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Research Article

The Relationship of POU1F1-HinfI Gene Polymorphisms on Milk Yields in Simmental Cattle

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Simmental

Abstract: As the global population grows, there is a need to produce higher yields in food, agriculture, and livestock. To achieve this, scientists are exploring new techniques and methods. However, it is crucial to select the right candidate genes and markers, especially in techniques like QTL and MAS in livestock, to ensure success. We conducted a study to determine allele frequencies and their association with milk yield in 70 Simmental cattle breeds in two lactations using the PCR-RFLP technique. The statistical analysis was conducted using the general linear model procedure with the least square method The study focused on the allele frequencies for the POU1F1-*HinfI* gene in Simmental cattle. The dominant B allele frequency for POU1F1/Hinf1 was 0.58, while the A allele frequency was 0.42. Simmental population was under HardyWeinberg Equilibrium (HWE) for the POU1F1-*HinfI* genotypes ($p>0.05$) The study found that genotype frequencies were in balance for POU1F1/Hinf1. No significant correlation between POU1F1-*HinfI* gene polymorphisms and milk yield was found, but they have been associated with growth and reproductive traits in various cattle breeds. The results could provide useful information for breeding programs aimed at improving the performance traits of Simmental cattle.

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1. Introduction

The determination of the best genotypes is hampered by the fact that the majority of economic features are influenced by polygenes and vulnerable to environmental fluctuations. In order to understand how the characteristics of the genes involved in a population are changed by non-genetic factors that might have an impact on a metric trait, statistical approaches are required. Transcription of the genes encoding the growth hormone (GH), prolactin (PRL), and thyroid stimulating hormone (TSH) subunits is dependent on the pituitary-specific transcription factor (POU1F1; also known as Pit-1), (Li et al.1990). These genes are involved with a wide variety of signaling pathways that are essential for a variety of physiological and developmental processes, including pituitary gland development (Li et al., 1990; Mullis 2007), mammary gland growth (Sjaunja and Olsson, 2005), expression of milk proteins, milk production, and secretion (Sjaunja and Olsson, 2005; Akers, 2006). A HinfI polymorphism is located within exon 6 and characterized by a silent mutation on HinfI (–) allele (Dierkes et al., 1998), and their effects were associated with growth traits in cattle (Silva et al., 2006). The available sources provide references to multiple studies that have explored the associations between POU1F1gene

polymorphisms and different traits in cattle. POU1F1 was discovered to be associated with milk yield, protein yield, protein percentage (Zhang et al., 2009; Özdemir, 2012), fat milk production (De Mattos et al., 2004), growth traits (Kai et al., 2006; Carrijo et al., 2008) body weight (Renaville et al., 1997), some feeding criteria, and carcass dimensions in cattle (Oprzadek et al., 2003; Ozdemir et al., 2018). It was also found to be significantly associated with variation in fertilization and embryonic survival rates (Khatib et al., 2009; Laporta et al., 2011).

The marker-assisted selection techniques (MAS), applied depending on the quantitative trait locus maps created depending on the proximity of the markers to the relevant gene region or quantitative features on the chromosomes, allow the application of molecular techniques in plant and animal breeding and enable to obtain quality products with the desired characteristics and yield in a short time. Today, thanks to the developing technological methods and applications, genome-level mapping, and genome-wide association studies can be performed in breeding studies (Das et al., 2021; Ozdemir, 2021; Reshma and Das, 2021). The allele frequencies of the studies on POU1F1/HinfI gene polymorphism on different breeds between 2012 and 2023, the breeds in which the studies were conducted, and some of their numbers are given in Table 1.

Table 1. Between 2012 and 2023, several studies have been conducted to investigate the frequency of the POU1F1/Hinf1 gene variant

Breeds		**Allele frequencies**		**References**
POU1F1/Hinf1	**N**	**A (A)**	**B (G)**	
Holstein calves	100	0.34*	0.66	(Fındık and Özdemir, 2022)
Anatolian Black cattle	104	0.36*	0.64	(Sakar and Zülkadir, 2022)
Turkish Grey Steppe	137	0.46*	0.54	(Cobanoglu and Ardicli, 2022)
Anatolian Black	105	0.91	0.09	(Cobanoglu and Ardicli, 2022)
East Anatolian	125	0.45*	0.55	(Cobanoglu and Ardicli, 2022)
Holstein Friesian,	50	0.32*	0.68	(Aytekin and Bayraktar, 2022)
Brown Swiss	50	0.10*	0.90	(Aytekin and Bayraktar, 2022)
Simmental	50	0.23*	0.77	(Aytekin and Bayraktar, 2022)
Iranian Holstein	134	0.32*	0.68	(Sadeghi et al., 2022)
Buffaloes	60	0.1*	0.9	(Zghairand and Hassooni, 2021)
Limousine	115	0.29*	0.71	(Pytlewski et al., 2022)
Holstein	147	0.25*	0.75	(Cañizares Martínez et al., 2021)
Polish Holstein Fresian (PHF)	1024	0.20*	0.80	(Pytlewski et al., 2018)
Holsteins	245	0.80	0.20	(Ozdemir et al., 2018)
Grati-Ongole Grade cattle	107	0.01*	0.99	(Hartati et al., 2018)
Slovak Simmental	288	0.23*	0.77	(Trakovická et al., 2015)
Holstein	100	0.25*	0.75	(Mohammadabadi et al., 2015)
Sahiwal breedcattle	77	0.19*	0.81	(Chauhan et al., 2015)
Slovak Spotted	110	0.30*	0.70	(Moravčíková et al., 2013)
Holstein	100	0.53	0.47	(Heidari et al., 2012)

*Allele gene frequencies similar to our study.

Understanding the associations between POU1F1 gene polymorphisms and traits in cattle can have implications for breeding programs. By identifying specific genetic variants associated with desirable traits, breeders can make informed decisions to select animals with favorable genetic profiles for breeding purposes.

İn this study, POU1F1/*HinfI* gene polymorphisms were identified in the Simmental cattle breed, and their association with milk yield of genotypes and their usability in animal breeding were investigated.

2. Material and Methods

2.1. Material

In a study blood samples were collected from 70 Simmental cattle breeds, which were reared under intensive conditions in a private farm in Erzurum, from the tail vein under veterinary control and

the samples were collected in vacutainer blood tubes containing K3EDTA anticoagulant.DNA samples were extracted from the collected blood using the QIAGEN Genomic DNA Purification kit (Gentra Puregene, USA), following the manufacturer's instructions.

2.2. Methods

The primer sequences used in the PCR process was shown in Table 2.

Table 2. POU1F1/*HinfI* gene PCR primers

Gene region	Reference	Primer sequences	PCR product
POU1F1/ *HinfI*	Ozdemir (2012)	F:5-ACTCGCTATTACACAATAGGAGCCT-3 R: 5-TCCTGCCAACTCCTCACCTCCC-3	260 bp

The PCR reaction mixture (10 mL) contained; 2 µLgenomic DNA, 0.25 µL F –Primer, 0.25 µL R-Primer, 2.5 µL of dNTPmix (D7595: Sigma, St. Louis, MO, USA, 0.25mM), 0.5 units of Taq DNA polymerase (D1806: Sigma), 5 µL of 10x PCR Buffer, 1.5 µL of 0.25 mM $MgCl_2$ and ddH_2O making the total volume of 30 µL were used for PCR amplification. The reaction conditions used in amplifications are given in Table 3.

Table 3. POU1F1/*HinfI* gene PCR conditions

Gene polymorphism	Initial denaturation	Denaturation Annealing	Extension	Number of cycles	Final extension
POU1F1	95 C/30 sec	61 C/45 sec	72 C/2 min	33	72 C/5 min

The POU1F1/*HinfI* gene was amplified using PCR, and the resulting PCR products were treated with 2-5 U of restriction enzymes and incubated at 37 °C for 12 hours. The digested products were separated on a 3.0% agarose gel for 2.0 hours at 50 Volts and visualized under UV light. The allele frequency of the base mutation of each gene was determined using POPGENE software (Francis et al., 1999) to check for Hardy-Weinberg equilibrium.

In order to investigate the influence of POU1F1/ *HinfI* gene polymorphisms on lactation and milk yield traits in Simmental cattle, a variance analysis was conducted between the polymorphic regions and milk yield records of animals in different lactations. The study was carried out on a private enterprise in Erzurum, where milk production records were systematically kept between 2017 and 2020. The statistical analysis used lactation milk yield, 305-day milk yield, lactation period, and daily milk yield as parameters to evaluate the association between genotype and milk yield traits. The data were analyzed using the general linear model in SPSS 25.0 software package (IBM Corp. 2017. SPSS 25.0 released 2017.). Environmental factors such as genotype, lactation order, and calving seasons were considered to have an impact on the relevant yield trait and were taken into account in the analysis.

According to the yield traits in the research, the following statistical model was employed.

$$Yijkl: \mu + ai + bj + ck + eijkl \quad (1)$$

Where

$Y_{\_ijkl}$ is any of the milk yield traits (lactation milk yield, 305-day milk yield, daily milk yield, and lactation period),

μ is the population mean,

a_i is the i^{th} genotype effect,

b_j is the effect of the j^{th} lactation order (j: 3; 1^{st} Lactation: 1, 2^{nd} Lactation: 2, 3^{rd} Lactation: 3),

c_k is the effect of the k^{th} calving season (k: 2; 1: winter-spring, 2: summer-autumn),

$e_{ijkl\_}$ is the error.

3. Results

The figures show the PCR amplification results and base sizes of PCR products Figure 1.

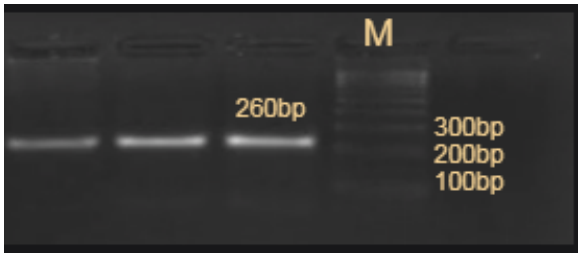

Figure 1. PCR results of the POU1F1 gene regions (260bp, with Standard GENESTA 100 bp DNA marker images, respectively).

A representative agarose gel image of the PCR-RFLP results for these polymorphisms under UV light is shown in Figure 2.

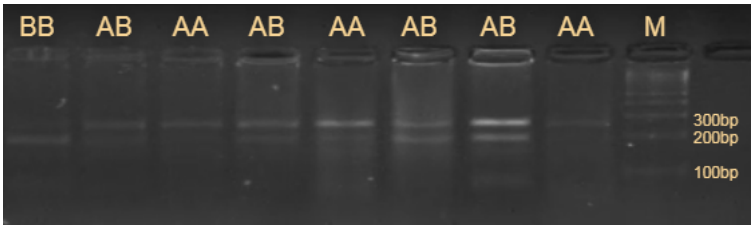

Figure 2. POU1F1/ *HinfI* restriction fragment size: AA; 260 bp, BB; 190 bp and 70 bp, AB; 260 bp, 190 bp, and 70 bp With Standard GENESTA 100 bp DNA marker.

Table 4. The genotype, allele frequencies, and H-W equilibrium for POU1F1/ *HinfI* genes

Genotype	N	allele A	allele B	Observed Frequency	Genotype Frequencies	H-W
AA	11	22	0	11	11.701	
AB	34	34	34	34	32.597	
BB	22	0	44	22	22.701	0.72^{ns}
Total	67	56	78	67		
p-value	134	0.42	0.58	134		

P-value for the test for Hardy-Weinberg equilibrium, in which P >0.05 indicates that the sampled population is in Hardy-Weinberg equilibrium, ns: nonsignificant. (1 degree of freedom), H-W: Hardy Weinberg.

Allele and genotype frequencies of the remaining 67 Simmental DNA samples were calculated after excluding three samples in which the cut region could not be detected as a result of RFLP analyses. As a result of determining allele and genotype frequencies in Simmental cattle, it was found that the AA, AB, and BB genotype frequencies for the POU1F1 gene were 0.164, 0.508, and 0.328, respectively. The Hardy-Weinberg genetic equilibrium test showed that the distributions of POU1F1 genotype frequencies were in balance (P > 0.05) in the studied breed (Table 4).

Table 5 presents the values of milk performance traits for daily milk yields, corrected milk yields, and lactation periods in 100 Simmental herds in different lactations depending on the POU1F1/*HinfI* genotype.

As a result of the milk yield analyses we made in the Simmental cattle breed, whose numbers vary at different rates and have more than one lactation data on average, cows produced 4985 kg of milk, 5323 kg of real milk, and 17460 kg of milk over a 305-day period, with a lactation period of 285444 days. The AB genotype in the polymorphic regions resulted in the highest milk yield, while the AA genotype in the POU1F1/*HinfI* polymorphic region resulted in the lowest milk yield. However, the difference in milk yield between the highest and lowest genotypes was not significant. The second season range had the highest overall averages for all yield values, while the first season range had the

lowest averages. When considering lactation order, individuals in their 9th lactation had the highest average 305-day milk yield of 5658 kg, while those in their 4th lactation had the lowest average of 4898 kg. The highest daily milk yield average of 18,537 kg was found in individuals in their 9th lactation, while the lowest average of 16,099 kg was found in those in their 4th lactation. The highest real milk yield of 5270 kg was measured in the highest 6 lactations, while the lowest average of 4764 kg was found in the lowest 4 lactations. Lactation periods were longest in the highest 4 lactations at 302,229 days, and shortest in the 7th lactation period at 253,206 days (Table 5). In this study, there was no statistically significant difference between the milk yield averages of 100 Simmental cattle in 5 lactations and the average of genotype, calving season, and lactation order factors ($P > 0.05$).

Table 5. Effects of POU1F1/*HinfI* genotypes on milk yields in Simmental cattle

Variation Sources		N	Lactation milk yield	305-day milk yield	Daily milk yield	Lactation period
			$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$
POU1F1/ *HinfI*	AA	11	4569.145±514.017	5027.714±403.634	16.479±1.324	284.510±25.558
	AB	63	5546.732±246.071	5651.558±193.229	18.545±0.634	304.344±12.235
	BB	26	4841.260±339.780	5291.175±266.814	17.355±0.875	267.477±16.894
		P	0.070	0.234	0.228	0.148
Calving season	1	62	4771.295±296.357	5128.166±232.716	16.806±0.764	281.655±14.735
	2	38	5200.130±313.769	5518.798±246.389	18.114±0.808	289.23±15.601
		P	0.254	0.187	0.178	0.685
Lactation order	3	3	4823.382±938.570	5222.156±737.017	17.129±2.418	285.844±46.667
	4	8	4764.374±580.059	4898.207±455.494	16.099±1.494	302.229±28.841
	5	17	5198.082±401.742	5447.053±315.470	17.87±1.035	294.281±19.975
	6	22	5270.972±361.534	5507.864±283.897	18.055±0.931	298.698±17.976
	7	25	4811.469±349.726	5374.027±274.624	17.620±0.901	253.206±17.389
	8	17	4963.763±409.244	5156.588±321.361	16.899±1.054	296.910±20.348
	9	8	5067.945±613.693	5658.480±481.905	18.537±1.581	266.939±30.514
		P	0.960	0.878	0.884	0.435
Total		100	4985.713±241.234	5323.482±189.430	17.460±.62	285.444±11.995

$\overline{X}$: General Means, $S_{\overline{x}}$: Standard deviation.

As a result of the milk yield analyses we made in the Simmental cattle breed, whose numbers vary at different rates and have more than one lactation data on average, cows produced 4985 kg of milk, 5323 kg of real milk, and 17.460 kg of milk over a 305-day period, with a lactation period of 285.444 days. The AB genotype in the polymorphic regions resulted in the highest milk yield, while the AA genotype in the POU1F1/*HinfI* polymorphic region resulted in the lowest milk yield. However, the difference in milk yield between the highest and lowest genotypes was not significant. The second season range had the highest overall averages for all yield values, while the first season range had the lowest averages. When considering lactation order, individuals in their 9th lactation had the highest average 305-day milk yield of 5658 kg, while those in their 4th lactation had the lowest average of 4898 kg. The highest daily milk yield average of 18.537 kg was found in individuals in their 9th lactation, while the lowest average of 16.099 kg was found in those in their 4th lactation. The highest real milk yield of 5270 kg was measured in the highest 6 lactations, while the lowest average of 4764 kg was found in the lowest 4 lactations. Lactation periods were longest in the highest 4 lactations at 302.229 days, and shortest in the 7th lactation period at 253.206 days (Table 5). In this study, there was no statistically significant difference between the milk yield averages of 100 Simmental cattle in 5 lactations and the average of genotype, calving season, and lactation order factors ($P > 0.05$).

4. Discussion

The POU1F1/Hinf1 gene has been studied in several populations of Simmental cattle, with varying results in terms of allele frequencies. In Table 1, we can see the frequencies of the A and B alleles in different populations, as reported in previous studies. Our study found that the frequency of the dominant B allele for POU1F1/Hinf1 in Simmental cattle was 0.58, while the frequency of the

recessive A allele was 0.42. This suggests that the B allele is more common in our population than the A allele.

Comparing our results to those in Table 1, we can see that the frequency of the B allele in our population is similar to that reported in some studies, while it is higher than in others. The frequency of the A allele in our population is also similar to that reported in some studies, while it is lower than in others. Overall, our study provides new information on the allele frequencies of the POU1F1/Hinf1 gene in Simmental cattle, which can be used to better understand the genetics of this population.

We performed a Hardy-Weinberg equilibrium test to assess the balance of genotype frequencies in the herd. Our results indicated that the genotype frequencies for POU1F1/Hinf1 were in balance ($P>0.05$), likely due to the closed barn environment, absence of external migration, and controlled breeding practices. Similarly in our study, it has been reported that POU1F1/*HinfI* genotype frequencies are in Hardy-Weinberg equilibrium in Holstein calves, Anatolian Black Steppe, and Turkish Grey cattle (Ozdemir et al., 2018; Cobanoglu and Ardicli, 2022; Fındık and Özdemir, 2022; Sakar and Zülkadir, 2022; Sadeghi et al., 2022), and similarly, genotype distributions are balanced in Holstein Friesian, Brown Swiss, and Simmental cattle breeds (Mohammadabadi et al., 2015; Aytekin and Bayraktar, 2022). On the other hand, in different cattle breeds such as Grati-Ongole, Bos Indicus, and Bali cattle (Bos javanicus), it has been reported that Pit-1 genotype distributions are not in Hardy-Weinberg equilibrium and there are deviations in HW equilibrium (Jakaria and Noor, 2015; Hartati et al., 2018).

In our study, due to the limited size of our Simmental breed and milk yield records, we were unable to establish any significant correlation between POU1F1/*HinfI* genotype polymorphisms and milk yield ($P>0.05$). Similar studies on different breeds, such as buffaloes, Slovak Simmental, and Holstein cattle, have also reported no significant association between these genotypes and milk yield (Heidari et al., 2012; Ebrahimi et al., 2015; Trakovická et al., 2015; Ozdemir et al., 2018; Thuy et al., 2018; Zghair and Hassooni, 2021). However, studies conducted in the Horasan region of Iran reported that the POU1F1/*HinfI* genotype polymorphism is linked to milk composition in Holstein cattle, affecting protein and fat percentages, and in Shawial cattle, affecting total milk yield and 300-day milk yield (Chauhan et al., 2015; Mohammadabadi et al., 2015; Sadeghi et al., 2022) Further research with larger sample sizes and more diverse breeds is necessary to better understand this relationship.

Studies investigating the association of POU1F1 gene polymorphisms have not only focused on milk yield but also on growth and reproductive traits in various cattle breeds. Specifically, research on Polish Black-and-White Holstein-Friesian and Limousine cattle and calves has found that Pit-1 gene polymorphisms are associated with body weight, age at sexual maturity, and reproductive parameters such as insemination index (Pytlewski et al., 2018; Pytlewski et al., 2022). However, studies on Pit-1/*HinfI* polymorphisms and growth parameters have yielded mixed results, with some studies reporting no association with birth weight in calves, growth in Black Anatolian cattle, and growth and reproductive parameters in Canchim cattle (Grossi et al., 2015; Fındık and Özdemir, 2022; Sakar and Zülkadir, 2022).

Conclusion

In association analysis studies published to determine the potential of the gene polymorphisms to be used as markers in cattle breeding, the above-mentioned genes appear to have significant implications for body weight, meat yield, and reproductive performance in general. There is still much to learn about the effects of POU1F1 gene polymorphisms on Simmental cattle performance traits. Future research should focus on expanding the sample size to increase the statistical power of the study. Additionally, studies should examine other genetic factors that may interact with these genes to influence performance traits. Finally, more research is needed to determine how these findings can be applied in practical breeding programs to improve Simmental cattle performance as markers in milk data analyses.

Ethical Protocol

Ethical Protocol was approved by the experimental animal ethics committee of Atatürk University with its session dated 27.10.2022 and decision numbered 257.

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Research Article

Nematophagous Fungi Isolated from Municipal Waste-contaminated Soil in Medan City, North Sumatera: Morphological Identification, Phylogeny Analysis and Assessment as Root-knot Nematodes Biocontrol

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Abstract: Root-knot nematodes (RKNs) are groups of nematodes that cause significant diseases in horticultural and field crops. Chemical pesticides used to control RKNs could pollute environmental resources and ultimately affect human health. Therefore, eco-friendly efforts are needed. Previous research revealed that nematode-trapping fungi (NTFs) as the biological enemies of nematodes has been observed suppressing the nematode population. This study aimed to isolate NTF species from municipal waste-contaminated soil in Medan City, Indonesia, and identified them using morphological and molecular analysis. Furthermore, their biocontrol potential against *Meloidogyne hapla* Chitwood (Nematoda: Meloidogynidae) was assessed. Soil sample covered seven districts with seven repeats for isolation and *in vitro* assessment against *M. hapla* was done on CMA and observed after 12-72 hours. Three isolates were successfully obtained and proven effective in suppressing *M. hapla* by 97.7% (isolate sH51 and sH52) and 89.27% (isolate sH53). Morphological identification on PDA and genetic analysis of ITS concluded that sH51 is *Drechslerella brochopaga* Drechsler (Ascomycota: Orbiliaceae) and sH53 is *Arthrobotrys thaumasius* Drechsler (Ascomycota: Orbiliaceae). Morphological analysis for isolate sH52 reveals it as *Arthrobotrys sinensis* but is limited to *Arthrobotrys* sp. based on phylogeny analysis thus additional gen needs to be sequenced for confirmation.

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1. Introduction

Root-knot nematodes (RKNs) are a group of several parasitic nematodes infecting various horticultural crops. Their infection leads to the formation of knots in plant roots which subsequently intervene in water and nutrient transportation systems and trigger more extensive breakdown such as canopy leaves yellowing, stunting, withering, hypertrophy, and decreased yield (Istiqomah and Pradana, 2017; Asyiah et al., 2021). The most well-known and significant of them is *Meloidogyne* spp. (Nematoda: Meloidogynidae). Most of the efforts to control RKNs especially in Asian countries are the

utilization of chemical pesticide such as carbofuran (Sim et al., 2019) which involves soil microbiomes intervention (Güven and Koç, 2020) and further promoting bioaccumulation and biomagnification (Mishra et al., 2020). Sustainable alternatives are employing various biological agents to inhibit the development of some stages in their life cycle (Mendoza-de Gives, 2022) without intervening with other non-target organisms in ecological systems such as natural metabolites (Göze Özdemir et al., 2021), bacteria (Migunova and Sasanelli, 2021), and fungi (Zhang et al., 2020).

