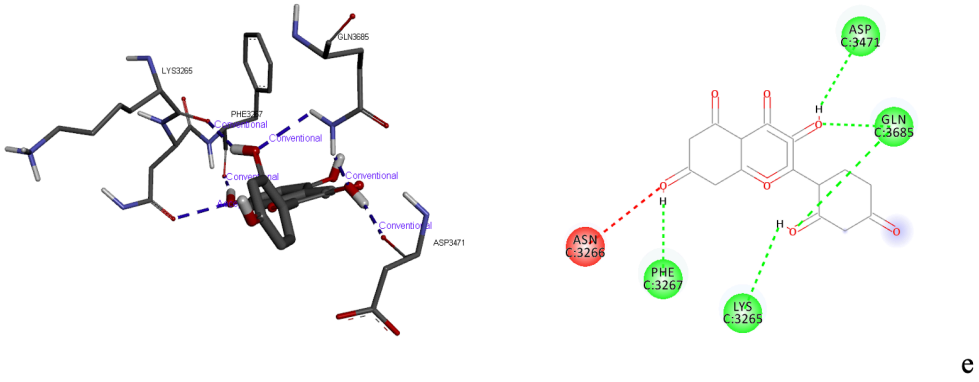

Figure 1. Morin's binding sites with clean receptor

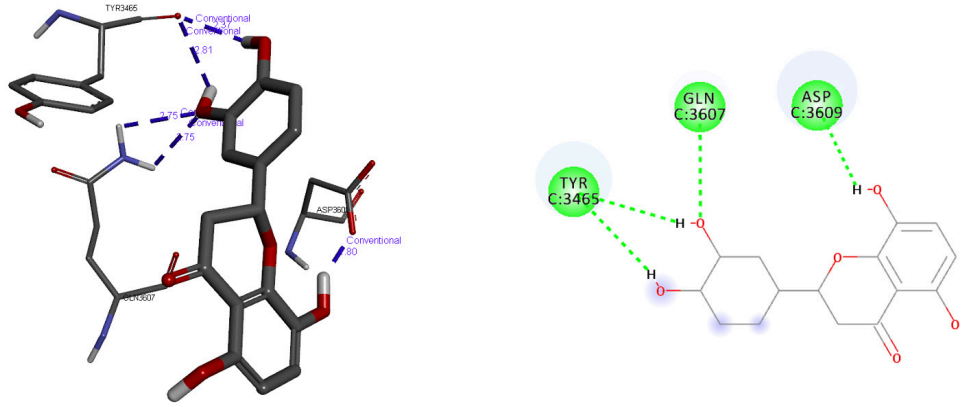

Figure 2. Hesperetin's binding sites with clean receptor

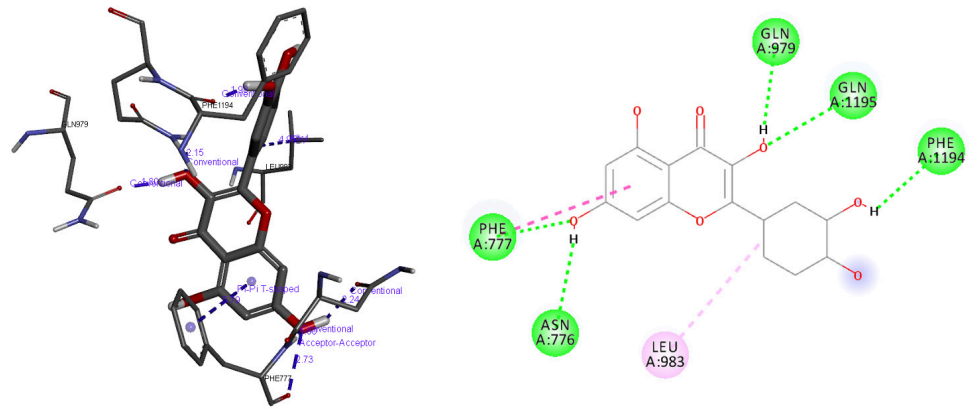

Figure 3. Quercetin's binding sites with clean receptor

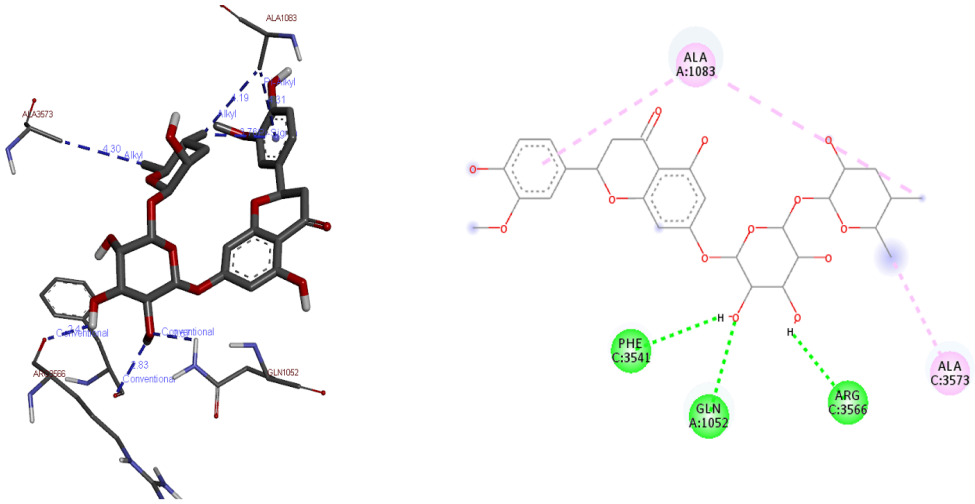

Figure 4. Hesperidin's binding sites with clean receptor

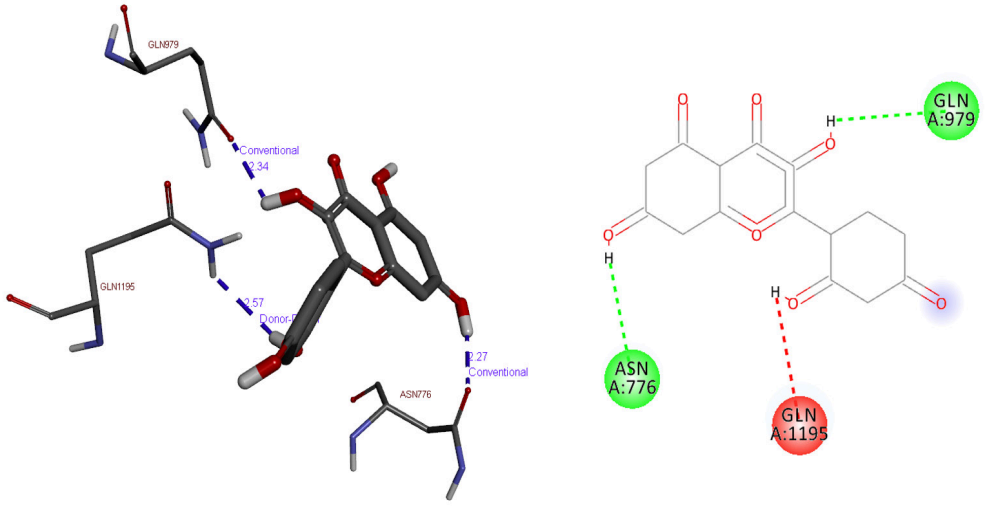

Figure 5. Morin's binding sites with uncleaned receptor

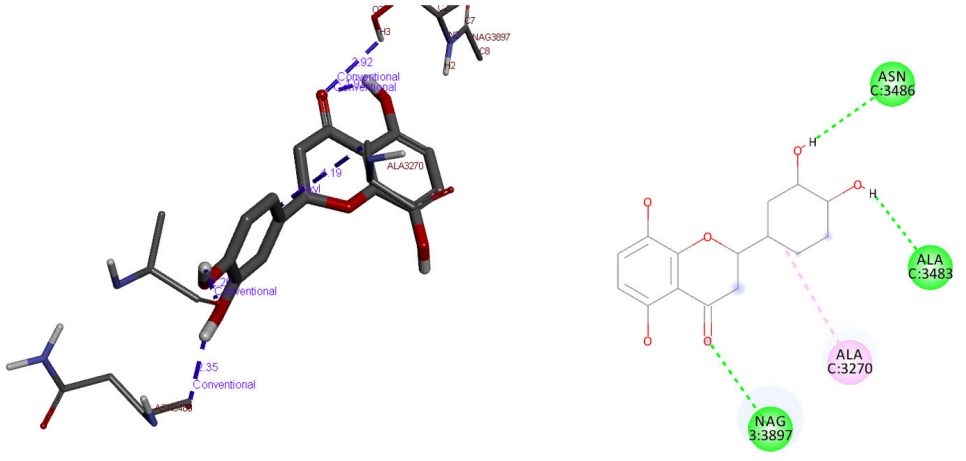

Figure 6. Hesperetin's binding sites with uncleaned receptor

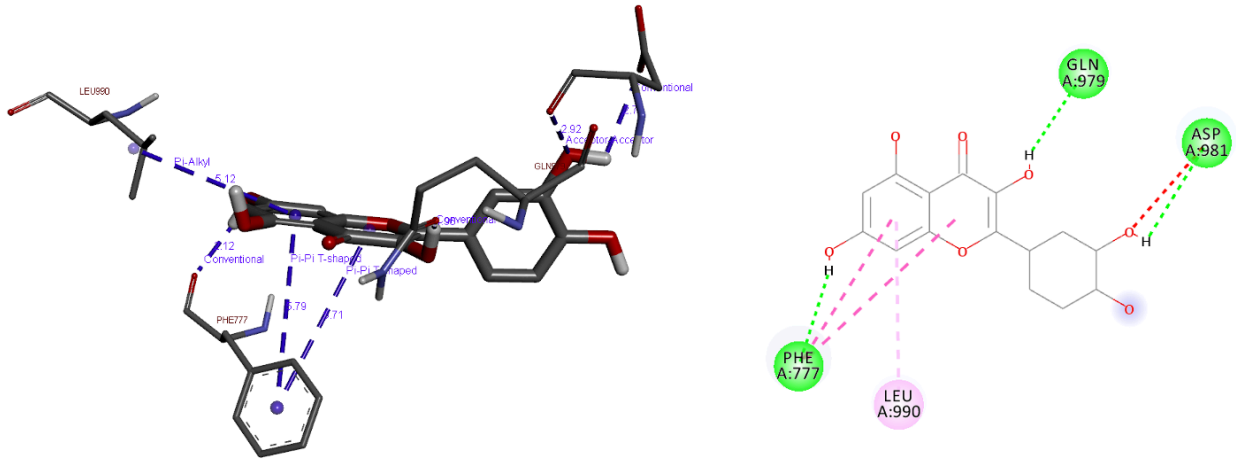

Figure 7. Quercetin's binding sites with uncleaned receptor

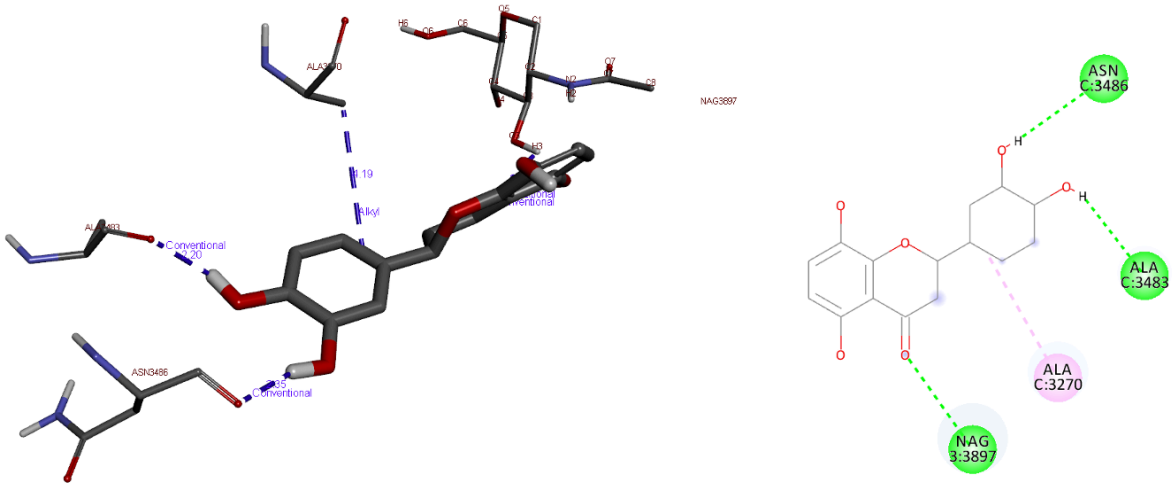

Figure 8. Hesperidin's binding sites with uncleaned receptor

CONCLUSION

Feline Infectious Peritonitis Virus is a highly lethal virus for cats, and efforts to develop drugs for its rapid, inexpensive, and effective treatment are ongoing. In this study, the potential outcomes of applying the general binding affinities exhibited by flavonoids against viral spike proteins to this virus were discussed, and the situation was elucidated through docking studies. Initially, a standard docking process was performed, where the virus spike protein was cleaned of its own ligands and water, and docking studies were conducted with 13 selected flavonoids, yielding surprisingly good results. Subsequently, the effect of these flavonoids on the viral protein in the presence of other ligands was investigated, and a second docking study was performed without removing the ligands from the protein. The results of both studies are presented in Table 1, indicating that flavonoids achieve a considerable binding score to the viral spike protein in both scenarios. At this stage, the study was taken a step further to investigate whether the flavonoid drug candidates act as allosteric modulators.

Allosteric modulators bind to the allosteric regions of proteins, altering their shape and function. This property is utilized to modulate the biological activity of proteins and offers great potential, particularly in drug discovery. They can induce conformational changes in the viral membrane spike protein, which may affect interactions with cell surface receptors (Markwell et al., 1985). These changes can influence the virus's ability to bind to and fuse with cells, thereby affecting infectivity (Clapham & McKnight, 2002). Furthermore, these modulators can weaken the interfaces

of the viral spike protein by targeting specific regions that directly interact with the body (such as ACE2 for COVID-19), thereby reducing viral infectivity. This targeting can provide a strategic advantage in antiviral defenses (Olotu et al., 2020).