Several biological agents possessing antagonistic interaction towards RKNs have been used to control their population such as fungal nematode-trapper (Sharma et al., 2021; Youssef and El-Nagdi, 2021). Nematode-trapping fungi (NTFs) are important species of fungi known for evolving a wide variety of traps to enamor and predate nematodes as a food source. Also, trap forms are various including constricting rings, adhesive knobs, columns, and networks (Su et al., 2017). Previously, several NTFs from Family Orbiliaceae (Phylum: Ascomycota) such as *Dactylella* sp., *Monacrosporium* sp., and *Arthrobotrys* sp. had been successfully isolated from soil (Hastuti and Faull, 2018). Furthermore, some species had been proven experimentally to be able to reduce the juvenile population of *Meloidogyne* spp. and reduce root cavities by more than 80% (Kang et al., 2019; Singh et al., 2019; Yusuf, 2019). *Arthrobotrys vermicola* Rifai was observed repressing nematodes by 99.8% and lessening root cavities by 60% (Tarigan, 2021). On the other hand, *Arthrobotrys oligospora* Fresen, *Candelabrella musiformis* Rifai and Cooke, and *Dactylaria eudermata* Drechsler showed an ability to reduce vermiform of *Meloidogyne incognita* Chitwood and root-knot in tobacco after 7, 15, 30 days (Hastuti and Faull, 2018). These results are extremely and sustainably satisfying; thus, NTF should be evaluated as a substitution for less environmental-friendly pesticides such as carbofuran. The objectives of the research were to isolate NTFs from municipal waste-contaminated soil in Medan, North Sumatera, Indonesia. Their ability to suppress *Meloidogyne hapla* Chitwood population was observed *in vitro*. Furthermore, these potential isolates would be identified morphologically and compared genetically with other previously recorded species through DNA sequencing and a phylogeny tree will be established.

2. Material and Methods

2.1. Soil sampling and isolation

Seven districts in Medan City, North Sumatera, Indonesia were selected and seven spots nearby areas contaminated by municipal waste were determined at each district. For each spot, the soil was sampled by making three plots sized 50 × 50 cm each and having a distance of minimally 10 meters between plots. A hundred grams of soil was removed from each plot at a 10-15 cm depth and the soil was mixed. Then, 100 g soil was removed from this mixture, wrapped with aluminum foil, and placed in a labeled sterile plastic container. All samples were preserved in an icebox at 4^{o}C for five days (Tarigan, 2021).

NTF isolation from soil utilized Chloramphenicol Water Agar (CHP-WA) formulated by solvating 10 grams of pure agar in 500 ml distilled water, sterilized at 120 ^{o}C, 1.02 atm for 15 minutes. The mixture was appended aseptically with 1 gram of Chloramphenicol. The previous soil mixture was inoculated one gram into CHP-WA and added with a few adult *M. hapla*. Cultures were incubated in dark storage for three days at 25 ^{o}C and examined everyday using a light microscope at magnificent 10x to see the formation of mycelium traps.

2.3. Antagonistic assessment of NTF isolates against *M. hapla In vitro*

The mycelium trapping *M. hapla* found on CHP-WA recultivated into Corn Meal Agar (CMA) for *in vitro* assessment. Approximately 1 000 adult *M. hapla* were added into the petri dish and counted using a light microscope at magnificent 10× after 12, 24, 36, 48, and 72 hours.

2.2. Macroscopic and microscopic observation of NTF isolates

The isolates were cultivated on PDA and incubated at 25 ^{o}C for 14 days (Hastuti et al., 1970). Macroscopic observations for potential isolates including characterization of colony color, morphology, conidia, and hyphae were conducted by using a light microscope with magnificent 10× and 40× (Winarto et al., 2019).

2.4. ITS alignment and phylogeny analysis

Selected isolate cultures were shipped to Macrogen, Inc. (Singapore) for internal transcribed spacer (ITS) isolation and sequencing. Cap3 Contig Assembly (Stothard, 2000) and Reverse Complement (Huang and Madan, 1999) were used for merging and reversing sequences. Subsequently, these sequences will be aligned using NCBI BLASTn (Zhang et al., 2000) and phylogeny trees were built using MEGA ver. 11 (Tamura et al., 2021).

3. Results

3.1. Antagonistic assessment of NTF isolates against *M. hapla In vitro*

Three potential NTF isolates were selected and designated as sH51, sH52, and sH53. *In vitro* assessment of the isolates against *M. hapla* on CMA are shown in Figure 1. Isolate sH51 and sH52 decreased *M. hapla* by 97.7% while isolating sH53 by 89.27% after 72 hours.

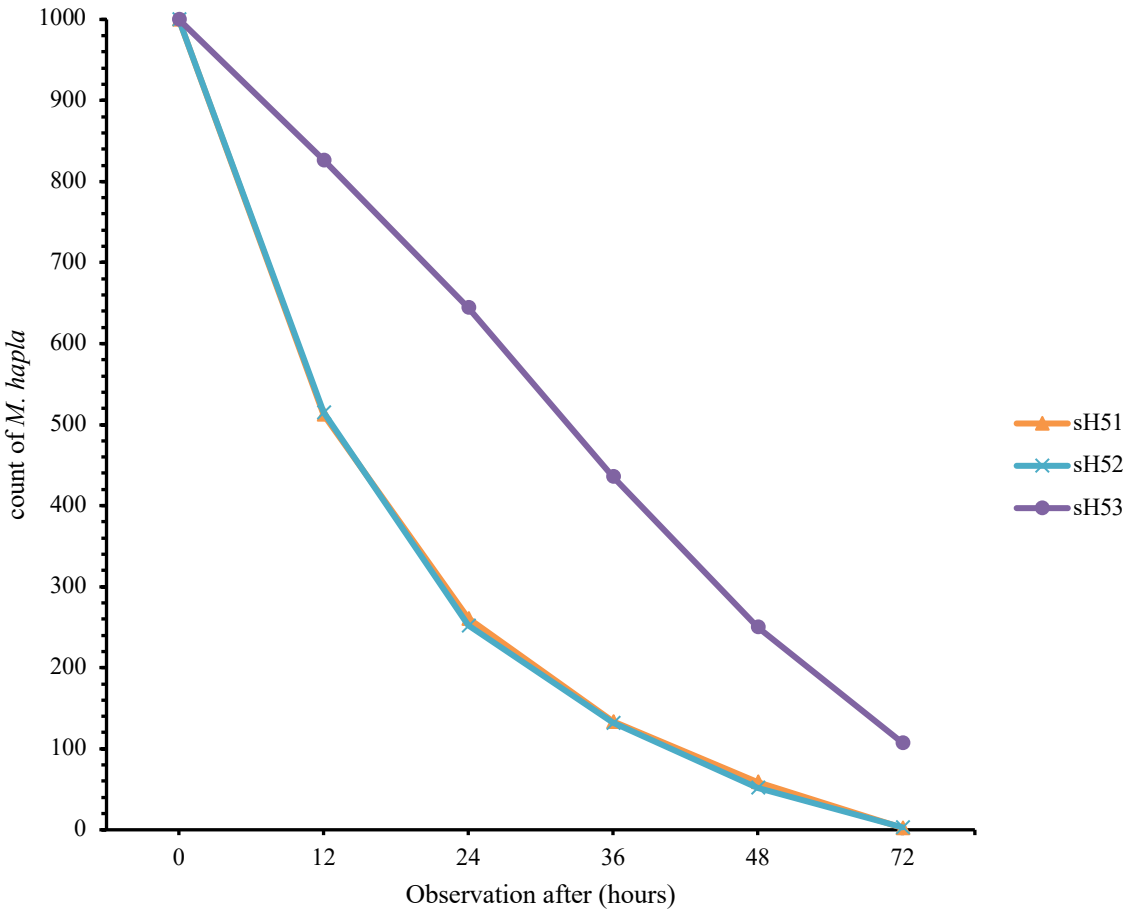


Figure 1. Observation of total count of *M. hapla* on CMA added with isolates after every 12 hours.

3.2. Observation and morphological identification of NTF isolates

Photomicrographs of the isolates were shown consecutively in Figure 2, Figure 3, and Figure 4. Isolate sH51 (Figure 2) at 25 ºC on PDA after seven days had a diameter of 4 cm, and upper and lower medium surfaces were whitish. Mycelium was sticky, branched, and septate. Conidiophores single, lined-vertically, colorless, septate. Conidia 5-9 (6) × 2.5-3.8 (3) µm, elongate ellipsoidal or cylindrical, hyaline, thin-walled, 3-septate. The trap formed a constricting ring. These data suggest that sH51 is identified as *Drechslerella brochopaga* Scholler, Hagedorn and Rubner (Ascomycota: Orbiliaceae) based on the same species described by Yu et al. (2014).

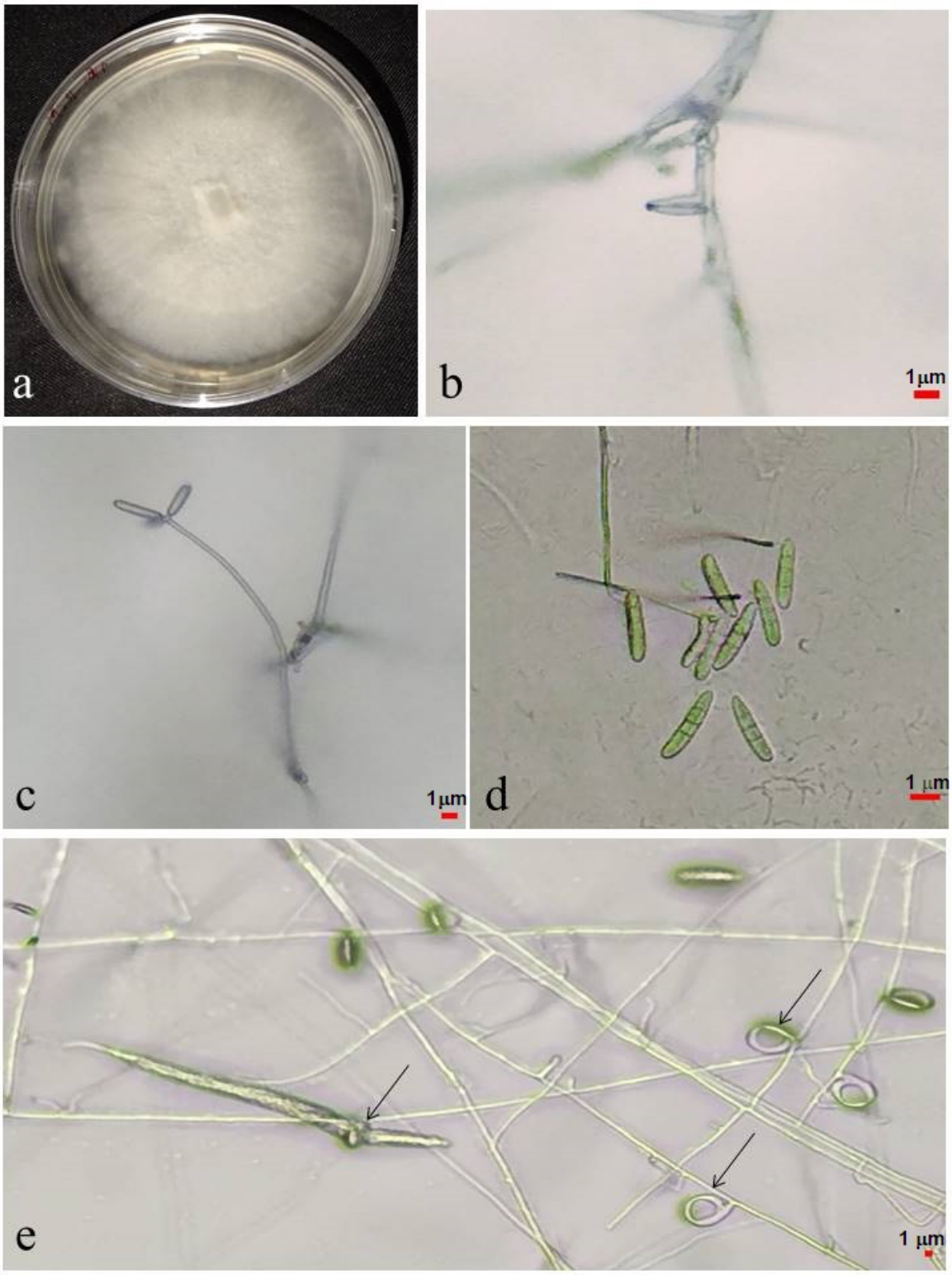


Figure 2. Observation of isolate sH51 on PDA: (a) colony, (b) early conidia, (c) elderly conidia, (d) conidiophore, (e) traps (arrow). Bars: 1 µm.

The colony of isolate sH52 (Figure 3) at 25 ºC on PDA after seven days had a diameter of 8 cm, whitish. Mycelium hyaline, branched and septate. Conidiophore erect, hyaline. Conidia 4-8 (8) x 7-13 (11) µm, hyaline, ellipsoidal or obovoidal, 1-3-septate. Nematode trap formed adhesive three-dimensional trap. These suggest that sH52 is identified as *Arthrobotrys sinensis* Scholler, Hagedorn and Rubner (Ascomycota: Orbiliaceae) based on the same species described by Yu et al. (2014).

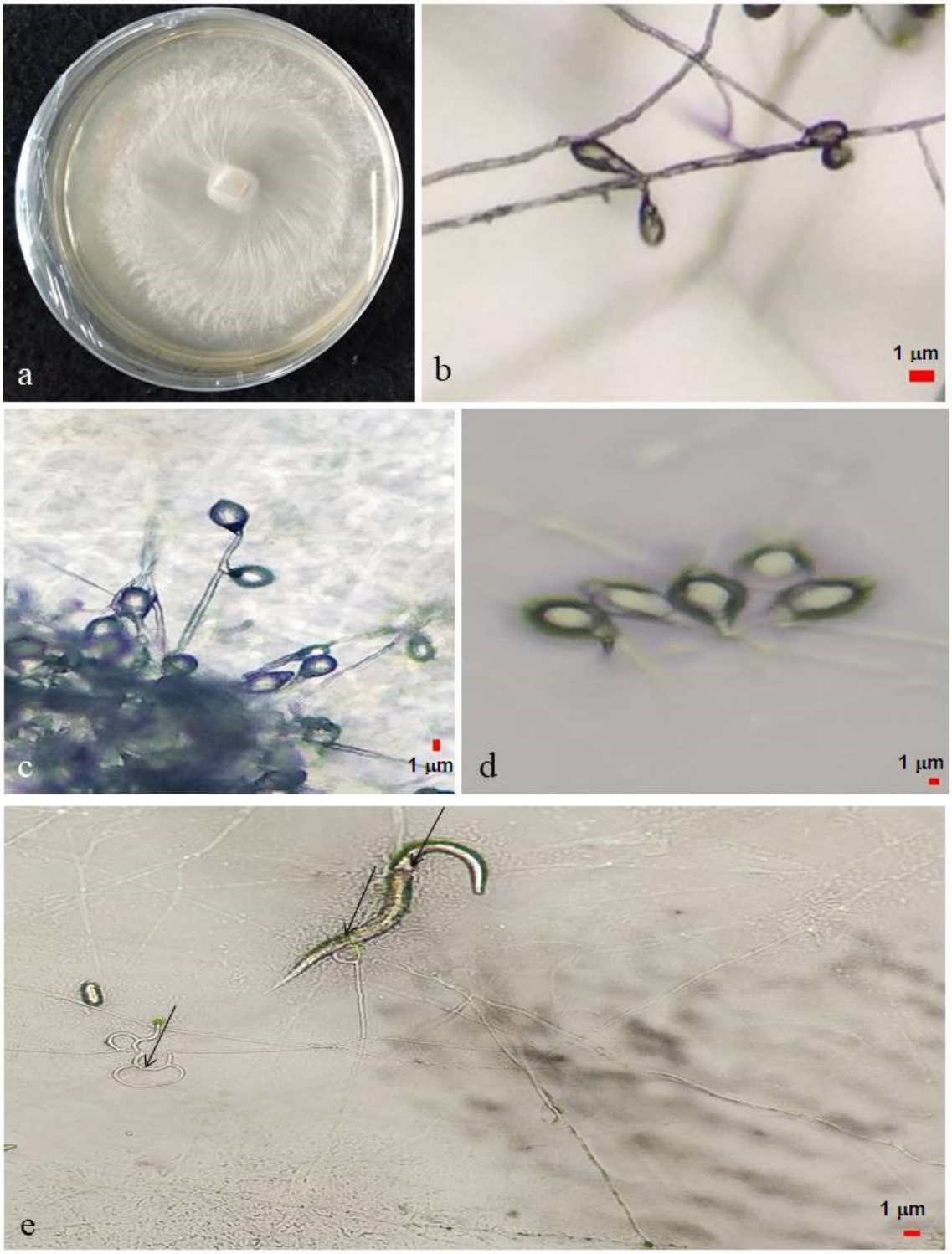


Figure 3. Observation of isolate sH52 on PDA: (a) colony, (b) early conidia, (c) elderly conidia, (d) conidiophore, (e) traps (arrow). Bars: 1 µm.

The colony of isolate sH53 (Figure 4) at 25 ºC on PDA after seven days had a 6 cm diameter, whitish. Vegetative hyphae hyaline, branched, septate. Conidiophore erect, hyaline. Conidia ellipsoidal or musiformis, hyaline, 1-4-septate, 3-5 (4) x 4-7 (6) µm, forming a three-dimensional adhesive trap. These data are similar to the *Arthrobotrys thaumasius* Schenck, Kendr and Pramer (Ascomycota: Orbiliaceae) described by Yu et al. (2014).

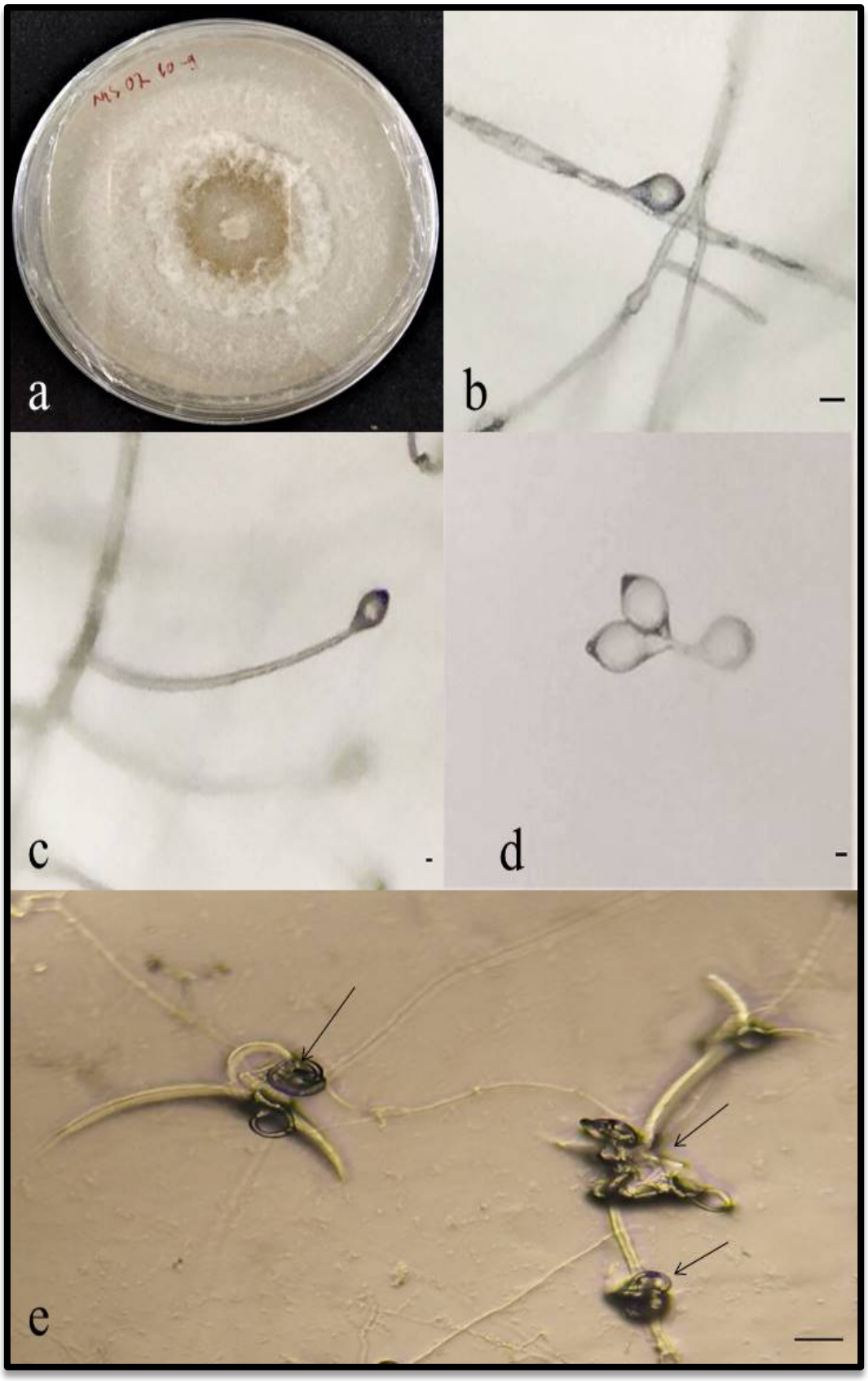


Figure 4. Observation of isolate sH53 on PDA: (a) colony, (b) early conidia, (c) elderly conidia, (d) conidiophore, (e) traps (black arrow). Bars: 1 µm.

3.3. DNA Sequence and Phylogeny Analysis

Isolates sequences obtained from Macrogen were aligned through BLASTn using the standard database (nucleotide collection), mega blast optimized and excluding models and uncultured genome. Ten species of the result for each isolate with the highest percent identity among others were collected and shown in Table 1.

Table 1. Top 10 of the highest percent identity of BLASTn match sequences

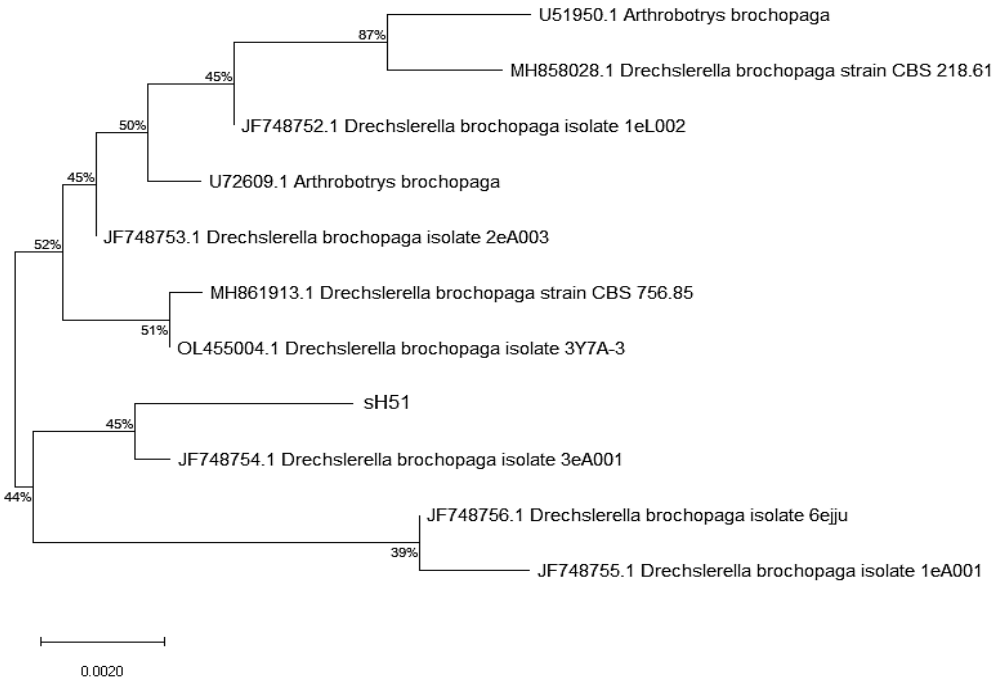
Scientific Name	GenBank Accession No.	Strain	Total Score	Query Cover	Percent Identity
Isolate sH51					
Drechslerella brochopaga	U72609.1		996	74%	100.00
Drechslerella brochopaga	JF748753.1	2eA003	909	68%	100.00
Drechslerella brochopaga	JF748752.1	1eL002	909	68%	100.00
Drechslerella brochopaga	U51950.1		1 022	77%	99.64
Drechslerella brochopaga	JF748754.1	3eA001	893	67%	99.59
Drechslerella brochopaga	JF748756.1	6ejju	797	61%	99.32
Drechslerella brochopaga	MH858028.1	CBS 218.61	1 024	78%	99.30
Drechslerella brochopaga	JF748755.1	1eA001	852	65%	99.16
Drechslerella brochopaga	MH861913.1	CBS 756.85	1 031	79%	99.13
Drechslerella brochopaga	OL455004.1	3Y7A-3	1016	78%	99.12
Isolate sH52					
Arthrobotrys sp.	MN014032.1	TWF898	1 000	79%	99.28
Arthrobotrys sp.	MN014031.1	TWF889	1 000	79%	99.28
Arthrobotrys sp.	MN014033.1	TWF1010	992	78%	99.10
Arthrobotrys sp.	MN014026.1	TWF761	998	79%	98.93
Orbiliaceae sp.	KX953548.1	SA228	1 046	83%	98.81
Orbiliaceae sp.	KX953601.1	SA323	837	67%	98.73
Arthrobotrys sp.	MN014024.1	TWF756	989	79%	98.58
Arthrobotrys sp.	MW131532.1	HK10	1 014	85%	97.34
Arthrobotrys thaumasia	MN717431.1	DS01	941	90%	94.03
Orbilia oligospora	MZ427476.1	JL1	937	90%	94.02
Isolate sH53					
Arthrobotrys thaumasia	MN014043.1	TWF1005	994	32%	99.82
Arthrobotrys thaumasia	MN014037.1	TWF588	981	32%	99.63
Arthrobotrys thaumasia	MN014039.1	TWF566	968	32%	99.62
Arthrobotrys thaumasia	EU977529.1	110	1 086	36%	99.50
Arthrobotrys thaumasia	EU977532.1	111	1 059	35%	99.49
Arthrobotrys thaumasia	MN014036.1	TWF579	963	32%	99.44
Arthrobotrys thaumasia	MN014035.1	TWF585	963	32%	99.44
Arthrobotrys thaumasia	AF106526.1	CBS 322.94	1 110	37%	99.19
Arthrobotrys thaumasia	MN947291.1	NPS014	1 026	35%	98.62
Arthrobotrys thaumasia	KX640093.1	NBS005	1 026	35%	98.62

Table 1 shows that based on the BLAST result for isolate sH51 (720 bp), all match sequences are *Drechslerella brochopaga*, which substantiates previous morphological identification (Figure 2). Isolate sH51 is closely related to the same species with accession no. U72609.1.

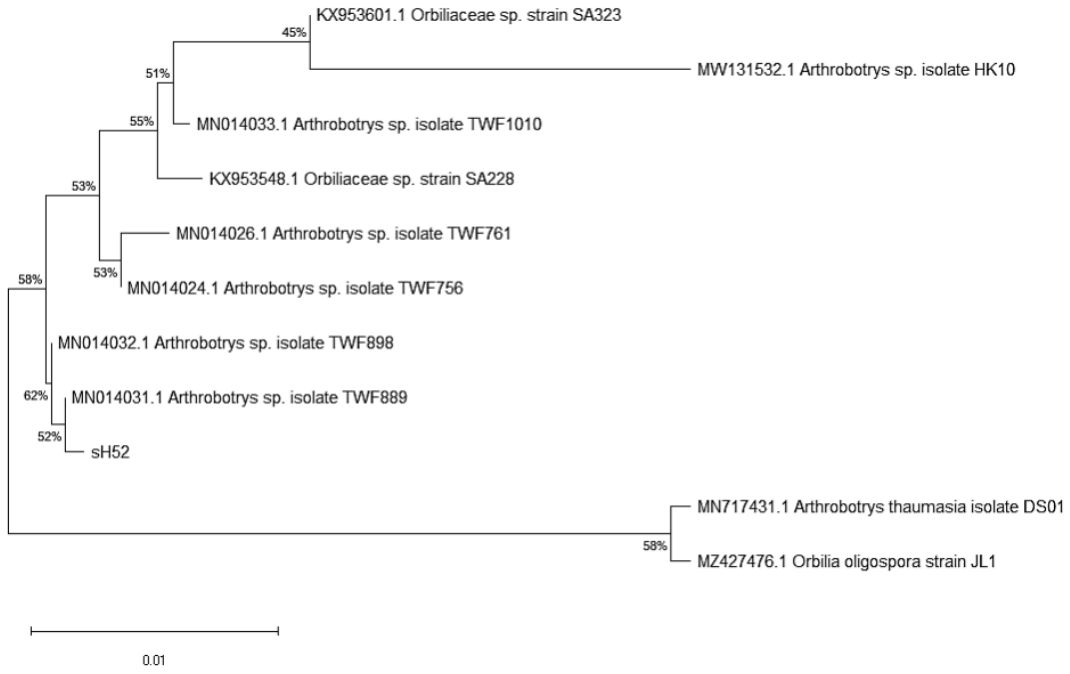
BLAST result definitely identifies isolate sH53 (1650 bp) as *Arthrobotrys thaumasia* which is in accordance with previous morphological identification (Figure 4).

Meanwhile, all compared sequences for isolate sH52 (701 bp) are diverse and its closest similarity is *Arthrobotrys* sp. var. TWF898 (accession no: MN014032.1) isolated in Taiwan. This result is also indefinite and insufficient to substantiate previous morphological identification (Figure 3) despite confirming the same genus.

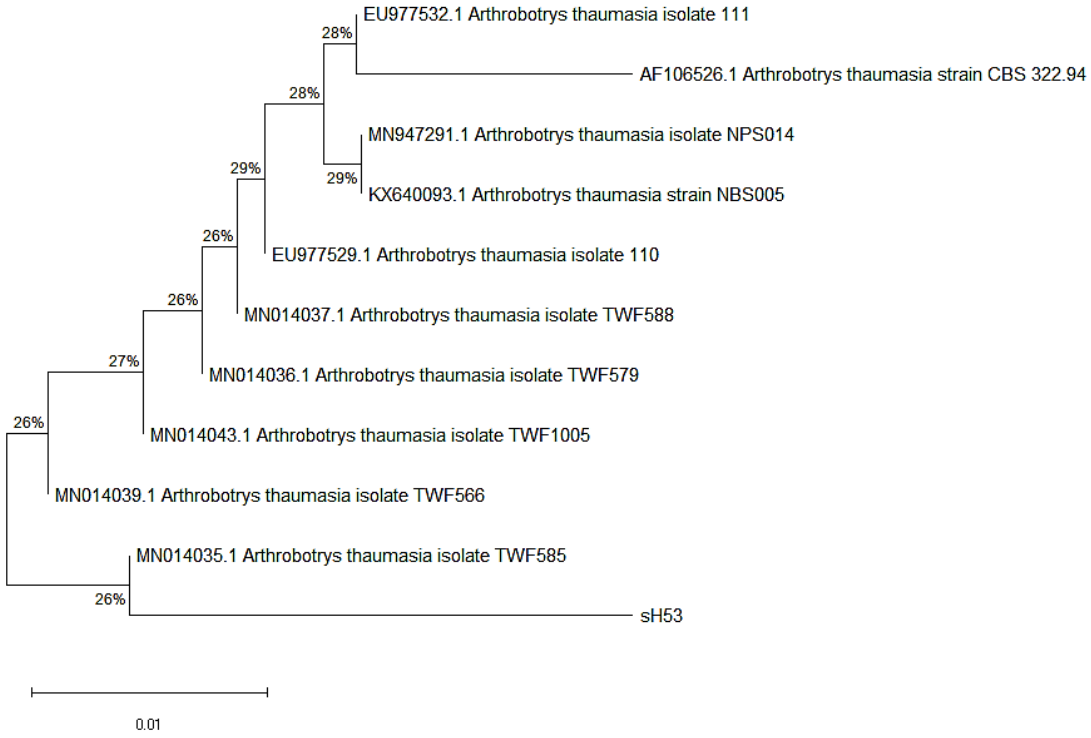
Subsequently, data in Table 1 was used to establish species-level neighbor-joining trees by using MEGA software with bootstrap replication 1000x for testing the reliability of BLAST results. Phylogeny tree results are shown in Figure 5.



(a)



(b)



(c)

Figure 5. Genetic relationship of (a) sH51; (b) sH52 and (c) sH53 with other most similar NTF species acquired from BLASTn results (percentages show site coverage of the sequences).

Figure 5a shows that the closest relationship for isolate sH51 is *D. brochopaga* strain 3eA001 (no. accession: JF748754.1) by 45%. Isolate sH53 in Figure 5c is closely related to *A. thaumasia* isolate TWF585 (accession no: MN014035.1) by 26%. Meanwhile, isolate sH52 is closely related to *Arthrobotrys* sp. strain TWF889 (MN014031.1) by 52%.

4. Discussion

Literature exploration revealed that this is initial research on the existence of *D. brochopaga* (isolate sH51) examined from municipal waste-contaminated soil in Medan City, North Sumatera, Indonesia. Previous studies identified *D. brochopaga* isolated from the soil of the oriental melon field (Cho et al., 2008; Singh et al., 2019) and leaf litter (Elshafie et al., 2006). Elshafie et al., (2006) and Xie et al., (2010) separately isolated *D. brochopaga* from soil and observed fungal constricting ring development which is a similar type of trap described in section 3.2 Figure 2.

Isolate sH53 can be identified assuredly as *Arthrobotrys thaumasia*. Previous reports also revealed isolating *A. thaumasia* from the soil sample in the same place (Hastuti et al., 2021) and neighboring regions in North Sumatera, Indonesia (Purba et al., 2022). However, Hastuti's isolate *A. thaumasia* DS01 (accession no: MN717431.1.) does not exist in the sH53 phylogeny tree (Figure 5c), which means they are distantly related. Other research isolating *A. thaumasia* from pasture soils, barn soils, and woodland soils in China revealed similar morphological characteristics (Wang et al., 2017).

Results for isolate sH52 are still uncertain. ITS genome sequenced does not adequately determinate its morphological and genetic identification to the species level consistently. Even though ITS is reliable for fungi identification in most cases, other regions such as large subunit (LSU) and small subunit (SSU), are also highly recommended to be sequenced to compensate for rampant cryptic speciation (Raja et al., 2017; Hastuti et al., 2021; Purba et al., 2022).

All isolates significantly suppressed *M. hapla in vitro* (Figure 1). Isolate sH52 and sH51 (*D. brochopaga*) suppressed *M. hapla* by 97.7% while isolate sH53 (*A. thaumasia*) showed suppression of 89.27%. Previous studies have also revealed that *D. brochopaga* and *A. thaumasia* isolated from soil in Korea reduce more than 70% of *M. incognita in vitro* (Kang et al., 2019). *D. brochopaga* was found to

be effective in controlling nematode, significantly increasing total chlorophyll content in leaves and activating root and shoot defense-related metabolic pathways (Singh et al., 2019). A study showed that *A. thaumasia* also suppressed the nematode population by 93% and supported plant growth when applied as a fungal suspension to tomato plants (Purba et al., 2022).

Most of the preceding studies discuss isolating NTF from farmland. This research provides basic information about novel reservoirs for acquiring NTF samples since farmlands are hardly available in urban areas. However, municipal waste-contaminated soil is very common, thus making it more accessible for following researches and supporting future sustainable urban agriculture.

Conclusion

Isolation of NTF from municipal waste-contaminated soil samples in Medan City, North Sumatera, Indonesia, successfully obtained three potential isolates that have an efficacious nematicidal impact against *M. hapla in vitro* by 97.7% (isolate sH51 and sH52) and 89.27% (isolate sH53) thus promising them as environmentally friendly bionematicide for crops. Morphological identification and ITS sequencing analysis determine that isolate sH51 is *Drechslerella brochopaga* and isolate sH53 is *Arthrobotrys thaumasia*. While morphological analysis for isolate sH52 reveals it as *Arthrobotrys sinensis* but is limited to *Arthrobotrys* sp. based on ITS sequencing and phylogeny analysis, thus additional gen regions need to be sequenced for confirmation.

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Research Article

Performances of F_3 and F_4 Bulk Populations in Cotton (*Gossypium hirsutum* L.)

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Abstract: The experiment was laid out in 2020 to compare the performance of thirty-seven F_3 and F_4 multi-parental bulk populations of cotton, including comparative cultivars, and to assess the heritability of traits studied. The differences within generations were significant for seed cotton yield, ginning out-turn, fiber quality, and nep fragments. The mean fiber strength of the F_4 generation showed significant performance compared with F_3. The broad sense heritability was high for ginning out-turn, fiber length, and fiber strength. The normal distribution for F_3 and F_4 generations due to non-significant skewness and kurtosis values indicated that there were no epistatic effects on the heritability of traits studied. Eight F_4 lines were selected for transfer to F_5 generation according to optimization in terms of desired traits.

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1. Introduction

Approximately 95% of the world's cotton production belongs to varieties within the *Gossypium hirsutum* L. (Chen et al., 2007; Fang et al., 2017). Türkiye produces 3.00–3.25% of the world's cotton annually (Anonymous, 2022). Cotton breeders' primary aim is high yield and fiber quality (Abdel-Aty et al., 2023). To improve cotton yield and fiber quality, appropriate breeding strategies are required to produce useful genetic variations and to identify desirable traits (Shahzad et al., 2022). Since high lint yield is the most important goal in cotton breeding programs, the unfavourable association between high yield and fiber quality traits has been ignored (Yang et al., 2023).

The success of the crop-breeding programme is determined by the selection process, and the bulk method is the third preferred method by public and private breeders in cotton (Bowman, 2000). Since the dominant gene variance is higher than the additive gene variance, applying the bulk method as an alternative to the pedigree method in early generations, such as F_2, F_3, and F_4, may be successful for breeding fiber quality traits (May and Green, 1994). It was reported that yield decreased from the F_3 generation to the F_5 generation, and the resulting significant inbreeding depression limited the success of selection in the early generations (Meredith, 1979). Furthermore, it was found that performance in early generations was not a predictor for later generations (Meredith and Bridge, 1973; Galanopoulou-Sendouca and Roupakias, 1999; Basal et al., 2017). In other studies, Khan (2003) and Khan et al. (2009)

determined that the plant families in the F_3, F_4, and F_5 generations gave superior values for fiber quality parameters than the standard varieties due to transgressive expansion and homozygotization.

High values for heritability in a broad sense indicated phenotypic variability due to genetic factors (Elhousary, 2023), whereas the low level of heritability shows the effect of non-additive genes and environment in the inheritance of studied traits. Regarding the broad sense heritability estimated for seed cotton yield, quite different results were found, ranging from low to high. (Balcı et al., 2020; Rehman et al., 2020). Skewness and kurtosis determine the presence of epistatic effects in the inheritance of the trait under study and provide information about the genetic background and structure of the population in the segregating generations (Savitha and Kumari, 2015).

Earlier studies were generally concentrated in early generations with very few hybrid combinations. For this reason, we conducted experiments with 37 multi-parental hybrid combinations in the later generations, such as F_3 and F_4, to estimate heritability and gene action patterns and determine the promising combinations to be transferred to the next generations.

2. Material and Methods

The F_3 and F_4 bulk populations used in the study were the progeny of 37 different cotton multi-parental cross combinations obtained from breeding studies conducted between 2013 and 2019 (Table 1). F_3 and F_4 populations originate from super lines obtained from cycle-1 of recurrent selection introduced by Balcı et al. (2021). We conducted the study at Cotton Research Institute, Nazilli, Aydın/Türkiye, in 2020. F_3 and F_4 generations of these 37 families and comparative cultivars, Gloria and ST-468, were arranged in Randomized Complete Block Design with three replications. Each population was grown in a single row of 12 m in length at 70 × 20 cm spacing. Twenty-five plants were randomly sampled from the middle 10 m section and were recorded seed cotton yield per plant (g) and ginning out-turn (%). Fiber fineness (FF; mic), fiber length (FL; mm), and fiber strength (FS; g tex^{-1}) were determined by HVI analysis. Nep count (number g^{-1}) and seed coat nep count (SCN; number g^{-1}) were measured by USTER® AFIS Pro 2 instrument.

Table 1. Designation of F_3 and F_4 bulk population

Code	Pedigree	Code	Pedigree
1	(Julia × ST-468) × (Gloria × Carisma) - I	**44**	(Carmen × ST-468) × (Gloria × Flash) - II
10	(Julia × ST-468) × (Gloria × Carisma) - II	**3**	(Carisma × Carmen) × (Gloria × Flash) - I
6	(Julia × ST-468) × (Gloria × Carisma) - III	**18**	(Carisma × Carmen) × (Gloria × Flash) - II
5	(Gloria × Flash) × (Gloria × Carisma) - I	**20**	(Carmen × ST-468) × (Gloria × Carisma) - I
13	(Gloria × Flash) × (Gloria × Carisma) - II	**22**	(Carmen × ST-468) × (Gloria × Carisma) - II
34	(Gloria × Flash) × (Gloria × Carisma) - III	**8**	(ST-468 × Claudia) × (Gloria × Flash) - I
15	(Julia × ST-468) × (Gloria × Flash) - I	**38**	(ST-468 × Claudia) × (Gloria × Flash) - II
24	(Julia × ST-468) × (Gloria × Flash) - II	**4**	(Carmen × ST-468) × (ST-468 × Claudia)
11	(Julia × ST-468) × (Gloria × Flash) - III	**7**	(Gloria × Flash) × (Gloria × Carisma)
16	(Julia × ST-468) × (Gloria × Flash) - IV	**9**	(Julia × ST-468) × (Carmen × Carisma)
31	(ST 468 × Claudia) × (Gloria × Carisma) - I	**12**	(Gloria × Flash) × (Gloria × Flash)
32	(ST 468 × Claudia) × (Gloria × Carisma) - II	**14**	(Julia × ST-468) × (ST-468 × Claudia)
42	(Carmen × ST-468) × (Gloria × Flash) - I	**17**	(Carmen × ST-468) × (Carmen × Carisma)
19	(Carmen × ST-468) × (Carisma × Carmen)	**36**	(ST-468 × Claudia) × (Julia × ST-468)
21	(Julia × ST-468) × (Carisma × Carmen)	**37**	(Carmen × Carisma) × (Gloria × Carisma)
26	(Carmen × Carisma) × (ST-468 × Claudia)	**39**	(Carmen × ST-468) × (Julia × ST-468)
29	(Julia × ST-468) × (Julia × ST-468)	**40**	(Gloria × Carisma) × (Gloria × Carisma)
30	(Carisma × Carmen) × (Gloria × Carisma)	**41**	(Carmen × Carisma) × (Gloria × Flash)
35	(Julia × ST-468) × (Carmen × ST-468)		

ANOVA was performed to compare the means of observed traits between and within generations. Distribution curves were formed for all observed traits, and skewness and kurtosis were estimated using the frequency distribution (Kapur, 1981). According to Singh and Chaudhary (1985), descriptive statistics were performed using the JMP® 14 statistical program (JMP, 2018). We calculated broad-sense heritability (h^2bs) using variance component analysis as follows;

Environmental variance (σ^2e) = Mean square of error
Genotypic variance (σ^2g) = (mean square of genotype - mean square of error) / replication
Phenotypic variance (σ^2p) = $\sigma^2g + \sigma^2e$

$$h^2bs = \frac{\sigma^2g}{\sigma^2p} \times 100 \text{ (Allard, 1960).} \quad (1)$$

Heritability was considered low (≤30%), moderate (30-60%), and high (60% ≤) (Srinivas et al., 2014).

3. Results and Discussion

Significant differences for all traits except seed coat neps within F_3 and F_4 indicated the opportunity for efficient selection among populations (Table 2). The effectiveness of breeding programs depends on the performance and variability among segregating populations (El-Shazly et al., 2023). Similar variability emphasized by El-Mansy (2005) and El-Shazly (2013) in later generations of cotton. The mean differences between F_3 and F_4 generations were significant for ginning out-turn, fiber fineness, fiber length, fiber strength, and nep fragments. Fiber strength increased in F_4 generations compared with F_3, whereas ginning out-turn and fiber length reduced, and fibers coarsed and nep fragments unfavourable increased in F_4 generations. In terms of the mentioned traits, it can be said that inbreeding depression is clearly manifested in the F4 generation (Meredith, 1979; Wu et al., 2010; Basal et al., 2017).