In summary, allosteric modulators can provide higher target selectivity compared to traditional orthostatic ligands, helping to reduce side effects and increase drug efficacy. In this study, the allosteric modulator status of flavonoids that successfully competed for binding with other ligands under allosteric effects was examined, and positive results were obtained. In particular, it was found that Morin, Hesperetin, and Hesperidin could not only be good drug candidates against the FIPV spike protein but also potential allosteric modulator molecules.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

No conflict of interest is declared by the author.

Author contribution

Bariş KURT designed the study, conducted the research, analyzed the data, and wrote the manuscript.

Funding

No financial support was received for this study.

Data availability

All data are provided in the manuscript.

Consent to participate

Not applicable

Consent for publication

Not applicable

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RESEARCH ARTICLE

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Inhibitory effect of plant essential oils on controlling *Alternaria* species

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Abstract

The use of natural products in the control of fungal diseases in plants is considered an alternative to synthetic fungicides due to their less negative effects on the environment. In this study, *in vitro* inhibitory effect of plant essential oils (PEOs) of black cumin, cumin, chamomile, cedarwood, and ginger were investigated for controlling two species of Alternaria, including *Alternari solani* and *Alternaria alternata* on tomato and cabbage under *in vitro* conditions, respectively. Aiming to evaluate the mycelial growth of the pathogen, mycelial discs were placed in Petri plates with 0, 500, 1000, 1500, 2000, and 2500 µL/L of PEOs. The experiment was carried out in a randomized plot design with three replications. Chemical analysis of PEO components were determined by Gas Chromatography and Mass Spectrometry methods. A total of 69 chemical compounds were determined in five different PEOs. As the main chemical compounds, Cuminaldehyde was detected in cumin PEO, Sesquithujene was found in ginger PEO, and Eucalyptol (1,8-cineole) was determined in black cumin, chamomile, and cedarwood PEOs. All five PEOs were found to inhibit the growth of *Alternaria* species in a dose-dependent manner, whereas cumin EO was determined more inhibitory effect against *A. solani* and *A. alternata*. Cumin PEO showed the highest effect against *Alternaria* species because it contains a Cuminaldehyde chemical compound. The lowest inhibition percentage was found in chamomile PEO compared to other PEOs. This study suggested that cumin PEO has the potential as an antifungal agent for controlling of Alternaria diseases.