Table 2. Mean values, heritability and comparisons of F_3 and F_4 populations

Gen.	SCY		GOT		FL		FF		FS		SCN		Neps	
	F3	F4	F3	F4	F3	F4	F3	F4	F3	F4	F3	F4	F3	F4
1	93.0	109.0	39.8	42.0	32.2	30.4	4.3	4.7	32.1	32.7	13.0	16.3	49.1	53.8
10	95.9	111.0	46.8	41.4	31.2	30.8	4.8	4.7	34.0	35.4	14.7	9.0	36.5	35.1
6	91.7	102.9	43.7	44.6	31.2	30.9	4.5	5.0	33.7	34.3	10.0	8.3	27.8	54.5
5	95.5	107.7	41.9	40.9	30.7	32.5	4.5	4.3	32.8	34.1	6.0	12.3	34.5	67.1
13	108.6	107.5	43.9	42.9	30.4	31.1	4.5	4.5	31.9	35.2	9.7	7.0	37.1	45.8
34	69.1	90.4	43.7	42.7	31.0	30.5	4.3	4.7	31.2	33.8	24.3	6.0	98.5	37.1
15	98.1	95.2	44.5	41.4	31.1	32.2	4.6	4.5	33.2	34.6	19.0	15.0	42.5	51.8
24	100.1	103.0	43.4	42.4	31.1	30.2	4.8	4.6	34.1	32.0	10.0	16.0	24.5	46.5
11	94.0	118.6	41.3	41.7	32.3	31.3	4.5	4.6	33.9	32.5	15.7	11.0	47.1	45.8
16	108.2	112.7	42.4	40.3	30.7	32.0	4.7	4.7	32.0	34.4	14.3	12.7	41.8	49.1
31	92.0	103.1	40.5	41.7	31.0	31.0	4.3	4.5	32.5	33.0	5.0	13.0	33.1	59.8
32	83.3	107.9	45.7	42.2	30.5	30.8	4.7	4.7	32.4	34.9	5.0	3.0	29.8	14.8
42	85.7	96.5	42.3	43.6	31.9	30.6	4.5	4.8	33.8	34.3	9.5	15.3	41.1	42.5
44	100.6	108.0	44.5	41.3	30.7	31.1	4.6	4.6	32.1	33.5	7.7	30.8	38.5	58.5
3	107.9	87.8	46.9	42.5	31.0	30.7	4.8	4.6	32.4	34.0	8.3	17.0	34.5	26.5
18	100.7	110.7	45.5	40.4	31.7	31.5	4.5	4.5	33.8	34.4	17.0	25.3	54.5	72.5
20	100.0	99.8	43.8	42.8	30.7	30.3	4.7	4.7	32.4	32.0	4.0	36.0	46.5	99.1
22	130.9	80.8	42.2	41.1	31.4	30.8	4.6	4.6	33.0	32.2	15.7	19.0	69.8	103.1
8	100.1	102.5	42.9	41.2	31.4	31.1	4.5	4.6	33.7	33.6	7.0	7.0	29.1	85.8
38	84.4	90.2	43.8	45.4	30.4	31.0	4.7	4.7	32.2	33.9	5.7	16.7	32.5	39.1
4	102.6	97.0	42.8	40.8	30.9	32.3	4.6	4.5	33.0	33.9	11.0	19.1	31.8	69.8
7	90.7	100.7	43.6	42.3	30.8	30.8	4.8	4.7	31.2	33.9	13.0	19.7	71.1	37.1
9	106.9	84.2	41.9	43.6	31.8	31.3	4.4	4.7	35.2	33.7	14.7	5.0	41.1	30.5
12	101.4	105.3	43.8	43.1	30.7	30.9	4.7	4.6	31.7	34.3	9.0	3.0	45.8	36.5
14	114.0	115.8	43.8	41.4	31.1	30.5	4.5	4.6	33.7	32.7	10.0	18.3	25.1	27.1
17	102.4	111.0	42.8	41.9	30.5	30.5	4.6	4.7	31.6	33.8	11.3	15.0	74.5	48.5
19	81.1	113.5	44.6	40.8	31.4	30.6	4.5	4.8	33.0	32.4	19.0	19.0	103.8	58.5
21	98.1	99.3	42.5	43.1	32.0	30.9	4.4	4.7	33.3	32.4	7.0	14.0	29.8	65.8
26	96.3	94.9	42.5	43.5	30.5	30.9	4.5	4.9	33.5	33.0	13.0	13.0	37.8	35.1
29	97.5	123.6	42.7	43.2	31.4	30.7	4.5	4.7	33.8	33.7	5.7	21.0	28.5	37.1
30	92.5	101.8	44.4	42.1	31.9	30.6	4.7	4.8	32.5	33.3	3.0	7.7	57.8	32.5
35	115.6	112.6	44.8	43.0	31.1	30.9	4.6	4.8	33.1	33.9	8.3	6.3	19.8	25.8
36	120.1	91.0	42.5	43.5	31.6	30.3	4.4	4.6	33.0	31.6	22.0	7.2	76.8	51.1
37	130.0	95.9	43.6	41.6	31.2	30.9	4.3	4.5	34.5	31.8	17.0	12.3	56.5	52.5
39	81.6	100.4	42.6	42.3	31.2	31.2	4.5	4.6	32.1	35.0	3.7	14.3	26.8	64.5
40	95.5	84.8	43.0	43.5	30.7	30.6	4.5	4.7	32.0	33.8	3.7	11.5	21.8	27.1

Table 1. Mean values, heritability and comparisons of F_3 and F_4 populations (continued)

Gen.	SCY		GOT		FL		FF		FS		SCN		Neps	
	F3	F4	F3	F4	F3	F4	F3	F4	F3	F4	F3	F4	F3	F4
41	94.7	105.8	42.6	41.8	31.2	30.2	4.5	4.6	33.5	31.1	5.7	17.0	62.1	28.5
ST-468	72.7		44.6		27.4		5.4		30.2		14.8		51.5	
Gloria	102.2		42.5		28.9		5.3		32.8		10.5		41.8	
F3 Mean	98.9		43.4		31.2		4.6		32.9		10.8		44.9	
F4 Mean	102.2		42.3		30.9		4.7		33.5		14.1		49.1	
LSD $_{(0.05)}$	25.9	23.6	1.8	1.9	0.7	0.8	0.3	0.3	1.6	1.6	ns	ns	40.8	36.4
F_3 vs F_4	ns		**		*		**		**		*		ns	
h^2bs (%)	36.76		76.88		76.40		52.38		71.34		19.32		53.41	

*: $P < 0.05$; **: $P < 0.01$; ns: non-significant, SCY: Seed cotton yield (g plant^{-1}); GOT: Ginning Out-Turn (%); FL: Fiber length (mm); FF: Fiber fineness (mic.); FS: Fiber Strength (g tex^{-1}).

F_3 and F_4 means showed that seed cotton yield per plant of both generations was similar to Gloria, whereas F_3 and F_4 generations produced significantly higher seed cotton yield than ST-468 (Table 1). Although the mean ginning out-turn of segregating populations in both generations was significantly lower than ST-468 cultivar, both generations had significantly stronger fibers than ST-468. Similarly, Azam et al. (2013), Kumar et al. (2014), and Fawad et al. (2022) compared F_3 populations with their parents and reported significant differences. In addition, both generations had significantly longer and finer fibers compared to the standard cultivars. The differences between populations and standard cultivars were non-significant for SCN and neps.

Heritability was estimated as low for seed cotton yield and seed coat neps; medium for fiber fineness and neps; and high for ginning out-turn, fiber length, and fiber strength (Table 1). It was concluded that environmental effects were higher than genetic factors in the inheritance of traits with low heritability. The high variance values observed in both generations for seed cotton yield and nep fragments confirmed the low and medium heritability levels. However, genetic variation in a broad sense of heritability can be due to additive as well as dominant and epistatic variation (Falconer and Mackay, 1996). Similar to the results of our study, non-additive gene effects were found to be higher for seed cotton yield (Lingaswamy et al., 2013; Usharani et al., 2016; Monicashree et al., 2017; Prakash et al., 2018). Zeng and Meredith (2010) found a moderately broad sense heritability for seed coat neps and reported that more than 50% of the variation was due to environment with genotype-environment interaction. In previous studies, broad sense heritability of 70% and above was found for fiber length (Fang et al., 2014; Gore et al., 2015; Huang et al., 2017; Ma et al., 2018) and ginning out-turn (Rehman et al., 2020; Ishaq et al., 2021). These results are in agreement with our study findings. Heritability ranging from high to low for different traits showed that bulk or modified bulk method should be applied for combination breeding. Evaluating breeding methods and selection criteria for improving fiber quality, May and Green (1994) concluded that the selection of F_2 bulk populations for fiber traits is more beneficial than selecting individual F_2 plants when initiating a line breeding program for quality improvement.

Non-significant skewness and kurtosis indicated that all traits had a normal distribution. Tabachnick and Fidell (2013) defined kurtosis and skewness values between -1.5 and +1.5 as normal distribution (Figures 1 and 2). Positive and negative skewness are indicators of complementary gene and duplicate (additive × additive) gene interactions, respectively. Negative or near-zero kurtosis showed the absence of gene interaction, while positive values are a signal of gene interaction (Choo and Reinbergs, 1982; Savitha and Kumari, 2015). In another study, Percy et al. (2006) found positive and high kurtosis values for seed cotton yield and fiber length but insignificant kurtosis values for fiber fineness. Our findings clearly showed that epistatic effects are not important in controlling all traits. Verhalen et al. (1971), Khan et al. (2003), and Khan et al. (2009) reported that epistatic gene effects were not significant in the genetic control of fiber quality traits. Although the longer right tail for seed cotton yield indicated that the expansion in the direction of high seed cotton yields continues, this aspect of the distribution is problematic in terms of nep fragments (Figure 1). These results show that simultaneous improvement between yield and neps is not possible, contrary to what was stated by Zeng and Meredith (2010). Therefore, seed cotton yield should be limited to a certain level.

In the F_4 generation, among 1174 plants, there were 247 superior plants with yield values 3 standard deviations above the population mean for seed cotton yield. This finding indicated that

approximately 21.04% of the population was suitable for high-yielding selection. The percentages of superior plants were 8.52% for ginning out-turn, 11.93% for fiber length, and 9.97% for fiber strength. 20.10% of the population was 3 standard deviations below the mean fiber fineness. Transgressive segregation could be said to cause this performance (Khan et al., 2009). Since the variance and, therefore, the standard deviation was high, this evaluation could not be made in terms of nep fragments. When optimization is desired in terms of all traits, 8 F_4 lines were selected for transfer to F_5 generation according to seed cotton yield above 90 g; ginning out-turn 42.2%; fiber length 30.2 mm; fiber strength 32.0 g tex^{-1}; fiber fineness between 4.5-4.7 mic.; seed coat neps below 21.0 and neps 64.5. Similar thresholds were proposed in the decision tree model developed by Çakmak et al. (2023). These lines were (Gloria × Flash) × (Gloria × Carisma); (Gloria × Flash) × (Gloria × Flash); (Gloria × Flash) × (Gloria × Carisma) – II; (Julia × ST-468) × (Gloria × Flash) – II; (Julia × ST-468) × (Julia × ST-468); (ST-468 × Claudia) × (Gloria × Carisma) – II; (Carmen × ST-468) × (Julia × ST-468) and (ST-468 × Claudia) × (Gloria × Flash) – II. These advanced lines were also the genotypes in which the negative association between yield and fiber quality traits was broken (Clement et al., 2015; Mızrak et al., 2020).

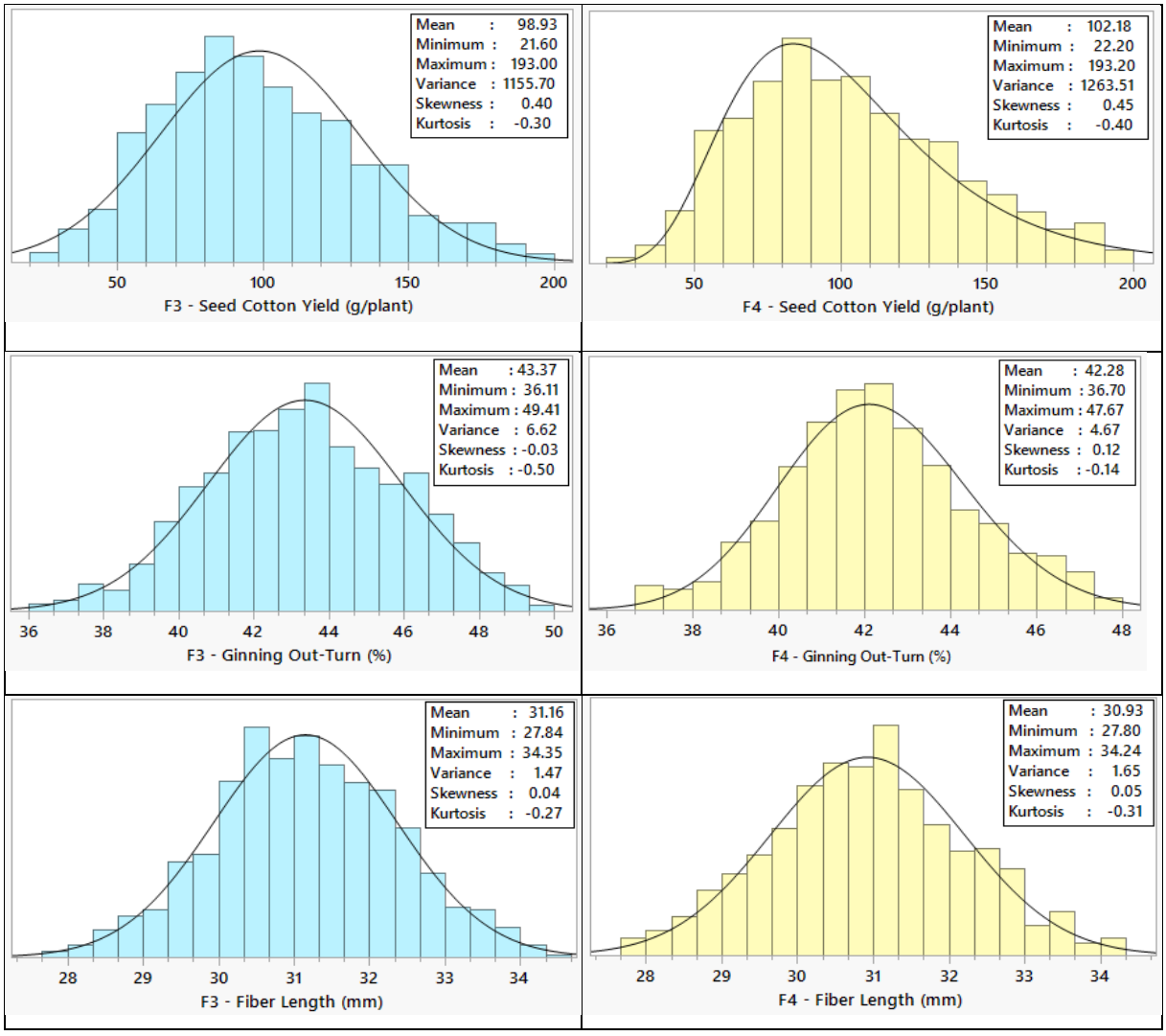

Figure 1. Normality distribution of yield and fiber quality in F_3 and F_4 generations.

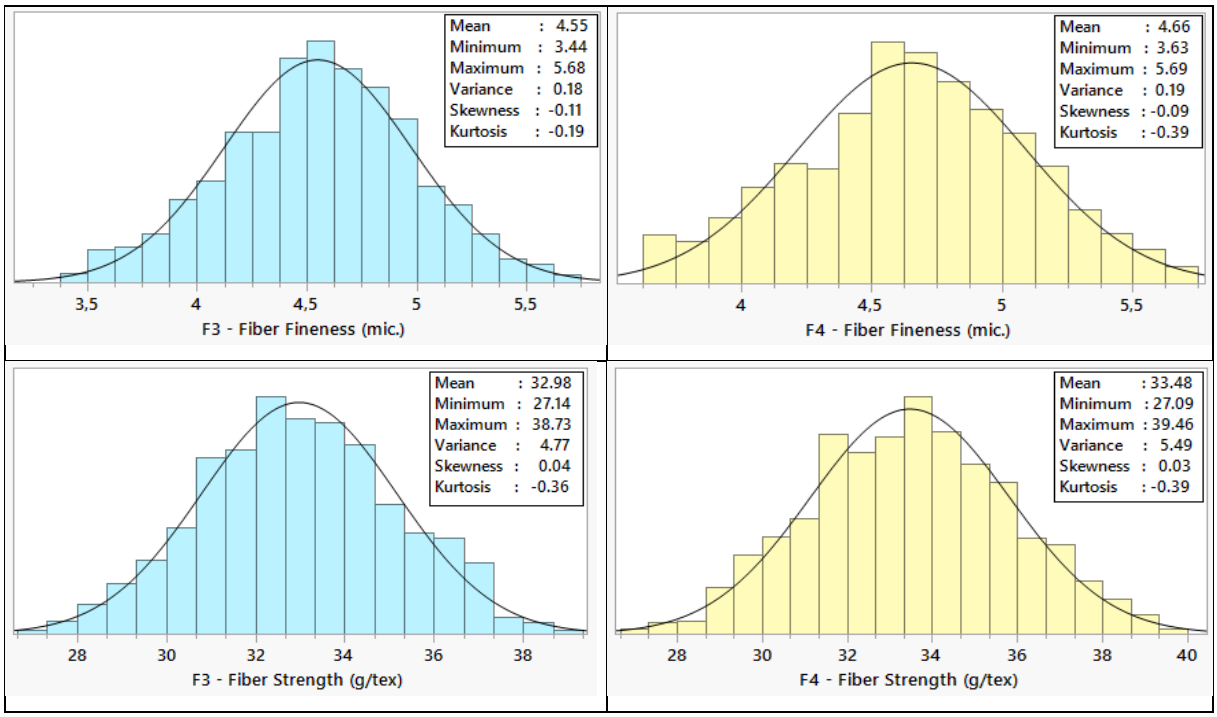

Figure 1. Normality distribution of yield and fiber quality in F_3 and F_4 generations (continued).

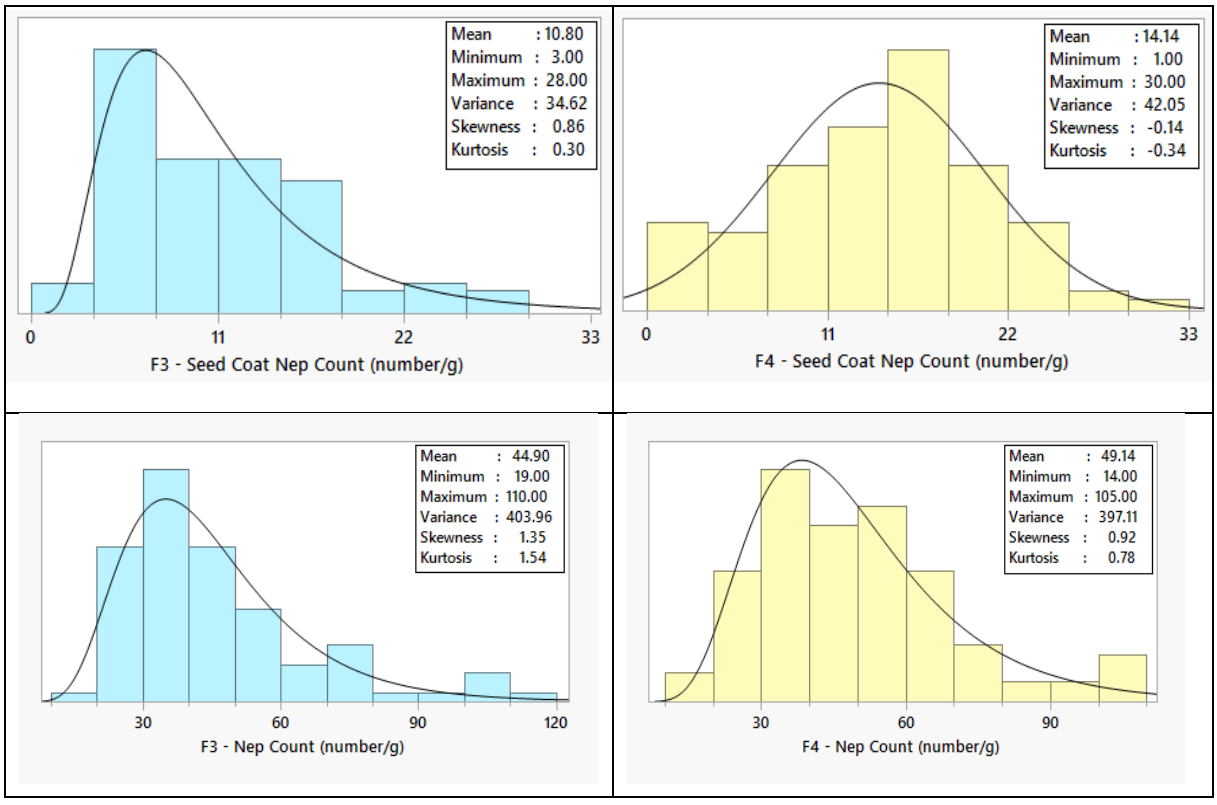

Figure 2. Normality distribution of nep fragments in F_3 and F_4 generations.

4. Conclusion

The results from the present study indicated the existence of sufficient variation for selection in multi-parental populations. Due to the continued high variation in seed cotton yield and nep fragments, single plant selection could be delayed until the F_6 generation. The most important goal for cotton breeders should be to combine high yields with improved fiber quality.

Contribution of the Authors

The authors declare that the contribution of the authors is equal.

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Research Article

Consumer Perception and Purchase Attitude towards Genetically Modified Foods during the Covid-19 Pandemic: the Case of Erzurum, Türkiye

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Abstract: The aim of the study was to determine consumers' perception and purchase attitudes towards foods with genetically modified organisms (GMOs) and the main factors impacting on their purchase decision in Erzurum province in Türkiye. The material of the research consisted of primary data obtained from face-to-face questionnaire fulfilled with 323 households residing in Erzurum in 2021 and intending to consume foods with GMOs during the Covid-19 pandemic, and then explanatory factor and cluster analyses were applied to determine the main factors affecting three homogenous consumer clusters' attitudes and beaviors towards foods with GMOs. The results of the study highlighted that high-income consumers were of willingness to buy foods with GMOs due to positive purchase motivation with orientation of media communication and product mixes, that middle-income consumers altered consciously their purchase models by preferring GMO-foods with lower price to traditional foods, and that low-income participants did not want to buy foods with GMOs owing to negative impacts on human health, environment safety and ethical issues. As a result, high and middle-income consumers attributed positive purchasing perception and attitudes toward foods with GMOs, but low-income those were of a negative perception for these.

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1. Introduction

The rapid increase in the world population and food supply security resulting from climate changes prevent people from reaching a balanced and healthy diet. Being able to reach to food products of people, it is possible with either to increase productivity in agricultural production or to expand useable agricultural fields. However, it is inevitable to increase the factor productivity per unit since it is not possible to expand potentially the cultivation areas. In order to obtain more products per unit area or to obtain more productive species with product diversity, organisms improving food attributes through genetic engineering, namely genetically modified organisms (GMOs), have taken over important missions for the last years (Gürbüzoğlu, 2016).

In USA, the first scientific studies conducted on GMOs started in 1980s (Uzogara, 2000), and the first genetically modified product in the world was obtained from tobacco plant applications. The

first plant with GMO to have been traded was tomato type called as Flavr Savr with a longer shelf life in the USA in 1994, and the number and variety of genetically modified plants has continued to increase dramatically until today.