Keywords: Essential oil, *Alternaria solani*, *Alternaria alternata*, Cuminaldehyde, Eucalyptol, Inhibition

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INTRODUCTION

The genus *Alternaria* was first introduced by Nees (1816), kingdom Mycota, phylum Ascomycota, class Dothideomycetes, order Pleosporales belongs to the family Pleosporaceae and is a ubiquitous genus of fungi that includes saprophytic, endophytic and pathogenic species (Saharan et al., 2016). The *Alternaria* genus has a wide variety of species around the world. Approximately 400 plant species are hosts of *Alternaria* species, including various species of crops, fruits, vegetables, ornamentals, and weeds (Leyva Salas et al., 2017; Meena et al., 2017; Gou et al., 2022). Among these species, *Alternaria solani* and *Alternaria alternata* cause early blight disease in many plants.

A. alternata is an endophytic species and is among the important seed-borne plant pathogens. *A. alternata* can produce more than 30 mycotoxins, and these toxins cause tissue necrosis with enzymes that degrade the cell wall (Ahmad & Sinha, 2002; Guo et al., 2019). The pathogen is usually carried by water, insects,

agricultural equipment, and infected seeds. This pathogen's spores can enter the plant's leaves, stems, and fruits (Tsuge et al., 2013). Early blight caused by *A. solani* Sorauer is an economically important and widely distributed worldwide disease on crops belonging to the Solanaceae family (Marak et al., 2014). The disease occurs on leaves, stems, petioles, and fruits in high humidity and temperature areas. The pathogen prefers mature tissues and is more frequent during the fruiting period, causing high economic losses (Foolad et al., 2000; Chaerani & Voorrips, 2006). Early blight causes 50% to 80% yield loss, especially in tomato production (Pandey et al., 2020).

Alternaria species have a wide host range, extreme variability, and a long and active vegetative phase, and are very difficult to control (Sharma et al., 2021). Various methods such as crop rotation, resistant varieties, soil fumigation, and fungicide application control the disease (Namanda et al., 2004; Kirk et al., 2005). Synthetic fungicides, used as both seed and spray treatments, include Captan, Ridomil, Strobilurin, Iprodione, Mancozeb, Carbendazim, Chlorothalonil (Swart et al., 1998; Khan et al., 2007; Horsfield et al., 2010; Karuna et al., 2012; Kumar et al., 2013). However, synthetic fungicides accumulate in soil, animals, and plants and negatively affect the ecosystem. Additionally, fungal agents become resistant to fungicides after a certain period and their combat effectiveness decreases (Smith, 2001). In this context, it has become necessary to research and develop alternative methods to chemicals that are friendly to human health and nature, in the control against fungal disease agents (Soylu et al., 2022).

Essential oils (EOs) obtained from different medicinal and aromatic plants, which do not contain chemicals, stand out as natural fungicides in the control against plant pathogens (Paulitz & Belanger, 2001). PEOs containing sesquiterpenes, monoterpenes, and oxygenated compounds have an antimicrobial effect (Regnault-Roger et al., 2012). These compounds cause the separation of lipid layers from the fungal cell membrane, disruption of cell membrane integrity and permeability by changing membrane structures, and metabolic deterioration in cytoplasmic and mitochondrial structures (Feng & Zheng, 2007; Nerio et al., 2010; Tian et al., 2012). Several studies have highlighted the importance of many plant families i.e. Asteraceae, Liliaceae, Apocynaceae, Apiaceae, Caesalpinaceae, Rutaceae, Piperaceae, Sapotaceae, etc., used as medicinal plants (Sheetal and Singh, 2008). Researchers have reported that different PEOs have antifungal effects against *Alternaria* species. (Feng & Zeng, 2007; Hadizadeh et al., 2009; Feng et al., 2011; Bayan et al., 2017; Moumni et al., 2021; Grati Affes et al., 2023; Porcino et al., 2023).

This study aimed was to evaluate the inhibitory effect of black cumin, cumin, chamomile, cedarwood, and ginger PEOs to control two species of *Alternaria* under *in vitro* conditions.

MATERIALS AND METHODS

Fungal isolates

In the experiment, highly pathogenic fungal isolates of *A. solani* (ET 66) and *A. alternata* (LAa 21) with known virulence and isolated from tomato and cabbage were obtained from the collection of fungal culture at Atatürk University of Agriculture Faculty, Department of Plant Protection and collection of fungal culture at Mustafa Kemal University of Agriculture Faculty, Department of Plant Protection, respectively (Camlica & Tozlu, 2019; Soylu et al., 2024). Fungal isolates were aseptically subcultured and purified by serial transfers onto Petri plates containing 25 mL of potato dextrose agar (PDA-Difco). Plates were incubated in the dark at 25±1°C for 7 days and culture was stored at +4°C in the refrigerator. *Alternaria* culture was prepared for the experiment and was left to grow in the dark at 25±1°C for 7 days in an incubation chamber before being used *in vitro* experiment.

Plant essential oils

PEOs used in this study were black cumin, cumin, chamomile, cedarwood, and ginger (Table 1). PEOs were obtained from Arpaş Arifoğlu Co. (İstanbul, Türkiye). PEOs were stored in sealed glass bottles at +4°C until further use (Amini et al., 2012).

Table 1. List of PEOs used in the study.

Scientific name	Family	English name	Brand name	Purity level (%)
Nigella sativa	Ranunculaceae	Black Cumin	Black Cumin Oil	100
Cuminum cyminum	Apiaceae	Cumin	Cumin Oil	100
Matricaria chamomilla	Asteraceae	Chamomile	Chamomile Oil	100
Cedrus atlantica	Pinaceae	Cedarwood	Cedarwood oil	100
Zingiber officinale	Zingiberaceae	Ginger	Ginger Oil	100

Chemical analysis and identification of PEO components

Five PEOs were analyzed by Gas Chromatography and Mass Spectrometry (GC-MS) (Shimadzu 2010 SE, Kyoto Japan; Süleyman Demirel University Innovative Technologies Application and Research Center). Compounds of PEOs were identified by a combination of the mass spectrum of the Wiley library (Wiley, New York, NY, USA) and the NIST mass spectral database (Semiz et al., 2016).

In vitro efficacy of PEOs on mycelial growth of Alternaria species

The antifungal effect of PEOs was done by contact phase against *Alternaria* species (Soliman and Badeaa, 2002). In the contact phase, different concentrations (0, 500, 1000, 1500, 2000, and 2500 µL/L) of PEOs were prepared by dissolving them in Tween 20 (1:1) and added to flasks containing molten PDA. The PDA was poured into 90 mm plastic Petri plates (20 mL). A fungal disc (5 mm in diameter) was cut from the edge of 7-day-old cultures of *Alternaria* species grown on PDA and was placed at the center of each Petri plate. The plates without the PEOs were used as control treatment. All Petri plates were incubated at 25±1°C for 10 days. The experiment was carried out in a randomized plot design with three replications. The diameters were measured when fungal mycelium covered one plate in the control treatment to calculate the inhibition effect. The mycelial growth inhibition was calculated by the following formula (Equation 1).

$$\text{Mycelial growth inhibition (\%)} = [(dc-dt) / dc] \times 100 \quad (1)$$

where dc and dt represent the mean diameter of the mycelial growth (mm) of the control and treated fungal isolates (Moumni et al., 2021).

Data analysis

The statistical analyses were accomplished with the JMP IN packet statistic program (SAS Institute, Carry, NC, 13.0 PC version). Analysis of variance (ANOVA) followed by LSMeans Differences Student's test ($P \leq 0.01$) was performed to evaluate differences between studied cases.

RESULTS AND DISCUSSION

Chemical analysis of PEOs

The major identified components of the PEOs of black cumin, cumin, chamomile, cedarwood, and ginger are listed in Table 2. In the study, 69 active components were determined in PEOs by GC-MS analysis. The major compounds in the PEOs of black cumin were Eucalyptol (1,8-cineole) (48.28%), alpha-Pinene (14.78%), beta- Pinene (9.07%), and Cymol (8.41%). Cuminaldehyde (31.44%), gamma-Terpinene (17.79%), beta-Pinene (16.03%), and Cymol (14.73%) were predominant components of cumin PEO. Major components of chamomile PEO belonged to Eucalyptol (1,8-cineole) (46.78%), alpha-Pinene (10.17%), and beta-Pinene (6.34%). Eucalyptol (1,8-cineole) (27.88%), Thujopsene (22.59%), alpha- Cedrene (10.08%), alpha-Pinene (9.33%), and alpha-Cedrol (8.23%) were determined in cedarwood PEO. Major components of ginger PEO belong to Sesquithujene (7-epi) (17.72%), Eucalyptol (1,8-cineole) (16.27%), Limonene (12.72%) and Camphene (12.33%).

Table 2. Chemical compound of PEOs determined by GC-MS analysis.

			Nigella sativa[c]	*Cuminum cyminum*[c]	*Matricaria chamomilla*[c]	*Cedrus atlantica*[c]	*Zingiber officinale*[c]
No	**Compound name**[a]	**LRI**[b]			**% of the oil**		
1	Tricyclene	924	0.77	0.04	0.46	0.41	1.44
2	alpha- Thujene	927	2.40	0.32	0.57	0.55	0.16
3	alpha - Pinene	933	14.78	1.68	10.17	9.33	7.01
4	beta- Fenchene	942	-	-	-	-	0.12
5	Camphene	953	4.77	0.24	3.66	3.07	12.33
6	Sabinene	972	2.23	0.32	1.69	1.28	0.51
7	beta- Pinene	978	9.07	16.03	6.34	5.26	2.11
8	4-Methyl-1-hepten-5-one	986	-		-	-	0.54
9	beta- Myrcene	991	-	0.69	0.75	-	1.55
10	Octanal	1006	-		-	-	0.14
11	Phellandrene	1007	-	0.44	0.18	-	0.39
12	DELTA.3-Carene	1009	-	0.05	0.27	-	0.04
13	alpha- Terpinene	1018	-	0.18	0.45	0.27	0.13
14	Cymol	1025	8.41	14.73	3.72	2.84	1.17
15	Limonene	1030	3.10	1.19	7.53	1.72	12.72
16	Eucalyptol (1,8-cineole)	1052	48.28	1.93	46.78	27.88	16.27
17	gamma-Terpinene	1058	3.36	17.79	2.59	2.21	0.70
18	trans-Sabinene hydrate	1088	0.52	0.03	1.42	0.52	0.20
19	alpha- Terpinolen	1096	-	0.13	-	-	0.28
20	Dimethylstyrene (alpha-para)	1104	-	0.52	-	-	0.65
21	Linalool	1114	-	-	2.91	-	-
22	Chrysanthenone	1133	-	-	0.38	-	-
23	Carveol	1152	-	0.10	4.09	-	-
24	Camphor	1157	2.32	0.09	0.99	1.60	0.87
25	4-Terpineol	1193	-	0.28	-	-	0.18
26	Dimethylbenzylcarbinyl acetate (DMBCA)	1200	-	0.35	-	-	0.62
27	alpha- Terpineol	1207	-	-	0.34	-	-
28	Perilla alcohol	1208	-	0.85	-	-	-
29	Dihydrocarvone	1210	-	0.11	-	-	-
30	p-Allylanisole	1210	-	-	0.85	-	-
31	Z-Citral	1238	-	-	-	-	1.88
32	Cuminaldehyde	1247	-	31.44	-	-	-
33	Carvotanacetone	1260	-	0.33	-	-	-
34	E-Citral	1268	-	-	-	-	2.25
35	Phellandral	1277	-	0.34	-	-	-
36	2-Undecanone	1294	-	-	-	-	0.17
37	2-Caren-10-al	1298	-	6.84	-	-	-
38	1-Phenylpropane-1,3-diol	1302	-	0.89	-	-	-
39	Thymol	1307	-	0.10	-	-	-
40	Carvacrol	1317	-	0.05	-	-	-
41	Citronellyl acetate	1363	-	-	-	-	0.37
42	Eugenol	1372	-	-	3.24	-	-

43	alpha- Copaene	1375	-	-	-	-	0.25
44	gamma- Cadinene	1388	-	0.09	-	-	-
45	Linalyl acetate	1392	-	-	-	-	0.93
46	beta- Elemene	1400	-	-	-	-	0.33
47	alpha- Zingiberene	1414	-	-	-	-	0.09
48	alpha- Cedrene	1414	-	-	-	10.08	-
49	beta- Cedrene	1423	-	-	-	1.47	-
50	Caryophyllene	1428	-	0.12	0.62	-	-
51	Thujopsene	1433	-	-	-	22.59	-
52	Germacrene B	1439	-	-	-	-	0.15
53	Farnesene ((E)-, beta)	1466	-	0.05	-	-	-
54	alpha- Cedrene	1483	-	1.32	-	-	-
55	Germacrene D	1490	-	-	-	-	0.45
56	Curcumene	1491	-	-	-	-	4.30
57	Alloaromadendrene	1503	-	-	-	-	0.52
58	Sesquithujene (7-epi)	1506	-	-	-	-	17.72
59	Cuparene	1515	-	-	-	0.68	-
60	alpha- Farnesene	1517	-	-	-	-	1.40
61	beta- Bisabolene	1519	-	-	-	-	3.80
62	beta- Sesquiphellandrene	1534	-	-	-	-	3.80
63	Carotol	1601	-	0.05	-	-	-
64	alpha- Cedrol	1614	-	-	-	8.23	-
65	Tetracosane	2400	-	0.07	-	-	-
66	Pentacosane	2500	-	0.08	-	-	-
67	Hexacosane	2600	-	0.07	-	-	-
68	Heptacosane	2700	-	0.06	-	-	-
69	Nonacosane	2900	-	0.02	-	-	-
			100	100	100	100	100

[a]Compounds listed in order of their elution, [b]LRI: Linear retention index, [c]GC-MS analysis results are shared in the article of Sağlan et al. (2022).

Differences observed in many studies may relate to different components of PEOs in terms of geographical origin, plant variety, and age, environmental and agronomic conditions, harvesting time, drying and extraction methods, and genetic difference (Yeşil Çeliktaş et al., 2007). The Antibacterial, antifungal, and antioxidant activities of cumin EO are attributed to the presence of Cuminaldehyde compounds (Ghasemi et al., 2019). chamomile EO has broad-spectrum antifungal activity by changing the cell membrane permeability of fungi (Seyedjavadi et al., 2020). Another study conducted indicated that EOs from *M. chamomilla* had moderate to weak effects against the mycelial growth of fungi (EL-Hefny et al., 2019). Many herbal shampoos and natural repellents contain cedarwood EO as an active ingredient (Anderson, 1995). Kačániová et al. (2022), reported that the EOs of cedarwood have significant antifungal activities. In this context, researchers found that Eucalyptol (1,8-cineole), and Thujopsene have high antifungal effects (Morcia et al., 2012; Mukai et al., 2019). Growth inhibition of phytopathogenic fungi was attributed to the presence of phenolic compounds such as Sesquithujene, Eucalyptol (1,8-cineole), and Limonene isolated from *Z. officinale* oil (Rahmah et al., 2013; Ayodele et al., 2018).