Especially the production fields of products with GMO for the food industry have been expanded, and their production periods have been shorted and thus the product quality has been also improved through the enzyme and fermentation processes facilitated by gene technology in the last decades (Shetty, 2008). A large number of crops with GMO such as wheat, corn, rice, potatoes, soybeans, tomatoes, sunflowers, zucchini, pumpkins and peanuts, some fish species, rapeseed, cassava and papaya have survived in today. Moreover, it was also reported that agricultural food products with GMO such as melon, watermelon, banana, strawberry, raspberry, cherry, pineapple, pepper and canola were of a much more contribution to mitigate the impacts of climate change and then to accelerate the natural adaptation process (Cummins and Lilliston, 2000).

According to the International Service for the Acquisition of Agri-biotech Applications (ISAAA) 2019, it was reported that the expansion rates of biotech agricultural product areas versus those of other agricultural fields had dramatically increased in developing countries such as Vietnam, the Philippines and Colombia. On the other hand, it was stated that the top five countries with the largest biotech product areas were the USA, Brazil, Argentina, Canada and India. It was also declared that those who adopted basic biotech products in these countries were 1.95 billion people in 2019, which correspond to 26% of the world's population. Furthermore, with Malawi, Nigeria and Ethiopia from the African continent in 2019, the number of countries growing biotech products increased from 24 to 27 (ISAAA, 2019). As a result of all these development trends, the products with GMO have been accepted as technological products providing faster adaptation to agricultural fields and increasing productivity at agricultural production.

In parallel with developments in the world, trial fields have been expanded for agricultural products such as potato, cotton, corn and soybean with GMO in Türkiye since 1999. Especially, it was stated that soybean is at the highest level with a share of 50% among the others including in corn (22%), cotton (12%), canola (5%) and other agricultural products (11%) (Sahin et al., 2018).

As a result of the production of the products with GMOs and the spread of their usage areas, possible natural risks on human health and environment have begun to increase. However, some studies conducted on the effects of the products with GMOs on human health and environment declared a positive outlook (economic and environmental benefits), whereas the others were also focused on negative and irreversible impacts (environmental, biosafety and ethical concerns, and bioterrorism) (Kaya, 2020).

Nowadays, issues including in the previously mentioned positive and negative motivation items continue to spark debate marked by contrasting the ideas about the GMO-food production, marketing and consumption. Foods with GMOs, therefore, led to reveal the opposite approaches between those supporting the use of GMOs in agriculture and those supporting not (Palmieri et al., 2020).

In marketing literature, it was also highlighted that the negative emotional attitude about foods with GMOs was associated to the lack of knowledge dealing with core of GM and its impacts on human organism (Boccia et al., 2018, Palmieri et al., 2020, Russo et al., 2020, Turan et al., 2022). Consumers' concerns about GMO foods and suspicious feelings towards new food technologies could lead to unsuccessful food product innovation under marketing mix, and thus their possible uses and designs are strongly affected from the consumers' perception and preferences changing their expectation of core benefit via GM technology under product mix. The lack of consumers' knowledge about innovative food products being applied new technologies such as GMOs, in fact, could cause an important barrier to their acceptance.

Previous studies indicated how consumers' positive or negative attitudes towards foods with GMOs could be an indicator of their intention to purchase these foods, and thus they could affect food pricing with GMOs (Lackowski et al., 2017). It was stated that consumers, indeed, not only purchased food with GMOs at more affordable prices but contributed to expanding also innovative foods penetrating to the food markets since they were able to decrease negative environmental affects via good agricultural applications (Ghozzi et al., 2018, Palmieri et al., 2020, Russo et al., 2020, Turan et al., 2022). On the other hand, it was also reported that consumers concerned GM technologies being capable to alter the natural/ecological food attributes, and it could have caused dangerous impacts on human health and environment (Boccia et al., 2018, Pechlaner, 2020, Arani et al., 2021, Adalja et al., 2022).

Consumers have caused to exhibit their asymmetrical behavior patterns under these two opposite paradigms in the literature (Kaya, 2020). According to various researches focused on consumers' attitudes and behaviors towards foods with GMOs, thus, it was reported that American consumers were accepting of foods with GMOs than European consumers interesting extremely to traditional and local food products, but Chinese consumers were fairly willingness to buy these products (Perito et al., 2019, Palmieri et al., 2020).

In fact, societies' perspectives towards foods with GMOs have been differently evaluated globally. There are consumers' different perspectives towards these products in Türkiye as a research region, and it is of great importance to reveal these differences on regional basis to be able to create innovative approaches for these products. As a result of all those, changings at the consumers' attitude and behaviors towards foods with GMOs during Covid-19 could have very important effects on production and supply decisions based on food industry and market strategies. In current research, thus, it was planned to explore the approaches of consumers residing in Erzurum, Türkiye and their consumption awareness and attitudes towards foods with GMO during the Covid-19 pandemic, and to determine the main factors affecting the consumers' purchase attitudes and behaviors towards these.

2. Material and Methods

2.1. Material

The main material of the research consisted of primary data obtained from face-to-face surveys with consumers residing in Erzurum, Türkiye in 2021. The secondary data of the study were also collected from the paper results and project reports about biotechnological product consumption, as well as from data of various statistical institutions and organizations such as TUIK, OECD, EFDA and FAO.

2.2. Methods

2.2.1. Determination of sample size

The sample size taking into account the main mass and consumption tendency of food with GMOs of the population whose variance rate is known under Standard Normal Distribution probabilities at 95% confidence interval was calculated in Equation 1 and 2 by using the Simple Random Sampling Method based on Main Mass Ratios (Newbold, 1995).

$$n = \frac{Np(1-p)}{(N-1)\sigma_p^2 + p(1-p)} \tag{1}$$

Where;
n: Sample size,
N: Main population (417 784 persons),
σ^2_p: Main mass variance ratio,
r: Deviation from the mean (5%),
$Z_{\alpha/2}$: Z table value at 95% confidence interval (1.96),
p: The probability of those preferring foods with GMOs (0.70).

$$\sigma_p^2 = \frac{r}{Z_{\frac{\alpha}{2}}} \rightarrow \sigma_p^2 = \left(\frac{r}{Z_{\frac{\alpha}{2}}}\right)^2 = \left(\frac{0.05}{1.96}\right)^2 = (0.0225)^2 \tag{2}$$

$$n = \frac{417\,784 * 0.7 * 0.3}{(417784 * (0.0255)^2) + (0.7 * 0.3)} = 323 \tag{3}$$

It was determined that the population size of Erzurum province was 417 784 persons based on Address Based Population Registration System (ABPRS) in 2021 (TÜİK, 2021). In order to determine GMOs food consumption probability of the consumers representing the main mass, a pilot study was conducted at the research region and probability of willingness to consume the foods with GMOs was founded to be 70% (p = 0.70). The sample size was calculated as 323 persons by considering this probability level.

2.2.2. Method applied in the preparation of the questionnaire forms

In the study, it was applied face-to-face questionnaire technique due to the difficulty and complexity of the questions planned for the survey form used in data collection by taking into consideration the questionnaire form approved by Igdir University Ethics Committee with 2022/13 number. The variables based on consumers' attitude and behaviors towards foods with GMOs were determined by accounting the variables used in domestic and foreign consumption researches. It was asked consumers participated in the survey to mark each statement on attitude scale determined by Likert Scale with 5-point (1: not any important, 3: neutral/undecided, 5: very important) (Kotler and Armstrong, 2004). Product attributes being consumers' purchase attitude and behavior determinates involve in their cognitive and visual perspectives related to foods with GMOs such as GMO-born legal regulations and some components of marketing mix (Table 1).

Table 1. Variables used in the research model

Code	Variable idendification
M1:	GMO term refers to genetically modified organisms
M2:	Foods with GMOs are organisms obtained by transferring a gene or genes from plants, bacteria, viruses or any other living thing to products' genetic structure trough techniques interfering to genes
M5:	Food with GMOs could be recognized by their shape
M6:	Food with GMOs could be understood by their price
M8:	I know what foods with GMOs are
M9:	It is considered that tomato is a food with GMOs
M10:	It is considered that strawberry is a food with GMOs
M11:	It is considered that corn is a food with GMOs
M12:	It is considered that soybean is a food with GMOs
M13:	It is considered that potato is a food with GMOs
M14:	It is considered that eggplant is a food with GMOs
M17:	Consumption of foods with GMOs is harmful for human health
M18:	Products with GMOs are of a negative impact on the environment
M19:	Foods with GMOs are harmful for all age groups
M23:	Foods with GMOs are healthier than other products
M27:	Unit cost for foods with GMOs is lower than the others
M31:	When buying food, I pay more attention to its advertisements
M33:	When buying food, I pay more attention to its package knowledge and materials
M35:	If food with GMOs is sold at the real markets, I try to buy it
M36:	I buy by ignoring whether it with GMOs is or not, when any branded food is cheap
M37:	If the price of food with GMOs is more attractive, I buy it
M38:	I buy foods with GMOs if they are of higher quality than others
M39:	If foods with GMOs are of higher nutritional value than foods without GMOs, and are sold at the same price, I buy a food with GMOs
M41:	The reason why the price of food with GMOs is cheap is that its supply amount is high
M42:	The reason why the price of foods with GMOs is cheap is that the production cost is low
M43:	The reason why the price of food with GMOs is cheap is that its penetration rate at the market is high

Table 1. Variables used in the research model (continued)

Code	Variable idendification
M44:	The reason why the price of food with GMOs is cheap results from lower demand
M46:	Foods with GMOs are healthier
M47:	Foods with GMOs should be consumed in a wider concept
M49:	We consume by being not had information about foods with GMOs, if I find out that I consume food with GMOs, I stop immediately its consumption
M53:	The Covid-19 pandemic has changed considerably my purchase habits
M54:	During the Covid-19 pandemic, I have token care not to buy foods with GMOs
M56:	I have bought foods with GMOs during the Covid-19 pandemic
M58:	The Covid-19 pandemic has created a difference in my cognitive memory to purchase the foods with GMOs
M59:	Even though the foods with GMOs is known to be harmful for human health, I have purchased them for stock purposes during the Covid-19 pandemic
M60:	Since the foods with GMOs is known to be harmful for human health, I don't buy them for stock purposes during the Covid-19 pandemic

2.2.3. Method applied in statistical analyses

In the first step, Explanatory Factor Analysis (EFA) was especially used to determine the factors related to the attitude and behaviors influencing on consumers' GMOs food consumption preferences under Covid-19 pandemics. EFA is a multivariate statistical dimension reduction technique trying to create a small number of unrelated, but conceptually meaningful new factors by bringing together variables that are related to each other (Civelek, 2020).

Hierarchical steps for the EFA were followed to test the suitability of the data, to determine the main factor number, to perform the rotation (transformation) techniques, to identify main factors, to calculate the explained and cumulative variances for each factor dimension, respectively. In order to investigate the data suitability of the sample mass according to the main population for the EFA, Kaiser-Meyer-Olkin (KMO) and Bartlett's test of Sphericity were used in the research. KMO, the adequacy criterion of the sample size should be in acceptable confidence interval (between 0.50 and 1.00). On the other hand, Correlation Matrix should be different from the Unit Matrix in Bartlett's test of Sphericity explaining the relationship among the variables depending on the correlation matrix calculated between each pair of variables.

Whereas determining the main factor number in the EFA, the factors with Eigenvalues greater than one or equal to one were taken into consideration statistically. Rotation technique was also used to be able to give easily the factor names, and to eliminate the variable overlaps in factor matrices. In the rotation process, the factors in the axes are rotated so that reducing the variable loads to optimal levels. Rotation could be applied in two groups as vertical (orthogonal) and oblique rotation. While it could be minimized the relationship among the factor dimensions in vertical rotation, it could be accepted the relative relations among them in oblique rotation. It could be used Varimax, Quartimax and Equamax Methods in vertical rotation techniques, but it could be used Direct Oblimin and Promax Methods in Oblique Rotation one. In this study, it was applied vertical rotation and Varimax Method by being assumed being minimal relationships among the factors. As a result, 60 variables impacting on the consumption perception and awareness of foods with GMOs in Erzurum were conducted by considering the hierarchical process steps explained for EFA.

In the second step, it was used Two-step Cluster Analysis, dividing a heterogeneous target mass into two or more homogeneous segments by taking into account their various characteristics such as socioeconomic, demographic and psychographic variables (Karagöz 2019). In the present study, two-step cluster analysis was used and target consumers were classified into three groups as low (less than ₺750), medium (₺750-₺1500) and high (over ₺1500) income groups. Low, middle and high income groups constituted 23.2% (75 households), 49.8% (161 households) and 26.9% (87 households) of the sample population, respectively.

3. Results

3.1. Consumers' cluster profiles and perceptions for foods with GMOs

The relationships between consumers' demographic and socioeconomic characteristics being tendency to consume foods with GMOs in Erzurum and three income segments were given in Table 2.

Table 2. Demographic and socioeconomic attributes of the participants in each cluster

Demographic and socioeconomic factors		Income groups			Total
		Low	Middle	High	
Gender	Men	27	80	57	164
	Women	48	81	30	159
Age	15-25 years	25	27	8	60
	25-35 years	31	101	39	171
	35-45 years	11	21	27	59
	45-85 years	8	12	13	33
Education	Primary school	6	1	2	9
	Middle school	4	4	2	10
	High school	21	31	14	66
	University	44	125	69	238
Marital status	Married	33	74	65	172
	Single	42	87	22	151
Number of children	0	46	93	23	162
	1-3	20	57	53	130
	3-5	8	8	10	26
	5-8	1	3	1	5
Occupation	Lawyer	0	5	4	9
	Teacher	1	10	8	19
	White collars	2	36	27	65
	Blue collars	33	40	11	84
	Retired	2	5	1	8
	Others	37	65	36	138
Where did you first hear about concept of GMOs?	Television	46	92	48	186
	Journal	1	3	1	5
	Newspaper	48	2	2	4
	Internet	18	43	23	84
	Other	9	21	13	44
Is it objectionable to buy foods with GMOs?	Yes	65	138	77	280
	No	10	23	10	43
Should foods with GMOs have a label?	Yes	72	157	86	315
	No	3	4	1	8
Do you inquire about GMOs while purchasing?	Yes	22	48	21	91
	No	53	113	66	232
Organic food budget	Yes	12	38	30	80
	No	63	123	57	243
Number of consumers in each group		*75*	*161*	*87*	*323*

In the sample population, men and women consisted of 50.77% and 49.23% of the participants. Although the rates of men and women at the middle-income group were almost equal to each other, they showed a higher density than the other segments. While 54% of the participants were in 25-35 age groups, this age group was mostly concentrated at the middle-income group. 74% of target mass and 78% of middle-income group graduated from any university.

On the other hand, whereas the marriage rate for sample mass was calculated as 53%, singles with 56% at the low-income group and marrieds with 75% at the high-income group were found a more intensive position. Although the ratio of childless families in total population was 50%, this density increased at low and middle-income groups. The density of households with 1-3 children at the high-income group was, however, determined as higher than the others. The occupational statuses of blue and white collars come to the fore in all consumers, but blue and white collars were of a higher rate at the low and high-income segments, respectively (Table 2).

It was found that 58% and %26 of the participants perceived GMO-food awareness from visual media and social media, respectively. Similarly, it was stated that the consumers at the middle and high-income groups were widely used the visual and social media communication channels, but those at the low-income group were mostly triggered by the newspapers and visual media. On the other hand, 87% of all consumers and 86-89% of the participants at each segment manifested that it was a legal obligation to be given information about the negative effects of foods with GMOs on human health and the environment. In addition, 96-99% of consumers considered that it was a necessary to be included GMOs information on the food labels. Furthermore, 72% of the target mass and those at each segment highlighted that they were of not any information about food with GMOs during purchasing at food markets, and 75% of those were not able to make a budget for organic foods, as well (Table 2).

The average monthly food and organic food expenditures of the participants were calculated as ₺1245.36 and ₺111.15. While the average monthly food and organic food expenditures were ₺886 and ₺52 at the low-income segment, it was determined as ₺1104 and ₺79.81 and ₺1815.52 and ₺220.11 at the middle and high-income groups (Table 3). As the income levels of the participants increased, a similar trend was observed for the conventional and organic food expenditures. In conclusion, it was analyzed that there was a positive relationship between consumers' income and food expenditure amounts.

Table 3. Monthly general and organic food expenditures under income groups

Expenditure types		Income groups						All consumers	
		Low		Middle		High			
		Mean	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
Food expenditure	+ < ₺750	513.64	159.35	532.76	146.47	616.67	229.49	529.63	156.93
	₺750-₺1500	1137.50	271.36	1148.21	247.57	1176.53	275.96	1154.97	259.40
	+ > ₺1500	4000.00	1732.05	2657.89	1364.76	3018.75	1369.59	2946.30	1392.39
	Total	*886.00*	*788.80*	*1104.66*	*809.34*	*1815.52*	*1262.27*	*1245.36*	*1010.85*
Organic food expenditure	+ < ₺150	100.00	0	83.33	28.86	2650	42.74	87.50	25.00
	₺150-₺250	200.00	28.86	213.89	0	265.38	0	228.95	55.30
	+ > ₺250	600.00	270.80	625.00	311.78	923.53	521.15	767.14	441.91
	Total	*52.00*	*153.87*	*79.81*	*203.28*	*220.11*	*425.45*	*111.15*	*280.77*

3.2. The EFA results related to consumers' consumption tendency toward foods with GMOs

Kaiser Normalization (KMO), sample adequacy criterion index comparing the observation and partial correlation coefficients, and explaining the consumers' attitude and behaviors toward foods with GMOs was calculated as 0.787 in Table 4. Bartlett's test of Sphericity statistics for the main factors related to consumers' attitude and behaviors, on the other hand, was calculated as $\chi^2_{630;\,0.05} = 4517.93$ ($p = 0.000$) and unit matrix hypotheses was rejected (p<0.001). These two statistics evaluating the sample data set indicated that the data set, therefore, on the factors affecting consumers' food consumption with GMO were at a good level for EFA.

Table 4. EFA results and fit statistics related to consumers' purchase perception and attitudes toward foods with GMOs

	F1	F2	F3	F4	F5	F6	F7	F8
Willingness to buy foods with GMOs during the Covid-19								
M39	0.771	0.117	0.111	0.165	0.088	0.014	0.112	0.150
M37	0.756	0.023	0.118	0.104	0.002	0.206	0.050	0.118
M59	0.729	0.086	0.104	0.038	0.134	0.099	0.143	0.024
M38	0.695	0.101	0.113	0.147	0.141	0.074	0.141	0.161
M35	0.695	0.073	0.007	0.130	0.106	0.133	0.015	0.099
M36	0.689	0.044	0.109	0.070	0.093	0.147	0.066	0.001
M46	0.671	0.018	0.165	0.025	0.045	0.162	0.092	0.080
M47	0.641	0.117	0.065	0.145	0.083	0.075	0.025	0.021
M27	0.618	0.045	0.188	0.044	0.038	0.035	0.044	0.001
M23	0.591	0.068	0.248	0.018	0.133	0.096	0.088	0.065
M56	0.508	0.058	0.097	0.084	0.138	0.304	0.200	0.139
Food preference with GMOs								
M10	0.108	0.812	0.017	0.030	0.114	0.092	0.027	0.081
M14	0.003	0.806	0.125	0.055	0.126	0.072	0.062	0.029
M9	0.007	0.800	0.015	0.028	0.042	0.078	0.011	0.155
M11	0.173	0.765	0.011	0.012	0.033	0.063	0.135	0.081
M13	0.008	0.765	0.011	0.121	0.122	0.027	0.028	0.092
M12	0.108	0.658	0.017	0.030	0.114	0.092	0.027	0,081
GMOs concern perception								
M17	0.195	0.093	0.859	0.077	0.043	0.034	0.035	0.065
M18	0.198	0.108	0.846	0.007	0.037	0.030	0.009	0.033
M19	0.251	0.009	0.844	0.026	0.008	0.000	0.026	0.009
Price mix for foods with GMOs								
M42	0.006	0.018	0.041	0.788	0.056	0.109	0.125	0.028
M43	0.083	0.044	0.141	0.781	0.035	0.038	0.025	0.020
M41	0.007	0.147	0.025	0.723	0.008	0.044	0.073	0.098
M44	0.193	0.081	0.064	0.573	0.142	0.357	0.059	0.080
Visual and cognitive GMOs perception								
M5	0.138	0.179	0.038	0.009	0.762	0.036	0.046	0.065
M8	0.049	0.160	0.000	0.130	0.715	0.192	0.155	0.064
M6	0.153	0.187	0.045	0.053	0.701	0.035	0.262	0.001
Change in favor of GMO food purchase during the Covid-19								
M54	0.057	0.113	0.109	0.135	0.023	0.666	0.202	0.075
M58	0.056	0.075	0.062	0.155	0.005	0.658	0.125	0.098
M53	0.018	0.083	0.088	0.051	0.176	0.588	0.063	0.043
***GMOs concern based on product* mix**								
M31	0.147	0.019	0.033	0.013	0.196	0.060	0.658	0.331
M33	0.069	0.049	0.043	0.003	0.075	0.260	0.640	0.123
M49	0.192	0.007	0.077	0.134	0.218	0.014	0.601	0.166
M60	0.296	0.014	0.063	0.083	0.050	0.236	0.515	0.179
GMOs concept awareness								
M2	0.086	0.063	0.099	0.117	0.099	0.014	0.007	0.814
M1	0.156	0.075	0.017	0.082	0.070	0.033	0.066	0.794
				Goodness of fit statistics				
Eigenvalues	6.060	4.369	2.416	2.061	1.785	1.583	1.513	1.468
Explained variance (%)	16.833	12.135	6.712	5.726	4.960	4.398	4.202	4.078
Cumulative variance (%	16.166	28.968	35.680	41.405	46.365	50.042	54.965	59.042
KMO (Kaiser-Meyer-Olkin) statistics								0.787
Bartlett's test of Sphericity								*[Chi-square ($\chi^2_{df:630}$) = 4517.926] (p = 0.000)*
Sample size (n)								323

In the present study, it was firstly tested whether or not 60 attributes presented at food attitude scale designed for the consumers' consumption tendencies toward foods with GMOs met the criteria assumed for EFA. The results of rotated component matrix showed that 24 variables were excluded from EFA due to the variable loads overlapped and the meaningless items in the component matrix under

each factor dimension. 36 variables impacting on the consumers' consumption tendencies towards foods with GMOs were then determined, and they were reduced to eight main factors explaining 59.04% of the total variance by taking into consideration the Eigenvalues greater than 1 for each factor (Table 4).

First factor explaining 15.14% of total variance was identified as willingness to buy foods with GMOs during the Covid-19, and this factor included the food attributes such as M23, M27, M35-M39, M46, M47, M56 and M59 (Table 4). Second factor responding to 10.54% of total variance was called as foods with GMOs, which contains agricultural products such as strawberry, eggplant, potato, tomato, corn and soybean. On the other hand, GMOs concern perception and price mix for foods with GMOs illustrating 6.85% and 6.47% of total variance were third and fourth factors being constructed by the food attributes with M17-M19 and M41-M44, respectively.

Fifth factor accounting for 5.32% of total variance referred to visual and cognitive GMOs perception covering the variables such as M5, M6 and M8. Change in consumers' purchase attitudes towards foods with GMOs during the Covid-19 with 5.04% explanatory rate consisted of sixth factor covering the variables such as M53, M54 and M58 in Table 4. Similarly, the factors related to GMOs concern based on product mix and GMOs concept awareness revealed seventh and eighth factors accounting for 5% and 4.65% of total variance, respectively. Indeed, there were much stringer relations among not only the variables integrating M31, M33, M49 and M60 for seventh factor, in turn, but also M1 and M2 for eighth factor.

3.3. Cluster analysis results for consumers' consumption tendency toward foods with GMOs

After exploring eight factors affecting the consumers' attitude and awareness towards foods with GMOs in Erzurum, and then cluster analysis was applied to these main factors. Target consumer mass was divided into three income groups consisting of low, middle and high-income segments according to their income levels, and then it was determined the main factors for each consumer mass toward foods with GMOs. The relative ratios of high, middle and low-income groups were calculated as 26.9%, 49.8% and 23.2%, respectively.

The results of cluster analysis highlighted that the consumers with low-income positioned with awareness of foods with GMOs by considering biotechnological processes concerns under the product mix. In the middle-income group, the consumers focused on their purchase behavior changings in favor of being bought foods with GMOs based on lower prices by being awareness of foods with GMOs during the Covid-19 pandemic. On the other hand, it was pointed out that high-income consumers espoused willingness to buy foods with GMOs during the Covid-19 despite product mixes designed or improved by being awareness of GMOs food concern via their visual and cognitive perception (Table 5).

Table 5. The results of two-step cluster analysis based on the EFA results for each income group

Main Factors	**Income groups**		
	Low	**Middle**	**High**
Willingness to buy foods with GMOs during the Covid-19	-0.23	-0.04	0.31
Food preference with GMOs	-0.02	0.04	-0.09
GMOs concern perception	0.10	-0.03	0.13
Price mix for foods with GMOs	-0.05	0.14	-0.05
Visual and cognitive GMOs perception	-0.01	-0.06	0.11
Change in favor of GMO food purchase during the Covid-19	-0.08	0.05	-0.03
GMOs concern based on product mix	0.10	-0.03	0.02
GMOs concept awareness	0.05	-0.07	0.13
Number of samples in each cluster (pieces)	*75*	*161*	*87*
The share of each cluster in the total sample (%)	*23.20*	*49.80*	*27.00*

4. Discussion

Consumers' perception toward foods with GMOs and their purchasing attitude and behaviors has always differed from society to society and from region to region. Although there were various studies conducted on foods with GMOs in Türkiye, they referred to the technical and statistical approaches being used to descriptive the current condition. In Türkiye and Erzurum, there has been

fairly little consumption studies conducted on consumers' attitudes and perceptions towards foods with GMOs considering the structural equation models.

In general, consumers' knowledge about foods with GMOs is low due to their similarity to non-GMO foods and a subjective knowledge of food production with GMOs (Wunderlich and Gatto, 2015). Indeed, the results of the study indicated that 72% of Turkish consumers in Erzurum knew nothing at all or a little knowledge about foods with GMOs. Their knowledge about foods with GMOs, moreover, received from television and the internet with 57.6% and 26%, respectively.

In similar studies, it was reported that 65 and 79% of US consumers (Hallman et al., 2013), 77.3% of Latvian consumers (Aleksejeva, 2014), 82.9% of Turkish nursing students (Turker et al., 2013), 81.4% of Polish students (Jurkiewicz et al., 2014) knew very little or nothing knowledge about foods with GMOs, but 28% of Italian consumers and 33.3% of Japanese consumers (McGarry et al., 2012) were moderately or very familiar with GMOs foods. On the other hand, Turker et al. (2013) and Aleksejeva (2014) highlighted that 77.3% and 63.6% of Latvian consumers and 21.7% and 74.3% of Turkish consumers, in turn, received information about foods with GMOs from the internet and television. Although consumers' primer and seconder knowledge sources about foods with GMOs at each country differed, their awareness knowledge levels depicted fairly similar rates.

By depending on consumers' knowledge about foods with GMOs, they have concerned about foods with GMOs on individual health and environmental impacts last decades, and thus consumers showing a negative attitude toward foods with GMOs have particularly concerned about safety, labelling, environmental impacts and ethical issues (Kadirhanoğullaru et al. 2021; Adalja et al., 2022). In fact, the results suggested that 87% of consumers in research area concerned from the negative impacts on human health and environment of foods with GMOs, and thus 97% of those agreed to be a legal necessity to be labelled with mandatory labels of foods with GMOs in contrary US and Japanese consumers.

High-income consumers gave bigger importance to willingness to buy innovative GMO foods in view of information provided from the food labels under their cognitive and visual perception through communication mix by trusting to knowledge sources related to GMO-foods, and thus they were of a positive purchasing motivation towards foods with GMOs during the Covid-19 pandemic due to accessible foods with augmented quality image, disruption at traditional food supply chain, differentiated foods at the markets, more resistant plants to pesticides, agricultural applications without hormone and antibiotics, solution expectations related to resource depletion and world hunger issues. The high-income consumers showed a positive trend towards GMO-foods during the Covid-19 period, moreover, because they believed that GMO-foods would not be at levels to be led to health concerns, and even if they do, they have easier access to health services. In fact, some studies conducted on this topic informed that consumers' cognitive and visual perception about biotechnological applications and foods with GMOs affected positively their awareness, attitude and purchase intention, and thus they accepted new genetic discoveries and progresses at food production (Pham and Mandel, 2019, Palmieri et al., 2020).

In the middle-income group, consumers changed directly their purchasing patterns in favor of food preference with GMOs during the Covid-19 pandemic. With the Covid-19 pandemic, there were very much important problems at food supply chain, and then target consumers gotten difficult to access to the conventional foods, and thus conventional food prices increased rapidly, but foods with GMOs decreased gradually at the agricultural food markets (Akay, 2021; Topcu, 2022a, Topcu, 2022b). In this case, middle-income consumers' food purchase models altered consciously from the conventional foods to foods with GMOs. In other words, due to the higher price sensitivity of middle-income consumers and their lack of health concerns, they changed their purchasing patterns in favour of GMO-foods. In the studies conducted by Palmieri et al (2020) and Pham and Mandel (2019), indeed, it was highlighted that the participants who were not concerned about foods with GMOs did not wanted to pay more for non-GMO foods, since consumer awareness of GMO-food labeling were fairly much low, and their GMOs knowledge was a considerably lack (Russo et al., 2020).

The low-income participants suffering from unknown food ingredients in product mix lacking GMO food labels, and being concerns about human health and environmental safety and ethical issues for GMOs food consumption resulting from biotechnological processes attributed strongly a negative attitude toward foods with GMOs. Unlike high and middle-income participants, low-income consumers were of a negative perception and attitudes toward foods with GMOs. The factors driving consumers to

negative perceptions determined to be adverse impacts on human health and environment, ethical issues, as well as lack of GMOs knowledge based on the information sources in similar various consumption studies. From authors conducted these scientifically researches, Giordano et al (2018), Palmeri et al (2020) and Arani et al (2021) highlighted that even if consumers in favor of pro-science expressed positive opinions for food with GMOs, they believed that when compared the benefits to be provided with the risks to be endured for these; there was a negative perception toward GMO-foods due to their possible long term risks.

In addition, Boccia et al (2018) and Russo et al (2020) reported that the lower consumers' scientific knowledge scores and trust to information sources, the lower their perception and intention to buy foods with GMOs. Furthermore, Bovay and Alston (2018) and Adalja et al (2022) pointed out that mandatory branding legislative process for foods with GMOs increased considerably consumer aware, and thus non-GMO foods sales increased fairly due to differences in consumer awareness tied to legislative activity. Similarly, Ghozzi et al (2018) and Saputri et al (2019) also indicated that the performance of non-GMO food chain was better than GMO ones, and GMO-free foods were more sustainable.

Conclusion

During the Covid-19 pandemic, in the research trying to determine consumers' perception and purchasing intention toward foods with GMOs, it was found that 72% of all consumers did not any research about foods with GMOs, that 86% of those were not willing to buy the foods designed with new discoveries in biotechnology field, that %99 of those were a mandatory requirement of GMO-food labeling.

On the other hand, it was also reached to interesting results with respect to the understanding of the main drivers of consumers' purchase intention and patterns in the cluster analysis. The results of the study indicated that high-income consumers were of willingness to purchase foods with GMOs under mandatory food labelling by being created cognitive perception and awareness through media communication tools. It was found that, moreover, the middle-income participants wanted to change their purchase patterns to prefer foods with GMOs with lower prices than conventional foods. By contrast with high and middle-income groups, low-income consumers declared to concern in view of human health, environment safety and ethical issues from food mix (especially core product) designed at biotechnology filed. High and middle-income consumers, therefore, attributed a positive purchase motivation toward foods with GMOs, but low-income consumers were of a negative buying perception for these. Consequently, it was determined that there were gradually the adverse relationships among consumers' income segments and their purchase perception and attitudes towards foods with GMOs.

Ethical Protocol

Ethical Protocol was approved by the Scientific Research and Publication Ethics Committee of Iğdır University with its session dated 23.08.2022 and decision numbered 2022/13.

Author's contributions

All authors included in this article contributed equally to each stage of the research.

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Research Article

Developing Sterilization and Lighting Systems for Sprouting Rooms Using Ozone and Optical Fibers

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Abstract: The increasing population has led to the widespread adoption of hydroponics. Hydroponic production of fresh green forage requires minimal space, does not use soil, and allows for rapid harvesting. A fully controlled sprouting room can yield a substantial amount of green fodder from a small area with less water consumption. This study aims to investigate the effectiveness of ozone on seed germination, seedling growth, and microbial sterilization during germinated barley processing. Additionally, the sterilization of the barley sprouting room was conducted using ultraviolet and infrared light, which provides optimal sprouting conditions.The study comprises three experimental variables: three levels of ozonized water (13, 26, and 39 mg L^{-1}) combined with three light sources (fluorescent, infrared, and ultraviolet) and three light duration times (8, 16, and 24 h). The measurements include shoot length, fresh yield weight, dry yield weight, conversion factor, chlorophyll content, N, P, K, crude protein, ash, and log reduction.The results indicated that the maximum values were observed when using ozonized water at 39 mg L^{-1}, Ultraviolet LED as a light source, and a sterilizing medium with a light duration time of 24 h. Conversely, the minimum values were observed when using ozonized water at 13 mg L^{-1}, fluorescent LEDs as a light source, and a light duration time of 8 h. Based on the findings, it is highly recommended to utilize the developed sprouting room throughout the year for the production of fresh forage.

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1. Introduction

By 2050, the world's population is expected to reach approximately 10 billion, with 66% residing in urban areas. This burgeoning population has led to an increasing demand for food and livestock feed (Ghorbel et al., 2021). Traditional farming methods are facing limitations in meeting the growing need for higher quality and quantity of crops (Grigas et al., 2019). As a result, hydroponic systems have emerged as a promising solution to address these challenges. In Egypt, the use of hydroponics to produce high-moisture content forage has garnered significant attention, especially in

arid desert regions where fodder scarcity is a concern (Mariyappillai et al., 2020). Furthermore, climate change has resulted in water shortages, prompting the adoption of hydroponic systems for sustainable organic animal forage production (Sharma et al., 2018). Hydroponics is a soilless method of cultivating plants, enabling the production of fresh green forage from various crops like maize, barley, oats, and cowpeas (Bakshi et al., 2017). Among these crops, barley grains (*Hordeum vulgare L.*) have long been utilized in animal feed (Adiban et al., 2021) and the malting industry (Madakemohekar et al., 2018). Barely sprouts provide animals with essential nutrients, including proteins, carbohydrates, minerals, vitamins, and water. They are grown without soil by germinating seeds in water or nutrients solutions, offering easily digestible and nutritious fodder for livestock. Sprouting facilitates year-round availability of green fodder, conserves water by minimizing evaporation, reduces labor, preserves soil for main crop cultivation, lowers reliance on chemical fertilizers, decreases meat and milk production costs, and ultimately boosts national income.

During sprouting, proteins are converted into essential amino acids, carbohydrates into sugars, and fats into essential fatty acids. Increased enzymatic activity further enhances these conversions, making sprouts easier to digest than dry seeds (Shit, 2019). The hydroponic green forage produced through sprouting takes a period of 8-15 days and requires a small land area (Mooney, 2005; Gebremedhin, 2015). Approximately 6 to 10 kg of fresh green sprouts can be generated from one kg of seeds throughout the year (Kruglyakov, 1989). The resulting forage comprises germinated seeds with intertwined white roots and green shoots, which are entirely consumed by livestock (seed, roots, and shoots). Barley seeds are preferred due to their affordability and widespread availability. The sprouts maintain a crude protein content of 16-17% with more than 85% in vitro digestibility, as well as high levels of vitamin E and beta-carotene, which promote animal fertility (Atlas, 2004). Hydroponic fodder production significantly reduces water usage. For instance, around 3 liters of water are needed to produce one kg of fresh hydroponic feed (Ramteke et al., 2019). Comparatively, 1.5-2 liters of water are required to produce one kg of hydroponically grown green barley forage, whereas conventional field conditions consume 73 liters per kg (Al-Karaki, 2010).

However, despite the benefits of hydroponic sprouting, there are challenges in maintaining the production, nutritional value, and economic yield of sprouts due to of microbiological infections and physiological deterioration (Randeniya and de Groot, 2015). To address these issues, low-dose irradiation and acidified sodium chlorite have been combined to achieve effective sterilization during germination (Nei et al., 2010). The utilization of UV radiation for air, surface, and material disinfection has gained popularity (Yang et al., 2020). The germicidal effect is caused by UV damage to the DNA or RNA of bacteria or viruses. Ozone (O_3) is an inorganic molecule composed of 3 oxygen atoms. It is an unstable gas and cannot be stored. Monroy et al. (2017) reported that ozone may trigger antioxidants that regulate hormone levels, particularly abscisic acid, to break seed dormancy and enhance germination.

The application of ozone technology has become vital for eliminating microbes, fungus, and viruses from various seeds instead of using chemical methods (Mohammad et al. 2019). Ozone sterilization technology has recently been successfully employed in the agricultural sector, either as an aqueous solution or as a gas (Loeb 2018). Abeli et al. (2017) Ozone sterilization technology has recently been successfully employed in the agricultural sector, either as an aqueous solution or as a gas. Vazquez-Ybarra et al. (2015) examined the effects of 0.53 and 59.40 mg L^{-1} ozone on lettuce (*Lactuca sativa* L.) plants in a hydroponic float system. After 10 weeks of growth, the shoot and root biomass, root length, and stem diameter of the plants treated with 2.66 and 3.96 mg L^{-1} significantly increased. Rodrigues et al. (2019) found that ozone exposure at a concentration of 25 g m^{-3} for 120 minutes had no effect on the physiological or biochemical processes of cultivar soybean seeds. According to Mohammad et al. (2019), ozonizing at 5 mg L^{-1} for 20 minutes had no negative effects on the size, color, or germination percentage of alfalfa sprouts. UV-C radiation, known for its high photochemical and biological activity, has been utilized to accelerate the germination of maize and sugar beet seedlings (Sadeghianfar et al., 2019). UV radiation is divided into three wavelength ranges: UV-A (320–390 nm), UV-B (280–320 nm), and UV-C (100–280 nm). Sadeghianfar et al. (2019) found that UV-C radiation had a significant effect on the photosynthesis of treated maize and sugar beet seedlings. Furthermore, UV-C irradiation was used to improve wheat seed germination and growth parameters (Rupiasih and Vidyasagar, 2016) and groundnut (Neelamegam and Sutha, 2015).

Different crops were subjected to infrared micro-spectroscopy to determine structural alterations in the xylem of the plants. Rico et al. (2015) reported a significant increase in root elongation in barley plants in the range of 1727–1760 cm^{-1}.

Hydroponics fodder production is considered a successful alternative method for sustainable livestock production (Ramteke et al., 2019). The chemical composition of hydroponically germinated barley fodder is affected by the harvesting duration, with the seventh day being the optimal harvest day for producing usable fodder (Akbağ et al., 2014). Chlorophyll is essential for photosynthesis (Konica Minolta, 2009) and serves as an accumulation point for rising nitrogen quantities in plants. Measuring chlorophyll content provides information about a plant's general health (Marsh, 2016). Sprouting under net houses recorded the highest values of physical characteristics and chemical analysis compared to the control room.

In Egypt, where the costs of lighting and sterilization of sprouting rooms are high and ineffective, the increase in construction fees for sprouting rooms poses an obstacle to the widespread application of such technology. There are few reports on the application of ozone, ultraviolet light, and infrared to reduce fungal infections in sprouted barley. The traditional method of sprouting rooms relies on ultraviolet tube bulbs distributed along the shelves, resulting in high construction costs and limited dissemination. Additionally, exposure to fungal infections producing toxic auxins adversely affects animal health when feeding on contaminated food. Regarding barley, various parameters such as shoot length, fresh yield weight, dry yield weight, conversion factor, chlorophyll content, N, P, K, crude protein, ash, and log reduction were evaluated. The sterilization efficiency of ozone, UV, and infrared on barley sprouts was assessed using colony-forming units (CFU). The development of this system has enabled the production of fresh forage almost year-round. The research also aims to design an optical device connected to a collection of optical fibers that will transmit light from a single source inside the sprouting room equally to all plants, while controlling the intensity and duration of lighting using a special electronic circuit. This will help reduce the number of bulbs and electrical capacity needed, ultimately lowering production costs.

The present study aims to explore the effect of different concentrations of ozonized water on seed germination and seedling growth. Additionally, the research aims to develop a multi-spectral light system and evaluate its effect on barley growth speed in sprouting rooms.

2. Material and Methods

2.1. Material

2.1.1. Overall structure of the sprouting room

This experiment was conducted in an environmentally controlled room with an average temperature of 23±1°C throughout the study period. The sprouting room was constructed using a steel stand (2 m length, 0.5 m width, and 2 m height) with two shelves placed 30 cm apart. Each shelf had a capacity of holding 12 Polystyrene trays (0.6 m length, 0.3 m width, 0.03 m height, and 0.18 m^2 area), which were obtained from the local market, as shown in Figure 1. The developed lighting system comprises optical fibers in the form of a flexible strip of LED bulbs known for their energy-saving properties. Three different types were employed to replace fluorescent lamps or tube UV lamps, as follows:

1. Fluorescent LED plant grow light strips with a full spectrum (4000K natural white).
2. Infrared flexible LED strip light (940nm, 72 watts).
3. Ultraviolet flexible LED strip light (405nm), all illustrated in Figure 1.

An electronic control circuit was designed to automate the timing of lighting and irrigation with ozone water. The timing circuit incorporates an Arduino Nano, programmed via a computer to logically manage the timing. A crystal LED screen has been added to display the timing programming commands for controlling the sprouting room. The electronic circuit can be programmed using the included control buttons. There is a pair of relays, one for operating the lighting circuit and the other for operating the developed ozone device, as demonstrated in Figure No. 3. The shelves were irrigated using water sprinklers attached to a motor, with a total irrigation rate was 500 ml per tray per day, ensuring adequate moisture for the seedlings. Tap water was used for irrigation without any additional nutrient solutions or additives, following the method suggested by Naik et al. (2015). The relative humidity was adjusted

to approximately 70% through air circulation. The hydroponic system occupied an area of 1 m^2 (Figure 1).

Raw barley grain samples (*Hordeum vulgare* L., "*Giza 128*") from the 2022 crops were purchased from the Barley Department, Agricultural Research Institute, Egypt, and stored at 5 °C until use. The grains were not subjected to any abrasion treatment. Before use, the viability of the barley grains was checked, and the germination percentage was found to be 95%.

The experiments were conducted during the winter season of 2023, from 15th December to 30th of March, at El-Serw Agricultural Research Station, Damietta, Egypt (latitude 31° 41' - 95° 73' N, and longitude 31° 81' - 40° 72' E). The minimum temperature ranged from 10.7 °C in January to 14.2 °C in March, while the maximum temperature ranged from 17.3 °C in January to 20.1 in March. The average nighttime temperatures in December, January, February, and March were 14.3, 12.3, 14, and 15.5 °C, respectively, while the daytime temperatures were 16.9, 14.9. 16.8 and 18.9 °C, respectively. A total of 1290 hours of sunshine duration were recorded, with the maximum average rainfall of 30.4 mm occurring in January. The average relative humidity during December, January, February, and March was 74%, 76%, 73%, and 66%, respectively.

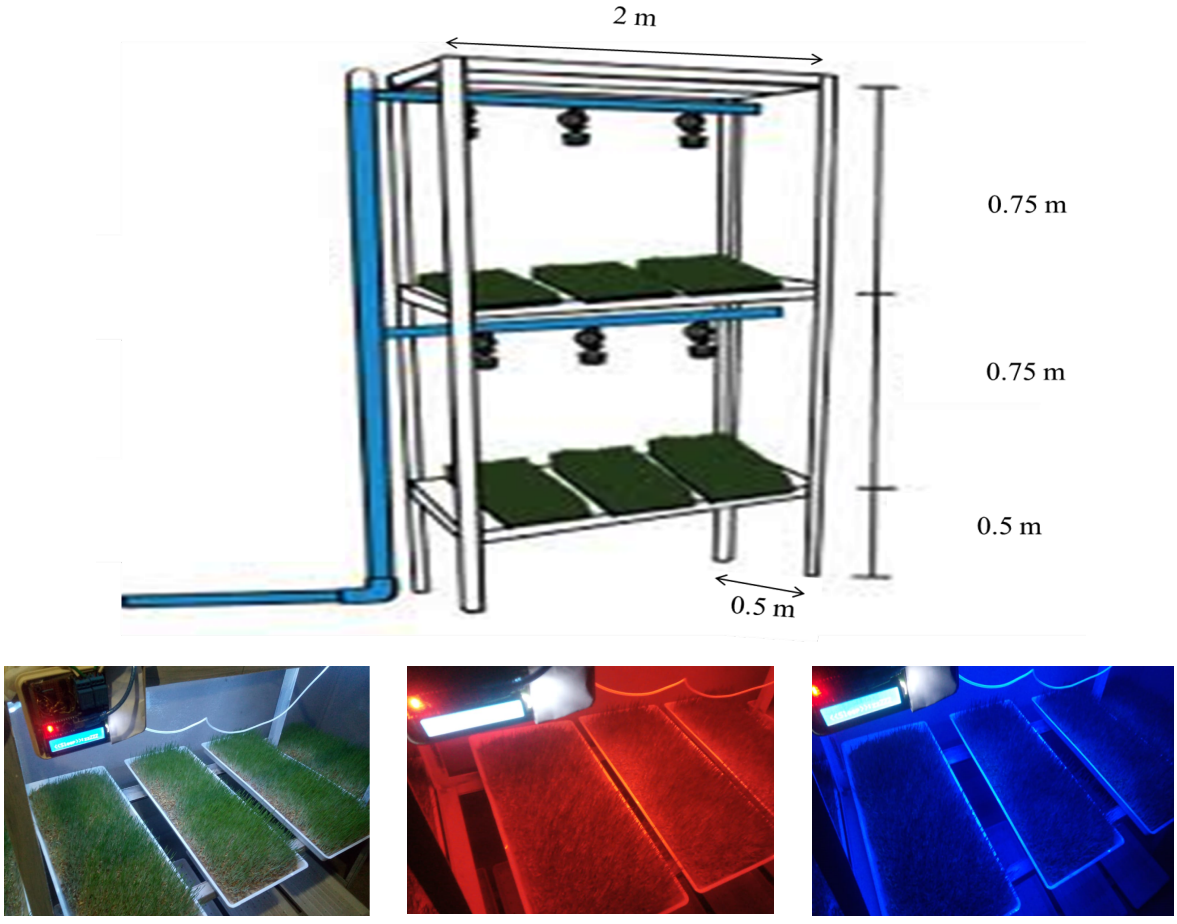
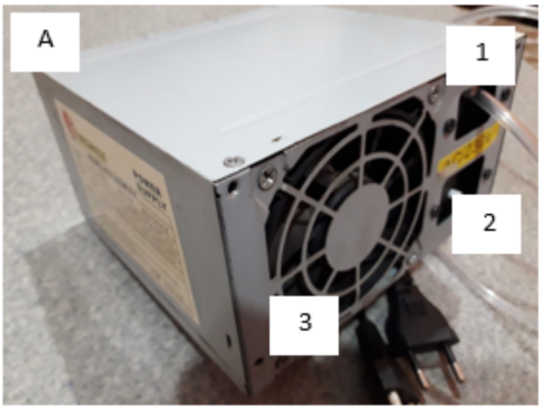


Figure 1. Barely sprouting room.