Inhibitory effect of PEOs on mycelial growth of *Alternaria* species

The effects of six various concentrations (0, 500,1000, 1500, 2000, and 2500 µl/L) of black cumin, cumin, cedarwood, chamomile, and ginger PEOs were evaluated for their inhibitory effects on *Alternaria* species mycelial growth using the contact phase technique. The inhibitory effect of five PEOs is summarized in Table 3. All of the tested PEOs significantly ($P \leq 0.01$) inhibited the mycelial growth of *A. solani* and *A. alternata* at all concentration levels compared to the control. Depending on the dose increase, each PEO used in the experiment reduced the mycelial development

of *A. solani* and *A. alternata* at different rates. The highest radial growth was found on control plates, while the lowest radial growth was found with 2500 µL/L cumin PEO against *A. solani* (ET 66 isolate) *and A. alternata* (LAa 21 isolate). High-dose (2500 µL/L) application of black cumin PEO showed 65.3% and 72.1% effects on tested *A. solani* and *A. alternata*, respectively. In other doses of black cumin PEO, inhibition of mycelial growth of *A. solani* was between 14.2% and 50.7%, while inhibition of mycelial growth of *A. alternata* was between 26.3% and 61.1%. The highest inhibitory effect in cumin PEO was determined against *A. solani and A. alternata* in the high-dose treatment at 80.3% and 87.6%, respectively, while 2000 µL/L dose application followed the next highest effect (64.5% and 69.5%). In other doses of cumin PEO, inhibition of mycelial growth of *A. solani* was between 17.2% and 64.5%, while inhibition of mycelial growth of *A. alternata* was between 25.0% and 69.5%. The highest antifungal effect in chamomile PEO was found at the rates of 64.1% and 69.4%, respectively, against *A. solani* and *A. alternata* in 2500 µL/L application. PEO of chamomile showed a potent inhibitory effect on the radial growth of *A. solani* (64.1%), *A. alternata* (69.4%) in 2500 µL/L application. In other doses of chamomile PEO, inhibition of mycelial growth of *A. solani* was between 12.8% and 49.7%, while inhibition of mycelial growth of *A. alternata* was between 21.0% and 65.0%. Application of 2500 µL/L in cedarwood PEO had an effect of 70.4% and 74.9% against *A. solani* and *A. alternata* pathogens, respectively. While other doses of cedarwood PEO inhibited the mycelial growth of *A. solani* between 14.6% and 57.8%, it inhibited the mycelial growth of *A. alternata* between 23.0% and 62.0%. While the 2500 µL/L treatment of ginger PEO showed the highest inhibitory effect against *A. solani* (65.1%), this effect was observed to be 70.5% in *A. alternata*. In other doses of ginger PEO, the radial growth of *A. solani* was reduced between 12.8% and 54.4%, while the radial growth of *A. alternata* was reduced between 16.3% and 58.2%. In the study, cumin PEO was determined as the most effective EO inhibiting mycelial growth of *A. solani* and *A. alternata*, compared to black cumin, chamomile, cedarwood and ginger PEOs. High-dose application of cumin PEO showed a higher inhibitory effect against *A. alternata* than *A. solani*, and the inhibitory effects of the other PEOs were found to be close to each other, depending on the *Alternaria* species and dose (Table 3).

In the present study, *N. sativa* EO showed moderate inhibition against *Alternaria* spp. Similar findings have also been reported by Patni et al. (2005) who tested different EO against *Alternaria brassicae* and *A. alternata*, respectively, and found good results in inhibiting the pathogen mycelial growth under *in-vitro* conditions. In the study conducted by Sitara et al. (2008) reported that *N. sativa* EO at 0.15% was significantly inhibition, but, it exhibited no inhibitory effect against *A. alternata*. In our study, *C. cyminum* EO showed the highest inhibitory effect against *A. solani* and *A. alternata*. Similar to our findings, Romagnoli et al. (2010) reported that the antifungal effect of cumin extract on *Alternaria* sp. is 19.6 % and 81.4% for 5 and 20 µL per disc, respectively. In a study performed by Kedia et al. (2014) *C. cyminum* EO showed an inhibitory effect against *Alternaria* sp. at the dose of 0.6 µL/mL concentration. Ghasemi et al. (2019) reported that *C. cyminum* EO has cuminaldehyde (31.44%) content as the main compounds that show antibacterial, antifungal, and antioxidant activities. Contrary to our findings, Mafakheri and Mirghazanfari (2018) reported that cumin EO exhibited a lower antifungal potential against *Alternaria* sp. In the study, *M. chamomilla* EO showed the lowest inhibitory effect compared to the other four essential oils. Sokovi'c and van Griensven (2006) showed that *M. chamomilla* EO has a weak antifungal effect, which is consistent with our results. Another study showed that *M. chamomilla* EO had moderate to weak effects against the growth of fungi (EL-Hefny et al., 2019). The obtained results were in agreement with those of Sazvar et al. (2022) who reported that the growth rate of *A. alternata* using *M. chamomilla* EO at a concentration of 200µL/L did not differ from the control application, but at higher concentration, the difference in the control application was significant. Our results showed that *C. atlantica* EO inhibited *A. solani* and *A. alternata* at varying rates. Similar to our results, Kumar et al. (2020) reported that *Curvularia lunata*, *A. alternata* and *Bipolaris spicifera* were susceptible to *Cedrus deodara* EO and formed different zones of inhibition showing its inhibitory effect. *C. atlantica* EO inhibited the growth of *Alternaria tenuissima* at concentrations of 1/250 and 1/500 (Chauiyakh et al., 2023). Eucalyptol (1,8-cineole) is the main chemical compound of *C. atlantica* EO. The inhibitory effect of Eucalyptol (1,8-cineole) against pathogenic fungi has been proved previously (Naz, 2011). The present findings show that *Z. officinale* EO has an inhibitory effect on the mycelial growth of *A. solani* and *A. alternata*. Consistent with our findings, Rizwana (2015) reported that various concentrations of *Z. officinale* EO had a moderate to high inhibitory effect on the mycelial growth of *A. alternata*.

Table 3. Inhibitory effect of 5 different PEOs on the mycelial growth of *Alternaria* species.

PEOs	Doses (µL/L)	*A. solani* (ET 66 isolate)		*A. alternata* (LAa 21 isolate)	
		Mycelial growth (mm)[1]	Percent inhibition (%)	Mycelial growth (mm)[1]	Percent inhibition (%)
N. sativa	0 (Control)	42.3 a*	0.0	42.4 a	0.0
	500	36.3 b	14.2	31.3 b	26.3
	1000	31.4 c	25.7	24.3 c	42.6
	1500	26.0 d	38.5	21.3 d	49.7
	2000	20.8 e	50.7	16.5 d	61.1
	2500	14.7 f	65.3	11.8 f	72.1
	$CV_{(0.01)}$	1.1		1.5	
C. cyminum	0 (Control)	42.3 a	0.0	42.4 a	0.0
	500	35.0 b	17.2	31.8 b	25.0
	1000	26.3 c	37.7	24.8 c	41.7
	1500	21.0 d	50.3	20.3 d	52.3
	2000	15.0 e	64.5	12.9 e	69.5
	2500	8.3 f	80.3	5.3 f	87.6
	$CV_{(0.01)}$	1.9		2.1	
M. chamomilla	0 (Control)	42.3 a	0.0	42.4 a	0.0
	500	36.8 b	12.8	33.5 b	21.0
	1000	32.3 c	23.7	27.3 c	35.6
	1500	27.1 d	35.9	21.9 d	48.3
	2000	21.3 e	49.7	16.8 e	60.5
	2500	15.2 f	64.1	13.0 f	69.4
	$CV_{(0.01)}$	1.9		2.3	
C. atlantica	0 (Control)	42.3 a	0.0	42.4 a	0.0
	500	36.1 b	14.6	32.7 b	23.0
	1000	31.0 c	26.6	28.3 c	33.4
	1500	22.4 d	46.9	21.6 d	49.1
	2000	17.8 e	57.8	16.1 e	62.0
	2500	12.5 f	70.4	10.7 f	74.9
	$CV_{(0.01)}$	1.6		2.8	
Z. officinale	0 (Control)	42.3 a	0.0	42.4 a	0.0
	500	36.8 b	12.8	35.5 b	16.3
	1000	32.0 c	24.3	29.6 c	30.3
	1500	26.3 d	37.9	24.3 d	42.6
	2000	19.3 e	54.4	17.8 e	58.2
	2500	14.8 f	65.1	12.5 f	70.5
	$CV_{(0.01)}$	1.9		2.0	

[1]The mean mycelial growth of *Alternaria* species was determined 10 days after inoculation. Based on three replicate plates, each observation. *Mean values followed by different letters within the column are significantly different according to the LSD Test (P≤ 0.01). CV: Coefficient of variation.

CONCLUSIONS

The results showed that PEOs of black cumin, cumin, chamomile, cedarwood, and ginger inhibited the mycelial growth of *Alternaria* species under *in vitro* conditions. The inhibitory effect against *Alternaria* species increased depending on the dose of All five EOs. The highest antifungal effect against *A. solani* (ET 66 isolate) and *A. alternata* (LAa 21 isolate) was obtained from high-dose (2500 µL/L) application of cumin PEO. The inhibitory effect of cumin PEO is thought to be due to the compound Cuminaldehyde. The second highest inhibitory effect against *Alternaria* species was detected in cedarwood PEO. The antifungal effect of cedarwood PEO is due to the compound Eucalyptol (1,8-cineole). The lowest inhibitory effect against *Alternaria* species was detected in the high-dose concentration of chamomile PEO. Therefore, cumin PEO can be used for controlling *A. solani*, and *A. alternata* and may be used as alternative control to synthetic chemicals. However, further studies are needed to explain the application time, dose, cost, and mechanism of action of selected PEOs.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

Author contribution

Oktay Erdoğan: conceptualization; investigation; methodology; data curation funding acquisition; writing-review & editing.

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Abbreviations

ANOVA: Analysis of variance, *A. solani*: *Alternaria solani*, *A. alternata*: *Alternaria alternata*, *N. sativa*: *Nigella sativa*, *C. cyminum*: *Cuminum cyminum*, *M. chamomilla*: *Matricaria chamomilla*, *C. atlantica*: *Cedrus atlantica*, *Z. officinale*: *Zingiber officinale*, GC-MS: Gas chromatography and mass spectrometry, LRI: Linear retention index, LSD: LSMeans Differences Student's test, CV: Coefficient of variation. PDA: Potato dextrose agar, PEOs: Plant Essential Oils, EOs: Essential Oils.

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RESEARCH ARTICLE

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A promising method for selecting imazamox-resistant sunflower plants

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Abstract

This study was conducted to investigate the potential of using the germination test as a model for screening imazamox resistance in sunflower plants . A standard germination test was performed by applying different doses of imazamox (control, 0.1, 0.2, 0.4, 0.8, and 1.6 mM) to imidazoline-resistant (IMI-R) and susceptible (IMI-S) sunflower hybrids. Germination percentage, mean germination time, germination index, shoot length, root length, seedling fresh and dry weight, and phytotoxicity percentage for seedling growth parameters were investigated. The results showed that imazamox concentrations slightly affected the germination percentage of two sunflower hybrids at only 1.6 mM. Increasing doses of imazamox led to an increase in the mean germination time and a decrease in the germination index for both sunflower hybrids, following a similar trend. Seedling growth parameters such as shoot length, root length, and seedling fresh weight were significantly decreased by increasing imazamox doses. In addition, the differences between IMI-R and IMI-S sunflower hybrids were very evident for these parameters. The IMI-S sunflower hybrid showed sensitivity in the presence of imazamox, while no changes in the IMI-R hybrid were determined up to 0.4 mM. The inhibition percentage was higher in the IMI-S hybrid than in the IMI-R. It was concluded that the optimal dose of imazamox for the selection of resistant plants during the early growth stage was found to be 0.2 mM. The germination parameters were not good criteria for imazamox resistance, while root length, shoot length, and seedling fresh weight should be considered as selection criteria for resistance to imazamox in sunflower.

Keywords: *Helianthus annuus* L., Herbicide, Resistant, Germination, Root length

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INTRODUCTION

In Türkiye, vegetable oil production depends mainly on sunflower (*Helianthus annuus* L.), which has been cultivated in arid and semi-arid regions under rainfed conditions. It has high adaptability and a high content (40-50%) quality oil in the seeds. Sunflower is affected by several adverse environmental conditions, such as drought, extreme temperature, salinity, weeds and broomrape (*Orobanche cumana* Wall.). It does not cope with the weeds due to its slow growth habits during emergence and early growth stages (Simic et al., 2011). In addition, the wide row spacing of 70 cm, which allows for lower planting densities across the field surface, makes it more susceptible to weed infestation. To solve weed management, IMI-resistant (IMI- R) sunflower hybrids have been developed, which allow the use of imidazolinone herbicides to control a wide variety of weeds in sunflower (Kaya and Kolsarıcı, 2005; Stefanic et al., 2023).

Imazamox, an imidazolinone (IMI) herbicide, is used post-emergent in leguminous crops and IMI-resistant wheat, sunflower, rice, lentil, and canola cultivars (Breccia et al., 2020). This herbicide effecctively controls both narrow and broadleaf weeds. The broomrape, a parasitic weed in plants belonging to *Asteraceae* species, is known as a sunflower parasite, which is one of the major factors destroying sunflower production (Mitkov et al., 2019). However, imazamox applications are highly effective in controlling broomrape along with other weed species (Mitkov et al., 2019; Shaner et al., 2019). For these reasons, the primary broadleaf, grass weed, and parasitic weeds from the genus *Orobanche* can be effectively controlled by producing hybrids of sunflowers that are resistant to imazamox (Fernandez-Martinez et al., 2009; Malidza et al., 2003).

Routinely, the seeds are sown and sprayed with imazamox herbicide at an appropriate dose when the plants are at V4-V8 stages in order to identify imazamox-resistant sunflower plants. Visual inspection is performed at 7 and 14 days after application (at score 0, there are no damages on the crop, and at score 9, the crop is completely destroyed) (Neshev et al., 2020). However, this method is an expensive, labor-intensive, and time-consuming process for early selection of IMI resistance. A pre-selection method should be developed to screen for resistant plants before field trials. For this reason, this study was undertaken to establish a simple, rapid, and effective model for the separation of imazamox susceptible and resistant sunflower plants using germination and early seedling growth under laboratory conditions.

MATERIALS AND METHODS

A standard germination test was performed to identify imazamox-resistant sunflower plants by comparing the germination and early seedling growth performance of resistant and susceptible sunflower hybrids at the Seed Science and Technology Laboratory, Eskişehir Osmangazi University, in 2024. Two commercial sunflower hybrids, the imidazolinone-resistant (IMI-R) hybrid SY Roseta CLP and the susceptible (IMI-S) hybrid SY Gibraltar, were used in this study.

The seeds were germinated at five levels of 0.1, 0.2, 0.4, 0.8, and 1.6 mM imazamox, prepared from Intervix Pro® with 40 g imazamox per liter. Distilled water was used for control. Four replicated fifty seeds from each sunflower hybrid were placed between three filter paper sheets with a dimension of 20 × 20 cm and each paper was watered with 7 mL of the respective imazamox solutions. After rolling filter papers, they were put into sealed plastic bags and transferred to an incubator at 25 °C for 8 days under dark conditions. Two millimeters of radicle protrusion was considered the germination criterion (ISTA 2018). Germination percentage (GP), mean germination time (MGT), and germination index (GI) were calculated as Equations 1, 2, 3, and 4:

$$GP\ (\%) = \frac{Germinated\ seeds\ at\ final\ day}{Total\ seeds} \text{ x } 100, \text{ (ISTA, 2018) (1)}$$

$$MGT\ (day) = \frac{\sum Dn}{\sum n} \text{ (ISTA, 2018) (2)}$$

where, n is the seed number germinated on day D, and D is the number of days from the beginning of the germination test.

$$GI = \frac{Number\ of\ germinated\ seeds}{Days\ of\ the\ first\ count} + \cdots + \frac{Number\ of\ germinated\ seeds}{Days\ of\ the\ final\ count} \text{ Salehzade et al. (2009) (3)}$$

On the last day (8th day), ten seedlings randomly selected from each imazamox level were sampled to determine shoot length (SL), root length (RL), and seedling fresh (SFW) and dry weights (SDW) after they were dried in an air oven at 80 °C for 24 h. The inhibition percentage was determined using the formula described by Archana et al. (2016).

$$\%\ inhibition = \frac{Control - Test\ solution}{Control} \text{ x } 100 \text{ (4)}$$

Statistical Analysis

The experiment was set up in a two-factor factorial in a completely randomized design with four replicates. Data were analyzed using the computer program MSTAT-C (v. 2.10, Michigan State University), and the differences were compared using the Least Significant Differences (LSD) test at the 5% level. A quadratic regression equation (was used to calculate the toxic level (50% reduction from control) of imazamox for each parameter (Harrison et al., 1976). In the regression equation, the dependent variable, y, was set to 50% of the related parameters in the control group.