2.1.2. General description of the ozone device

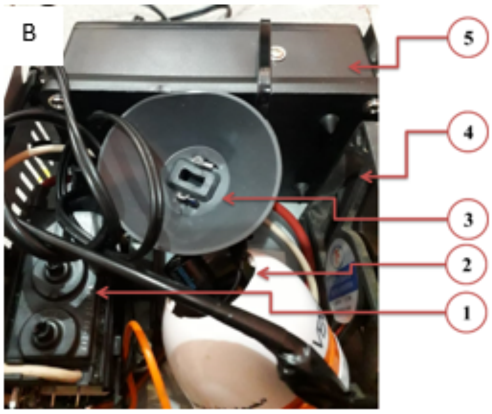
The locally manufactured ozone generation device is illustrated in Figure 2. The concept behind the ozone-generating device involves transforming the air or gas intended for treatment from an electrical insulator into a conductor of electricity, subsequently ionizing it through exposure to high electrical voltage. This process leads to the release of electrons, which gain acceleration due to the high electric voltage, resulting in collisions among them. These collisions break the bonds connecting the two atoms in an oxygen molecule, allowing one of the oxygen atoms to bond with a third atom, creating

ozone gas. This ozone gas offers numerous sterilization benefits. This device is capable of generating two types of ozone. Firstly, it produces ozone gas directly, which is then pumped through an air pump for sterilization purposes, as depicted in Figure 2B (No. 5). The ozone-directive pump is equipped with a switch that allows the user to choose between two speeds to control the amount of ozone gas emitted from the device. Secondly, the ozone generator generates dissolved ozone in the water through a hose connected to the water source, as shown in Figure 2A (No. 1 and 2). The core of the ozone generator includes a high-voltage transducer (Figure 2B, No. 1) that generates a significant electric charge, of up to 50,000 volts. This electric charge passes between two electrodes, the cathode, and the anode, resulting in the formation of an electric arc (Figure 2B, No. 3). This electric arc ionizes the oxygen and converts it into ozone. The device is further equipped with an electronic circuit to regulate the voltage entering the electrical transformer, as shown in Figure 2B (No. 2). Additionally, it incorporates an internal fan (Figure 2A, No. 3) to draw air from the surroundings and direct it internally into the device. Once the ozone gas is generated, an internal pump (Figure 2B, No. 5) functions to withdraw the ozone gas and direct it to the respective outlets, either as direct gas or dissolved in water. The device is further equipped with an electronic circuit to regulate the voltage entering the electrical transformer, as shown in Figure 2B (No. 2). Additionally, it incorporates an internal fan (Figure 2A, No. 3) to draw air from the surroundings and direct it internally into the device. Once the ozone gas is generated, an internal pump (Figure 2B, No. 5) functions to withdraw the ozone gas and direct it to the respective outlets, either as direct gas or dissolved in water. The device is further equipped with an electronic circuit to regulate the voltage entering the electrical transformer, as shown in Figure 2B (No. 2). Additionally, it incorporates an internal fan (Figure 2A, No. 3) to draw air from the surroundings and direct it internally into the device. Once the ozone gas is generated, an internal pump (Figure 2B, No. 5) functions to withdraw the ozone gas and direct it to the respective outlets, either as direct gas or dissolved in water.



A. Homemade ozone device

(1) Ozone outlet for water; (2) Ozone outlet directly; (3) Air suction fan



B. Components of the ozone device

(1) High-voltage transformer; (2) Electronic circuit; (3) Ozone generator cathodes; (4) Air suction fan; (5) Ozone pump

Figure 2. Homemade and Components of the ozone devices.

2.1.3. Generation of ozone treated water

A flask with a stopper and two holes was filled with irrigation water. One hole served as an inlet line for injecting ozone, while the other acted as an exit line to discharge any excess ozone gas. To achieve an aqueous ozone concentration of 13 mg L^{-1}, ozone gas was injected into the water for approximately 90 minutes. To prevent the dispersion of excess ozone into the air, it was passed through a second flask containing a 2% potassium iodide solution, following the method described by Mohammad et al. (2019). The ozone-treated water was prepared by bubbling the irrigation water with ozone gas for the required time to attain the desired concentration for each test. The spectrophotometric method, as outlined by Bader and Hoigne (1981), was used to determine the levels of ozone in the water used for treating barley grains and sprouts.

The following formula was employed to calculate the ozone concentration:

$$O_3 \text{ concentration } (mg\ L^{-1}) = 100\left(\frac{\Delta A}{f \times v \times b}\right) \rightarrow \quad (1)$$

Where ΔA is the difference in absorbance between the sample and blank solutions, b is the path length of the cell in the spectrophotometer (cm), v is the volume of the sample or blank (mL), and f is the factor corresponding to an aqueous ozone absorption coefficient of 0.42. The system was automated using a digital timer, as shown in Figure 3.

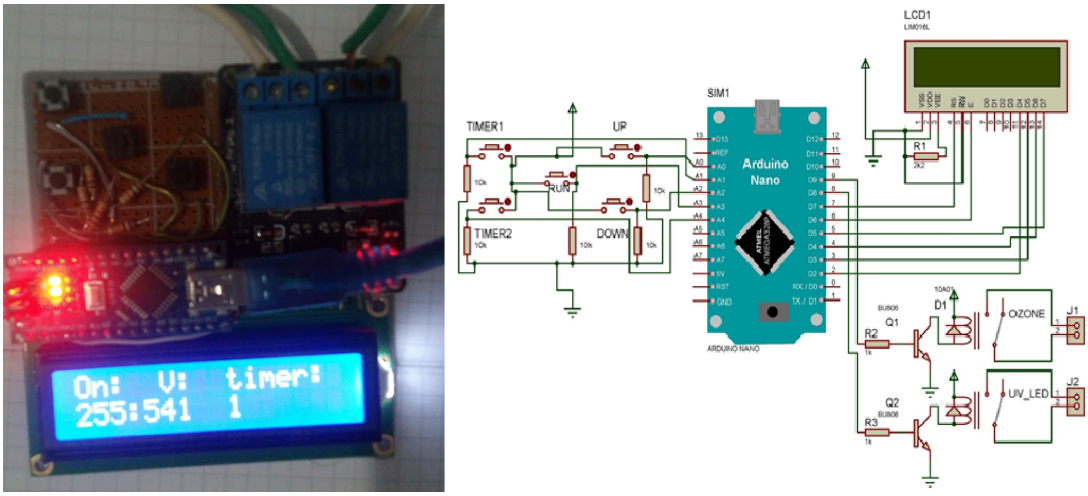


Figure 3. Digital timer circuit and its components.

2.2. Methods

At this stage and before seeding, about ten kg of barley seeds (*Hordeum vulgare L.*, *Giza 128*) were washed to eliminate floating materials (straw and immature seeds) for uniform growth. The seeds were then soaked in ozonated water for approximately 12 hours. After soaking, the seeds were placed in a wet gunny bag and incubated in a dark place to facilitate early germination. Trays were cleaned and disinfected using ozone gas generated from the ozone device, rather than undergoing chemical sterilization with a 0.1% sodium hypochlorite solution (5%), followed by a rinse with tap water. The soaked seeds were evenly spread out in the trays at a rate of 0.750 kg per tray with a thickness of 1.5-2 cm, following the sprouting method performed by Gebremedhin (2015). Small holes in the trays allowed excess water to drain, preventing water stagnation. A transparent plastic cover was placed over the trays to maintain seed moisture during germination. The germination process took 8 days to produce shoot sprouts.

2.2.1. Evaluated variables

Three variables were studied:

a) Ozone treated water (mg L^{-1}): Three levels of ozone treated water were used: 13, 26, and 39 mg L^{-1}.
b) Type of light source: Three light sources were utilized: fluorescent, infrared, and ultraviolet LED.
c) Light duration time (h): Three Light duration times were examined: 8, 16, and 24 h.

2.2.2. Measurements

2.2.2.1 Vegetative characteristics of barely sprout yield

After 8 days of seeding, the fresh fodder was harvested, and the following data were recorded: Shoot length (cm), total fresh yield (kg m^{-2}), total dry yield (kg m^{-2}), conversion factor [ratio of the produced yield to the initial planted seed weight (kg kg^{-1})], and chlorophyll content (mg m^{-2}). Chlorophyll content was measured using a nondestructive fluorometer analysis (Manetas et al., 1998). The CCM-300 chlorophyll meter, produced by OPTI-SCIENCES, Inc. (Hudson, NH), uses a

chlorophyll fluorescence ratio to measure the chlorophyll content within plant leaves (Opti-Sciences Inc., 2011).

2.2.2.2 Chemical, quality, and microbiological analysis of barely sprout yield

Fresh samples (leaves and roots) were collected, oven-dried at 70 °C in an air-forced oven for 48 hours, weighed, and stored for chemical analysis. Dried samples were turned into powder and digested in H_2SO_4 according to Allen (1974), and the nitrogen content was determined using the Kjeldahl method described by FAO (1980). The crude protein was calculated by multiplying N by 5.83 (Merrill and Watt 1955). According to AOAC (2000), samples were ignited in a muffle furnace (Protherm, PFL 110/10 MODEL) at 550 °C for four hours to determine the amount of ash. Potassium was determined using flame photometry (Ryan 1996).

The method described by Koide et al. (2009) was used to count the total number of bacteria on the barely sprouts. After germination (Day 7), all of the collected barely sprouts were weighed into 200 mL of sterile physiological saline solution. To eliminate the microbial cells from the sprouts' surface, the flask was forcefully shaken by hand. After that, 1 mL of the solution was diluted to 10 mL with sterile physiological saline solution. For the enumeration of the total bacterial count, 1 mL of the mixture was serially diluted (1:10) in a sterile saline solution. Then, 100 mL of the diluted solutions were evenly distributed in triplicate on LB plates and incubated at 37 °C to count the CFU of bacteria. Log colony-forming units per gram (log CFU g^{-1}) were used to express the microbial count. The log reduction, which was calculated using the following formula, was used to evaluate the sterilization ability of the new technique.

$$Log\ reduction = \log\left(\frac{CFU_{control}}{CFU_{treated}}\right) \quad (2)$$

2.2.2. Statistical analysis

The experiments were replicated three times. A completely randomized experimental study design was used for data analysis. The Costat Program (Oida, 1997) was used to determine the statistical significance of the variables under consideration based on the probability ($P<0.05$).

3. Results and discussion

3.1. Factors affecting some vegetative characteristics of barely sprout yield

The results presented in Figures 4 to 6 illustrate the relationship between ozonated water, the type of light source, and light duration time on various vegetative characteristics of sprouting barely fresh fodder, including shoot length, fresh yield weight, dry yield weight, conversion factor, and chlorophyll content. The findings indicate that fresh green fodder can be produced after 8 days of seeding in sprouting rooms from the tested barely crop (*Giza 128*). As the levels of ozone-treated water increased, there was an indirect increase in the vegetative characteristics of sprout yield.

Notably, the mean best values for vegetative characteristics were observed at an ozone-treated water level of 39 mg L^{-1}. Specifically, shoot length was 19.33±3.89 cm, fresh yield weight was 35.97±4.04 kg m^{-2}, dry yield weight was 7.60±1.06 kg m^{-2}, conversion factor was 8.63±0.97 kg kg^{-1}, and chlorophyll content was 44.58±2.09 mg m^{-2}. In contrast, the mean minimum values for vegetative characteristics were observed at an ozone-treated water level of 13 mg L^{-1}, with shoot length being 17.44±3.90 cm, fresh yield weight being 30.08±4.24 kg m^{-2}, dry yield weight being 6.44±1.50 kg m^{-2}, conversion factor being 7.20±1.02 kg kg^{-1}, and chlorophyll content being 35.96±2.41 mg m^{-2}.

Regarding the light source, the highest mean values of vegetative characteristics were observed when using Ultraviolet LED as a light source and sterilizing medium. Specifically, shoot length was 22.22±1.65 cm, fresh yield weight was 37.63±2.98 kg m^{-2}, dry yield weight was 8.21±0.77 kg m^{-2}, conversion factor was 9.03±0.71 kg kg^{-1}, and chlorophyll content was 42.56±3.66 mg m^{-2}. Conversely, the lowest mean values of vegetative characteristics were observed when using fluorescent lamps as a light source, with shoot length being 13.74±1.58 cm, fresh yield weight being 28.72±2.92 kg m^{-2}, dry

yield weight being 5.81±0.65 kg m^{-2}, conversion factor being 6.89±0.70 kg kg^{-1}, and chlorophyll content being 37.74±4.05 mg m^{-2}.

Furthermore, increasing the light duration time resulted in higher vegetative characteristics. The highest mean values were observed at a light duration time of 24 h, with shoot length being 19.37±3.55 cm, fresh yield weight being 34.69±4.79 kg m^{-2}, dry yield weight being 7.27±1.21 kg m^{-2}, conversion factor being 8.33±1.15 kg kg^{-1}, and chlorophyll content being 41.11±4.10 mg m^{-2}. In contrast, the minimum mean values were observed at a light duration time of 8 h, with shoot length being 17.37±4.00 cm, fresh yield weight being 32.06±4.69 kg m^{-2}, dry yield weight being 6.61±1.17 kg m^{-2}, conversion factor being 7.69±1.12 kg kg^{-1}, and chlorophyll content being 39.48±4.53 mg m^{-2}. The control group had significantly lower mean values for all vegetative characteristics, with shoot length being 10.40±1.30 cm, fresh yield weight being 20.56±2.67 kg m^{-2}, dry yield weight being 4.11±1.01 kg m^{-2}, conversion factor being 4.93±0.82 kg kg^{-1}, and chlorophyll content being 28.43±1.51 mg m^{-2}.

These results can be attributed to the beneficial effect of ozone-treated water as a nonchemical method for removing microbes, fungus, and viruses from sprouting barely. This is in line with previous research by Monroy et al. (2017) and Mohammad et al. (2019), which highlighted the regulatory role of ozone in hormone levels, particularly abscisic acid, leading to enhanced barley growth. Additionally, changes in ozone-treated barely sprouts may be attributed to the stress induced by certain doses of O_3, which triggered an adaptive response and improved the barley's resistance to environmental challenges.

The observed shoot length in barley is consistent with the findings of Al-Hashmi (2008). The values of total fresh yield align with previous reports by Shtaya (2004). Peer and Leeson (1985) have reported a negative relationship between dry matter content and fresh weight yield, which limits the consumption of green fodder by animals due to its low dry matter content.

The production conversion ratio, calculated as the quantity of new fodder produced per unit of seed utilized, ranged from 4 to 8 times the values reported by Morgan et al. (1993). Al-Ajmi et al. (2009) and Al-Hashmi (2008) also reported a ratio of 2.76 to 3 kg of green forage per kg of barely seed, which is lower than that reported by other researchers.

The higher production of chlorophyll has been linked to nitrogen accumulation and overall plant health (March, 2016). The higher level of chlorophyll found in these plants suggests that they were more vigorous and metabolically active.

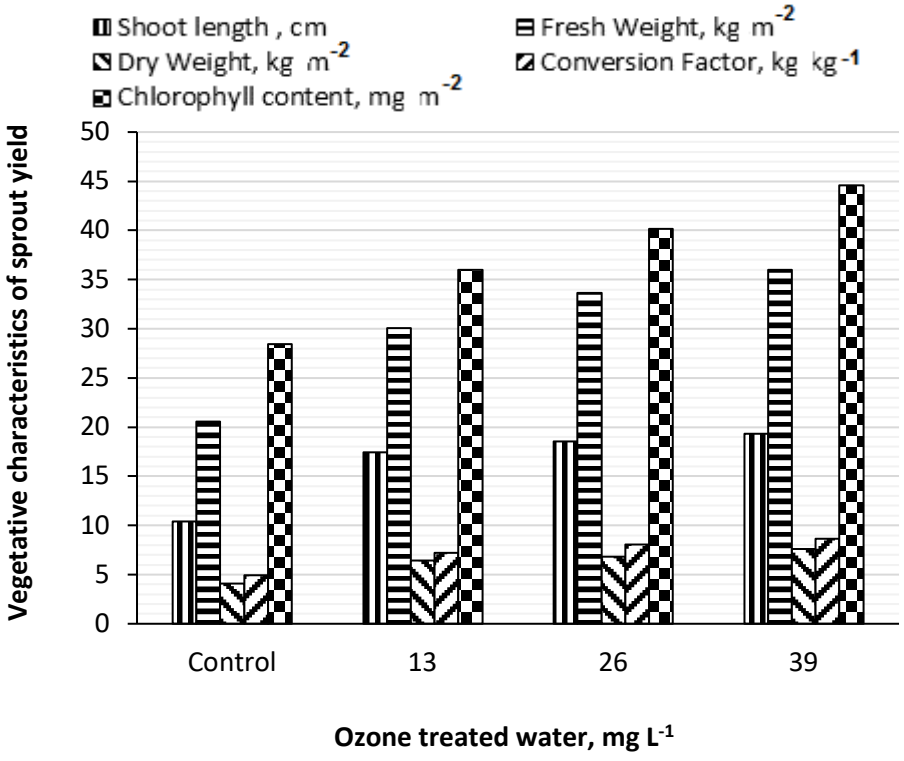


Figure 4. Effect of ozone treated water (mg L^{-1}) on vegetative characteristics of barely sprouts yield.

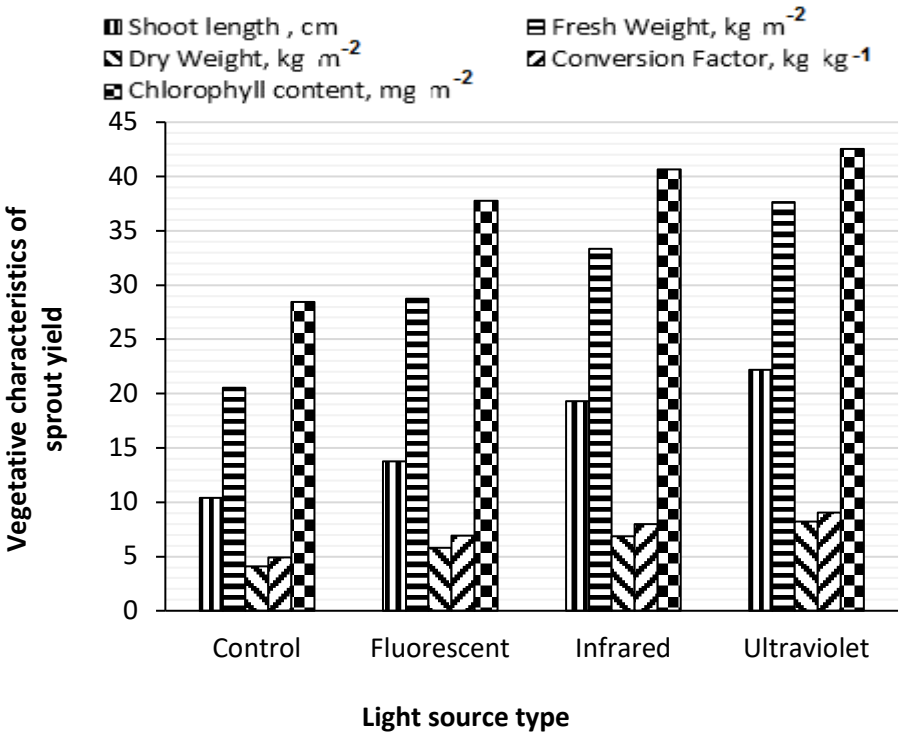


Figure 5. Effect of light source type on vegetative characteristics of barely sprout yield.

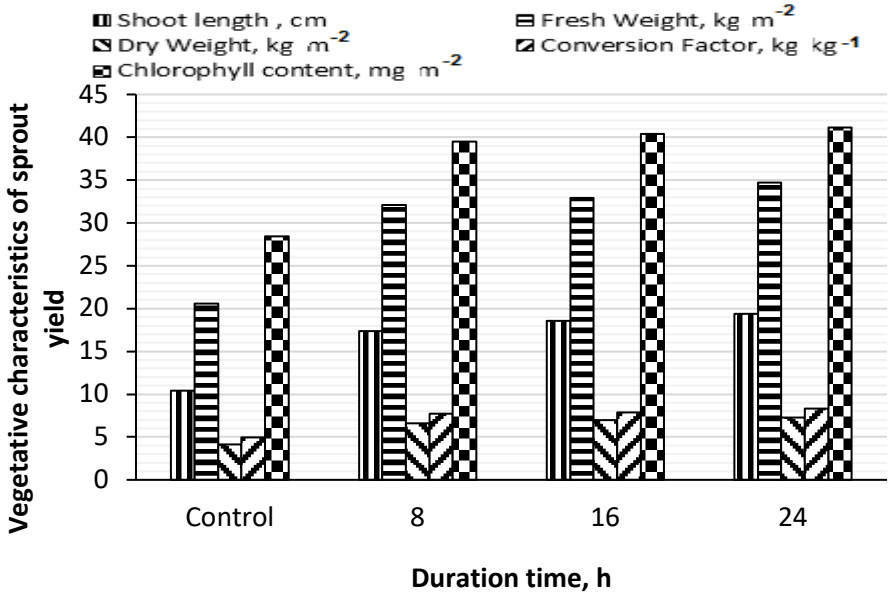


Figure 6. Effect of duration time (h) on vegetative characteristics of barely sprout yield.