RESULTS

A significant interaction between sunflower hybrids and imazamox doses was found for all parameters except germination percentage (Table 1). Germination percentages of the sunflower hybrids were similar, but increased imazamox doses significantly decreased at 1.6 mM. The mean germination time of the IMI-R and IMI-S hybrids was different and prolonged with increasing doses of imazamox. A similar trend was observed for two hybrids, with IMI-R germinating faster than IMI-S (Figure 1A). Also, a higher germination index was calculated in IMI-R at all levels of imazamox, but it was reduced by increasing imazamox (Figure 1B). There was a significant difference in shoot length between IMI-S and IMI-R sunflower hybrids at all doses of imazamox. Shoot length was sensitive to imazamox treatments because differences between sunflower hybrids were evident. The shoot length of IMI-S was greatly reduced in the presence of imazamox, however, it was significantly decreased in the IMI-R hybrid at 0.4 mM (Figure 1C). The toxic levels of imazamox were calculated as 1.04 mM and 0.57 mM for IMI-R and IMI-S hybrids, respectively, using the regression equations in Figure 1C. Imazamox caused a reduction in root length of sunflower hybrids. In the presence of imazamox, the root length of the IMI-S hybrid was apparently decreased, but a significant reduction in IMI-R hybrid was observed at 0.4 mM. In both sunflower hybrids, the minimum root length was obtained at the imazamox dose of 1.6 mM, which was similar to each other (Figure 1D). A 50% reduction in root length was estimated to be 0.92 mM for IMI-R and 0.24 mM for IMI-S hybrid. The seedling fresh weight varied depending on the reduction in root and shoot length,. IMI-R sunflower hybrid produced a higher seedling fresh weight than IMI-S, while it was adversely affected at higher levels than 0.4 mM (Figure 1E). The toxic concentrations of imazamox for the fresh weight of the seedlings of the IMI-R and IMI-S hybrids were 0.52 mM and 0.55 mM, respectively. There was a significant difference between sunflower hybrids regarding seedling dry weight in control. However, imazamox increased the dry weight of the IMI-R sunflower hybrid and exhibited heavier dry weight under high doses of imazamox.

As expected, inhibition percentages for shoot length, root length, and seedling fresh weight of IMI-S were higher than those of the IMI-R hybrid (Figures 2A, B and C). At 0.1 mM imazamox, the IMI-S hybrid had the highest inhibition percentages. However, the inhibition percentage of the IMI-R hybrid was significantly increased at 0.4 mM, and higher concentrations of imazamox induced the inhibition percentage.

Table 1. Analysis of variance and main effects of imazamox doses on the investigated characteristics of IMI-S and IMI-R sunflower hybrids.

Factors	GP (%)	MGT (day)	GI	SL (cm)	RL (cm)	SFW (mg plant^{-1})	SDW (mg plant^{-1})
Hybrids (A)							
IMI-S	86.6	2.20^{a}	20.8^{b}	3.31^{b}	2.15^{b}	2585^{b}	519^{a}†
IMI-R	86.5	1.97^{b}	24.3^{a}	6.55^{a}	7.77^{a}	4086^{a}	480^{b}
Imazamox doses (B)							
Control	85.7^{b}	2.01^{c}	22.5^{b}	6.80^{a}	8.15^{a}	4432^{a}	499^{b}
0.1 mM	91.0^{a}	2.01^{c}	25.0^{a}	5.36^{c}	6.51^{b}	3503^{c}	489^{b}
0.2 mM	88.5^{ab}	1.99^{c}	24.9^{a}	5.98^{b}	6.65^{b}	3702^{b}	503^{ab}
0.4 mM	87.5^{ab}	2.13^{b}	22.2^{b}	4.38^{d}	3.88^{c}	3188^{d}	494^{b}
0.8 mM	85.5^{b}	2.16^{ab}	21.1^{c}	4.15^{d}	2.84^{d}	2742^{e}	498^{b}
1.6 mM	81.2^{c}	2.23^{a}	19.6^{d}	2.89^{e}	1.73^{e}	2449^{f}	516^{a}
Analysis of variance							
A	*ns*	**	**	**	**	**	**
B	**	**	**	**	**	**	*
A×B	*ns*	*	**	**	**	**	**

significant at 1%, *ns*= non-significant, †= Letter(s) connected with the means denote significance levels at P<0.05.

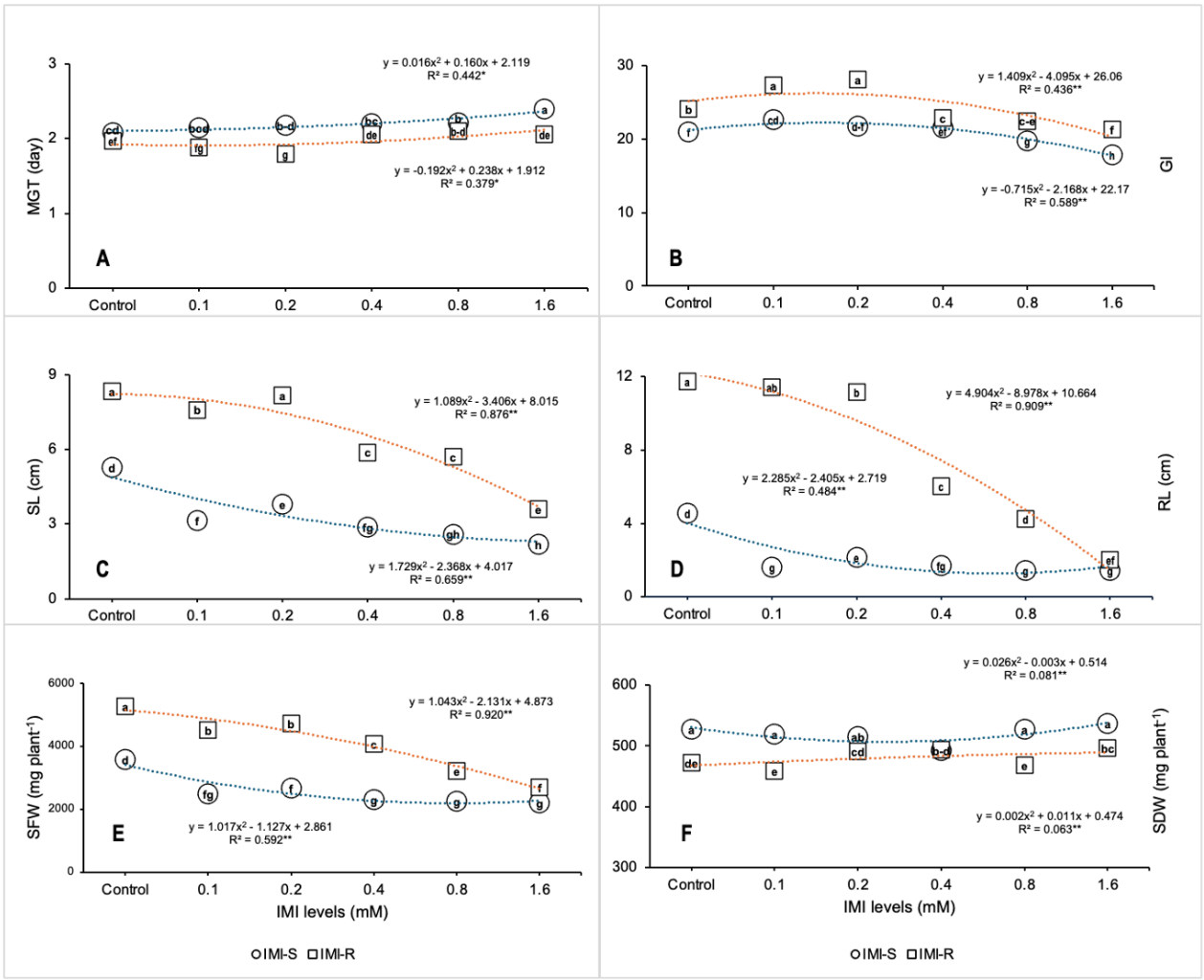

Figure 1. Changes in the investigated parameters of IMI-S and IMI-R sunflower hybrids exposed to increasing imazamox doses.

Table 2. Inhibition percentage of shoot length, root length, and seedling fresh weight of IMI-S and IMI-R sunflower hybrids

Factors	Inhibition %		
	SL	RL	SFW
Hybrids (A)			
IMI-S	37.1[a]	52.7[a]	27.9[a]
IMI-R	21.4[b]	33.8[b]	22.5[b]
Imazamox doses (B)			
Control	-[f]	-[f]	-[f]
0.1 mM	24.6[d]	33.8[d]	22.5[d]
0.2 mM	14.9[e]	28.9[e]	17.9[e]
0.4 mM	37.3[c]	55.4[c]	29.2[c]
0.8 mM	41.1[b]	66.0[b]	37.9[b]
1.6 mM	57.6[a]	75.5[a]	43.7[a]
Analysis of variance			
A	**	**	**
B	**	**	**
A×B	**	**	**

significant at 1%, *ns*= non-significant, †= Letter(s) connected with the means denote significance levels at P<0.05.

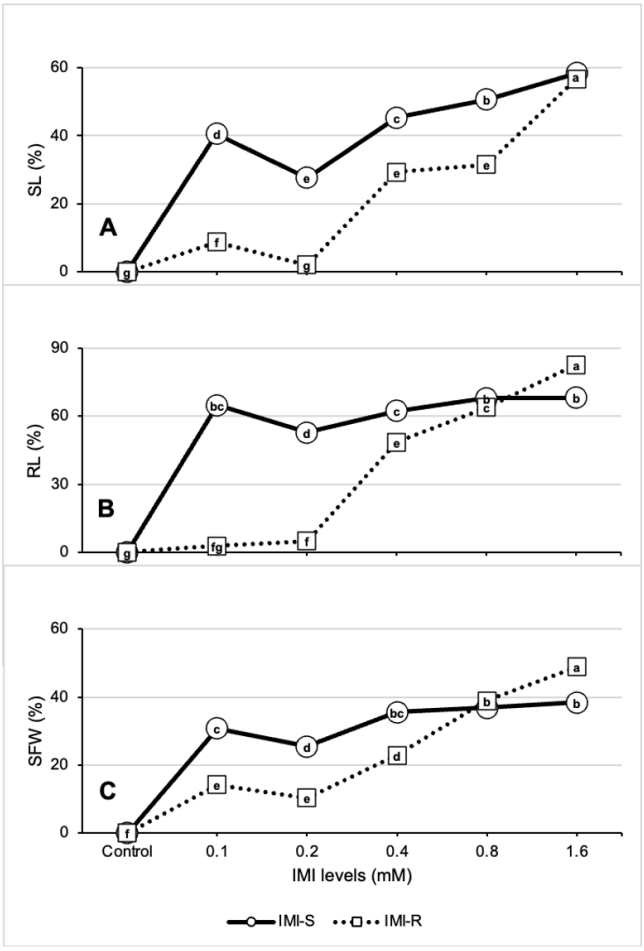

Figure 2. Inhibition percentages of shoot length, root length and seedling fresh weight of IMI-R and IMI-S sunflower hybrids under different imzamox concentrations.

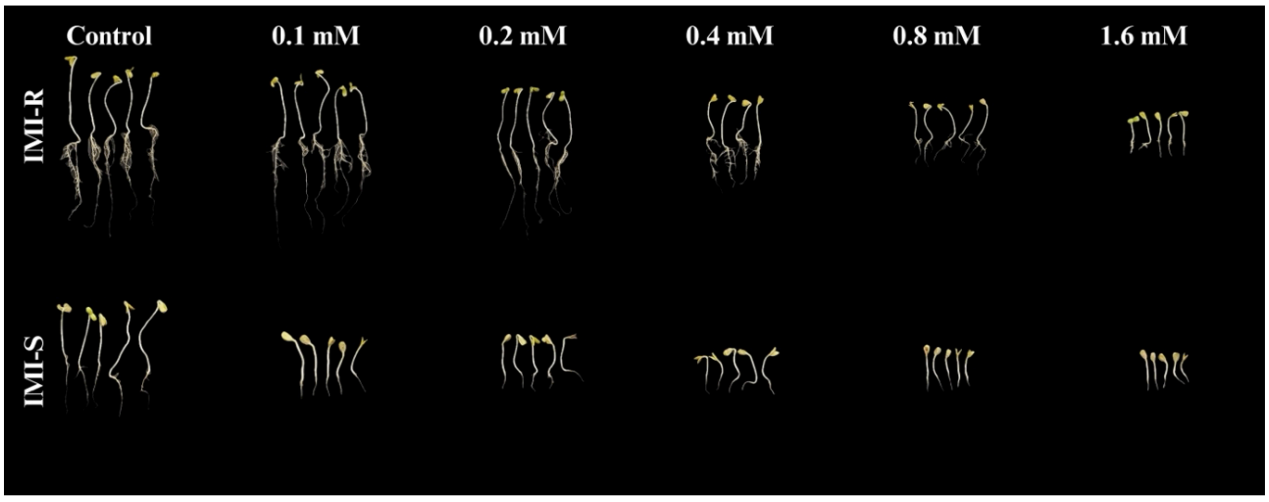

Figure 3. Changes in seedling growth of IMI-R and IMI-S sunflower hybrids subjected to different doses of imazamox.

DISCUSSION

This study showed that the germination percentage of IMI-S and IMI-R sunflower hybrids was not affected by low and medium doses of imazamox but was slightly inhibited by an overdose (1.6 mM). However, the mean germination time was delayed and the germination index was reduced as the imazamox concentration was increased. These parameters showed similar behavior in IMI-S and IMI-R sunflower hybrids, but there was no significant separation between resistant and susceptible hybrids. Our results confirmed the findings of Haliloğlu et al., (2022) in wheat, who found that germination percentage was not changed by imazamox doses under different germination mediums. These results suggest that imazamox toxicity begins after germinating seeds absorb water through the roots.

Seedling growth characteristics, including shoot length, root length, and seedling fresh weight, were severely inhibited in the presence of imazamox in the IMI-S sunflower hybrid. In contrast, the IMI-R hybrid exhibited no observable appearance up to a dose of 0.4 mM imazamox. The sensitivity of root growth in sunflower IMI-S was pronounced, with depletion observed at 0.1 mM imazamox. A clear difference was determined between the root lengths of the IMI-S and IMI-R hybrids (Figures 1D and 2B). This result is consistent with the findings of Breccia et al., (2018), who found that imazamox-sensitive wheat cultivars showed a sharp decrease in root length in the presence of imazamox. Similarly, shoot length responded to imazamox and successfully separated the IMI-R from the IMI-S hybrid. The availability of imazamox resulted in decreased shoot length in the IMI-S hybrid, while it was reduced at 0.4 mM in the IMI-R hybrid. Imazamox reduced the fresh weight ofseedling , but the response of sunflower hybrids varied basedthe length of shoot and root. Similar findings were observed in wheat by Breccia et al., (2018) and Haliloğlu et al., (2022), who found that root and shoot growth were inhibited by increasing doses of imazamox. Coversely, this study did not report any dead seedlings. Therefore, the duration of the experiment was terminated at 8 days because the sunflower seedlings in the control started to deteriorate, which was not enough time for the seedlings to die.

The inhibition percentage for root length, shoot length, and seedling fresh weight was higher in the IMI-S than in the IMI-R hybrid, and they provided a more accurate selection of imazamox-resistant plants from susceptible ones (Figure 2). Surprisingly, the inhibition percentages of IMI-R increased continuously as imazamox concentrations increased.

CONCLUSION

These results indicated that germination parameters could not be used as selection criteria for sunflower plants resistant to imazamox. Seedling growth was much more sensitive to imazamox, and the IMI-S hybrid was more affected by imazamox than the IMI-R hybrid. Root length, shoot length, and seedling fresh weight should be successfully evaluated to identify imazamox-resistant genotypes in sunflower germplasm. In addition, the percentage of phytotoxicity for seedling growth characteristics could be used as valuable criteria for imazamox resistance. Imazamox concentrations of 0.2-0.4 mM should be preferred for screening the sunflower genotypes.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared that there is no conflict

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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Data availability

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