3.2. Factors affecting chemical, quality and microbiological analysis of barely sprout yield

The results presented in Figures 7 to 9 demonstrate direct relationships between the tested factors (ozonated water, type of light source, and light duration time) and the measured chemical, quality, and microbiological analysis of sprouting barley yield. These findings have important implications for the optimization of fresh fodder production and the enhancement of the nutritional and microbiological quality of sprouting barley.

Regarding the chemical composition, the study revealed that the ozone-treated water significantly influenced the nutrient content of the sprouting barley. The most significant mean values of nitrogen (N), phosphorus (P), potassium (K), crude protein, ash, and log reduction were observed at an ozonated water level of 39 mg L^{-1}. The respective mean values were 3.20±0.04%, 0.77±0.03%, 2.05±0.06%, 18.64±0.24%, 3.43±0.19%, and 4.21±0.34. In contrast, the minimum mean values for these chemical parameters were observed at an ozone-treated water level of 13 mg L^{-1}, with the respective mean values being 2.98±0.13%, 0.54±0.05%, 1.39±0.25%, 17.39±0.74%, 2.22±0.31%, and 2.53±0.32. Additionally, the choice of the light source and light duration time also had a significant impact on the chemical composition of the sprouting barley. The highest mean values of N, P, K, crude protein, ash, and log reduction were obtained when using Ultraviolet LED as a light source and sterilizing medium. Conversely, the lowest mean values were observed when using fluorescent lamps as a light source. Moreover, increasing the light duration time was found to increase the mean values of N, P, K, crude protein, ash, and log reduction. The highest mean values were observed at a light duration time of 24 h, while the lowest mean values were observed at a light duration time of 8 h. These findings suggest that the combination of ozone-treated water, Ultraviolet LED as a light source, and longer light duration time contributes to the enhanced nutritional content of the sprouting barley, making it a more valuable feed source for livestock.

The study also compared the crude protein content obtained in this research with previous studies. The results were found to be comparable to the range reported by Sneath and McIntosh (2003) for the composition of sprouted barley, which ranged from 11.38% to 24%. This further validates the use of ozonated water and optimized light conditions to enhance the nutritional value of sprouting barley and ensure its suitability as a high-quality feed option.

In terms of microbiological analysis, the study demonstrated that non-treated sprouting barley had a significantly higher total number of bacteria. This highlights the importance of implementing effective measures to control microbial contamination during the sprouting process. Different authors have previously used chemical substances to treat microorganisms, but the study findings are consistent with Conceico et al. (2016) and Ferreira et al. (2016), who observed that such products may not completely remove the presence of microorganisms. The application of ozonated water in sprouting barley offers advantages over chemical treatments, as it significantly reduces the need for such chemicals, leading to a more environmentally friendly and sustainable approach to fresh fodder production.

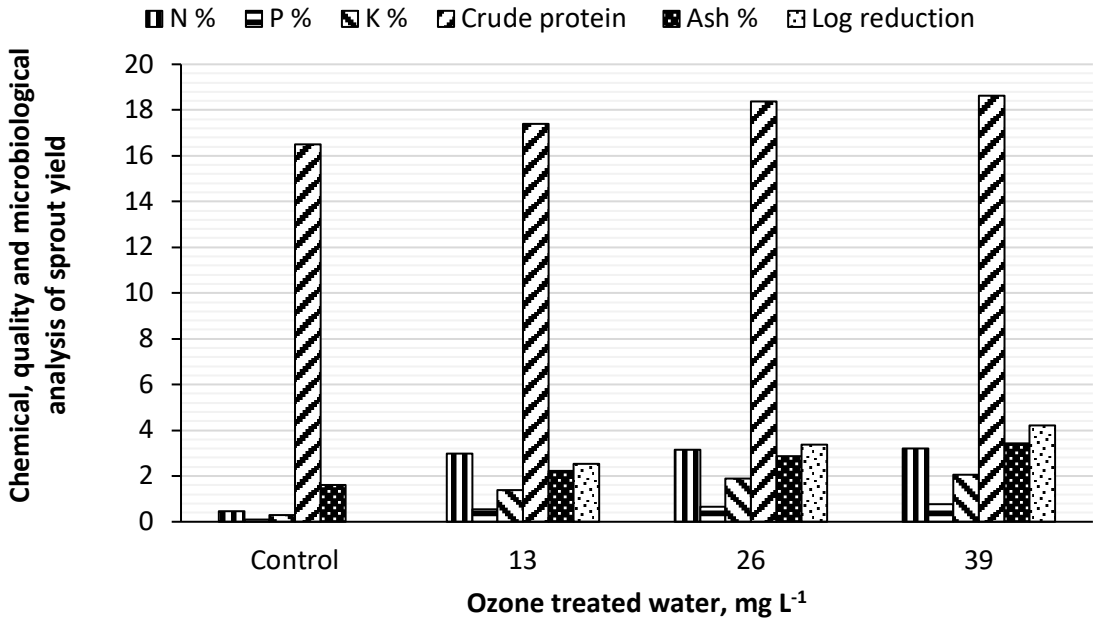


Figure 7. Effect of ozone treated water (mg L^{-1}) on chemical, quality and microbiological analysis of barely sprout yield.

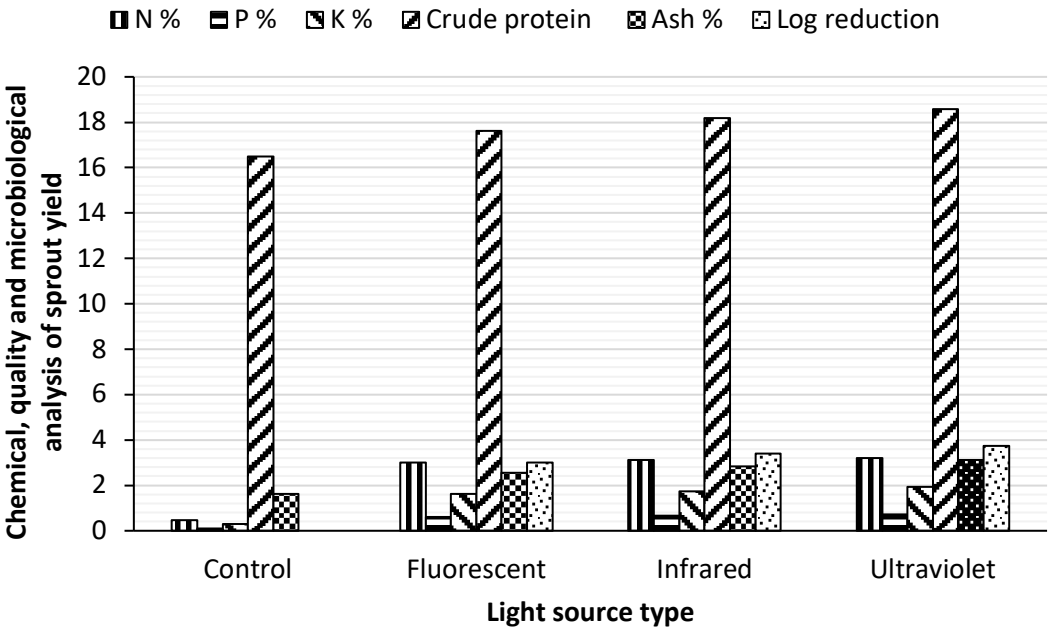


Figure 8. Effect of light source type on chemical, quality and microbiological analysis of barely sprout yield.

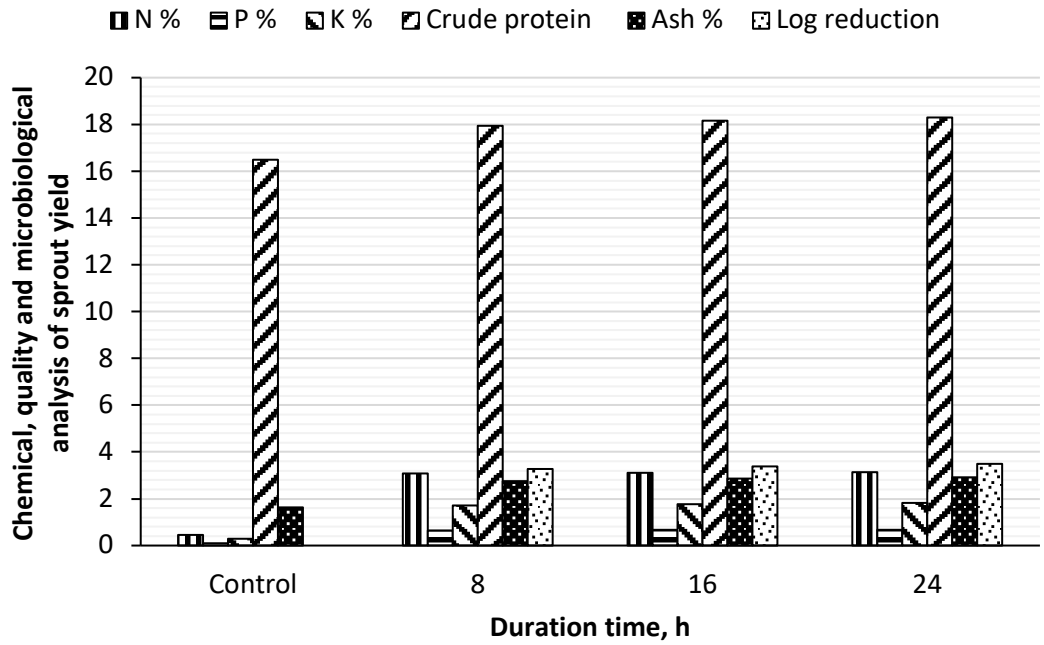


Figure 9. Effect of duration time (h) on chemical, quality and microbiological analysis of barely sprout yield.

The results presented in Table 1 and 2 provide valuable insights into the impact of various factors on the characteristics of barely sprout yield. The ANOVA analysis clearly demonstrates that different levels of ozone treated water have a significant effect on vegetative characteristics, as well as the chemical, quality, and microbiological analysis ($p < 0.001$). It is evident that the levels of ozonated water played a crucial role in influencing the growth and development of the sprouting barely. Specifically, the third level of ozonated water yielded the most favorable results, as indicated by the significant difference between the first level and both the second and third levels ($p < 0.05$). These

findings suggest that increasing the level of ozonated water positively correlates with improved vegetative characteristics of the sprouting barely, leading to enhanced yield and quality.

Moreover, the type of light source utilized in the sprouting process also exhibited a significant impact on the vegetative characteristics of barely sprout yield. Among the different light sources tested, the Ultraviolet LED emerged as the most effective in maximizing the vegetative characteristics. The results highlight the importance of choosing the appropriate light source for sprouting rooms, as it can significantly influence the growth and overall development of the sprouting barely.

Furthermore, the duration of light exposure demonstrated a noteworthy effect on all the measured parameters. The length of light exposure directly influenced the vegetative characteristics, with the third duration level producing the most favorable outcomes, while the first level yielded the least desirable results ($p < 0.05$). This finding emphasizes the importance of providing adequate light exposure to achieve optimal growth and yield of sprouting barely.

Table 1. Means and standard errors for some vegetative characteristics of sprout yield affected by studied factors

Factors	Level	Shoot length, cm	Fresh weight, kg m^{-2}	Dry weight, kg m^{-2}	Conversion factor	Chlorophyll content, mg m^{-2}
Control		10.40±1.30	20.56±2.67	4.11±1.01	4.93±0.82	28.43±1.51
Ozone treated water, mg L^{-1}	13	17.44±3.90^{a}	30.08±4.24^{a}	6.44±1.50^{b}	7.20±1.02^{b}	35.96±2.41^{b}
	26	18.52±3.76^{ab}	33.62±4.07^{ab}	6.85±0.83^{ab}	8.07±0.98^{ab}	40.15±2.55^{ab}
	39	19.33±3.89^{a}	35.97±4.04^{a}	7.60±1.06^{a}	8.63±0.97^{a}	44.58±2.09^{a}
p-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Type of light source	Fluorescent	13.74±1.58^{a}	28.72±2.92^{c}	5.81±0.65^{b}	6.89±0.70^{c}	37.74±4.05^{b}
	Infrared	19.33±1.57^{b}	33.31±3.24^{b}	6.86±0.87^{ab}	8.00±0.78^{b}	40.67±3.96$^{a b}$
	Ultraviolet	22.22±1.65^{a}	37.63±2.98^{a}	8.21±0.77^{a}	9.03±0.71^{a}	42.56±3.66^{a}
p-value		<0.0001	<0.0001	<0.0201	<0.0001	<0.0001
Light duration time, h	8	17.37±4.00^{b}	32.06±4.69^{b}	6.61±1.17^{b}	7.69±1.12^{c}	39.48±4.53^{b}
	16	18.56±3.95^{ab}	32.92±4.53^{ab}	7.00±1.30^{ab}	7.90±1.09^{ab}	40.37±4.37^{ab}
	24	19.37±3.55^{a}	34.69±4.79^{a}	7.27±1.21^{a}	8.33±1.15^{a}	41.11±4.10^{a}
p-value		0.0121	0.0131	0.0152	0.0116	0.0201

Table 2. Means and standard errors for chemical, quality and microbiological analysis of sprout yield affected by studied factors

Factors	Level	N %	P %	K %	Crude protein %	Ash %	Log reduction
Control		0.46±0.07	0.11±0.01	0.29±0.03	16.50±0.27	1.62±0.15	0.00±0.02
Ozone treated water, mg L^{-1}	13	2.98±0.13^{b}	0.54±0.05^{b}	1.39±0.25^{b}	17.39±0.74^{b}	2.22±0.3^{b}	2.53±0.32^{c}
	26	3.15±0.06^{ab}	0.67±0.05^{ab}	1.88±0.15^{ab}	18.37±0.37^{ab}	2.88±0.2^{ab}	3.38±0.29^{b}
	39	3.20±0.04^{a}	0.77±0.03^{a}	2.05±0.06^{a}	18.64±0.24^{a}	3.43±0.1^{a}	4.21±0.34^{a}
p-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Type of light source	Fluorescent	3.02±0.15^{b}	0.61±0.1^{b}	1.63±0.34^{b}	17.63±0.86^{b}	2.56±0.56^{b}	3.01±0.70^{b}
	Infrared	3.12±0.09^{ab}	0.66±0.1^{ab}	1.75±0.34^{ab}	18.19±0.50^{ab}	2.85±0.5^{ab}	3.39±0.72^{ab}
	Ultraviolet	3.19±0.07^{a}	0.71±0.0^{a}	1.94±0.24^{a}	18.59±0.39^{a}	3.12±0.46^{a}	3.74±0.70^{a}
p-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Light duration time, h	8	3.08±0.15^{b}	0.64±0.11^{b}	1.71±0.35^{b}	17.94±0.87^{b}	2.75±0.75^{b}	3.27±0.76^{b}
	16	3.11±0.12^{ab}	0.66±0.10^{ab}	1.78±0.33^{ab}	18.15±0.70^{ab}	2.86±0.55^{ab}	3.38±0.76^{ab}
	24	3.14±0.10^{a}	0.68±0.10^{a}	1.83±0.32^{a}	18.31±0.56^{a}	2.92±0.55^{a}	3.48±0.77^{a}
p-value		0.0112	0.0121	0.0107	0.0128	0.0171	0.0182

Conclusion

In conclusion, the application of ozonated water, type of light source, and light duration time had significant effects on the vegetative characteristics of sprouting barely fresh fodder. The results demonstrate the potential of ozone as an effective nonchemical method for improving sprout yield and enhancing barley growth. Additionally, the use of Ultraviolet LED as a light source and extending the light duration time contributed to increased vegetative characteristics. These findings provide valuable insights for optimizing the production of fresh fodder through hydroponics, promoting sustainable livestock

production and supporting animal health. Further research and optimization of the ozone and light treatment parameters are warranted to enhance the efficiency of the sprouting rooms and ensure consistent production of high-quality fresh forage.

Overall, the direct relationships observed between ozonated water, type of light source, light duration time, and the chemical, quality, and microbiological analysis of sprouting barley yield demonstrate the potential of this approach for optimizing fresh fodder production. The results provide valuable insights for the agricultural sector, particularly in the context of hydroponics fodder production. The use of ozonated water and the appropriate choice of light source and duration can significantly enhance the nutritional value and microbiological safety of sprouting barley, supporting sustainable livestock production and animal health. The study's findings contribute to the advancement of hydroponics technology and provide practical guidance for improving fodder production systems. Further research and development in this field can lead to more efficient and reliable methods for year-round fresh forage production, benefiting both farmers and the livestock industry as a whole.

In conclusion, the results indicate that the levels of ozonated water, type of light source, and light duration time all play vital roles in determining the vegetative characteristics and overall quality of barely sprout yield. The study suggests that the highest level of ozonated water, Ultraviolet LED as the light source, and the longest light duration time are the most favorable conditions for achieving the best results in terms of yield and quality. These findings provide valuable guidance for optimizing the production of fresh forage using hydroponics and can contribute to more sustainable and efficient livestock production practices. Additionally, the use of ozonated water offers environmental benefits by reducing the reliance on chemical treatments, which is a promising step towards eco-friendly agricultural practices.

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Research Article

Effects of Different Harvest Stages on Forage Yield and Quality of Soybean Cultivars Grown as Second Crops

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Abstract: In this study, the effect of three different harvesting stages [full bloom stage (R2), full pod stage (R4), and full seed stage (R6)] on forage yield and quality of three soybean (*Glycine max* (L.) Merr.) cultivars (Adasoy, Derry and Yeşilsoy) were evaluated under Mediterranean climate conditions in Adana, Türkiye in second crop seasons. Plant height, green herbage yield, dry matter yield, crude protein (CP), crude protein yield (CPY), leaf and stem ratio, dry matter intake (DMI), acid detergent fiber (ADF), neutral detergent fiber (NDF), digestible dry matter (DDM) and relative feed value (RFV) were determined. The results showed that the average plant height of three soybean cultivars was 106.5-203.5 cm and green herbage yield was 190 42-603 50 kg ha^{-1}. The highest values were obtained from cv. Derry at R4 and R6 harvest stages. In both years, the highest CPY values were determined from the R6 harvest stages. Obtained ADF, NDF, DMI, DDM, and RFV values were found to be between 32.8-47.1%, 41.1-59.3%, 2.0-3.6%, 52.1-63.3%, 83.0-180.2%, respectively, and the best results were obtained from the R6 harvest stage of cv. Yeşilsoy. According to these results, in second crop conditions, while cv. Derry came to the fore of soybean yield, cv. Yeşilsoy stands out in terms of quality. As a result, it is thought that it is appropriate to harvest soybean in the R6 harvest period, the use of soybean as a green herbage should be expanded and its addition to feed rations can provide positive contributions.

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1. Introduction

High-quality feed production plays an important role in the development of the livestock industry. Feed costs, which constitute approximately 70 percent of the production cost in livestock enterprises, are an important factor determining profitability. Considering that the profitability of the enterprise is affected by the yield and quality of the feed used, it is a great necessity to feed the animals with quality roughage. There is no problem in producing quality feed in the countries where animal husbandry is developed. However the problem of quality feed production in our country increases year by year (Ozkan and Demirbag, 2016). Grass and straw cereals grown for grain are very poor in protein. The amount of stem straw obtained from cereals in Türkiye is 40 million tons with a 40% harvest index,

and about 10 million tons of these are used for animal feeding (Sancak, 2011). However, it is known that these feeds are not quality roughage. Legumes are of increasing importance as a source of plant protein for both human and animal consumption (Voisin et al., 2014; Henchion et al., 2017). Legumes as forage crops are quality roughages rich in protein.

With the increase in soybean varieties developed for feed purposes in recent years, the production of soybean for roughage has also increased (Anderson et al., 2019). Roughage made from soybean attracts attention as a valuable protein source that can be used as an alternative to expensive protein sources in ruminant nutrition (McCandlish et al., 2017). Soybean is preferred in animal production for meat and dairy purposes because of its high digestibility, low fiber, high protein, and energy content (McPeake et al., 2010). In the south of the US, forage yields of soybean ranged between 1.1 and 5.4 mg ha^{-1} with 150 to 190 g kg^{-1} CP and 740 to 790 g kg^{-1} in vitro digestible dry matter and therefore adopted as a high-quality forage (Northup and Rao, 2015; Baath et al., 2018). Additionally, soybean is a highly productive plant. Studies conducted at different locations with Mediterranean climates demonstrated that it is possible to produce an average of 9 300 and 11 300 kg ha^{-1} at R4 and R6 stages, respectively, with soybean forage averaging 13.3% CP, 8.2% DP, and 60.6% IVDMD (Acikgoz et al. 2007). In addition, soybean, as a legume plant, provides additional nitrogen to cereal crops used in rotation due to its nitrogen fixation ability. For these reasons, studies are needed to include soybean in the production system. In this study, it was aimed to determine the yield and quality of three soybean cultivars (Adasoy, Derry, and Yeşilsoy) according to the harvesting stages in order to improve forage soybean production and diversity.

2. Materials and Methods

2.1. Study site and experiment treatments

This study was conducted in the experimental fields of Eastern Mediterranean Agricultural Research Institute (EMARI) (Türkiye, South East, and 36 ° 85' north latitude, 35 ° 34' east longitude and altitude of 12 m) second crop season in the years of 2014 and 2015. The organic matter level of the soil in texperimental area has been observed to be low with a percentage of (1.07%) but the phosphorus (P_2O_5) content was intermediate (36.0 kg ha^{-1}) and potassium (K_2O) level was sufficient. The soil structure was slightly alkaline and limy with pH=7.8, and clayed-loam. This region has a Mediterranean climate. Table 1 shows the average temperatures, rainfall, and humidity for both the 2014 and 2015 growing seasons as well as long-term (1950-2015) averages for the region.

Table 1. Temperature, precipitation, and relative humidity during the 2014-2015 growing period and their long-term average (1950-2015)*

	Average temperature (°C)			Precipitation (mm)			Relative Humidity (%)		
Months	LT	2014	2015	LT	2014	2015	LT	2014	2015
June	25	23	24	17	3	0	67	72	69
July	28	28	28	10	0.25	0	71	72	69
August	28	29	30	8	0.25	9	71	70	62
September	26	26	28	16	80.5	41	65	64	66
Total	109	106	110	51	84	50	273	276	266
Mean	27	27	28	13	21	13	68	70	67

*LT: Long term, The data of Adana Meteorological Station Province between 1950-2015 (Anonymous, 2016b).

The experiment was carried out using four replications in a split plot design with cultivars as main plots and harvest stages as subplots. The main plots had three soybean cultivars (Adasoy, Yeşilsoy, and Derry) and three harvesting stages [full bloom stage (R2), full pod stage (R4) and full seed stage (R6)] as subplots applied. Adasoy, Derry, and Yeşilsoy cultivars are registered as grain, forage, and silage, respectively and they are in the maturation groups of fourth, sixth, and fifth, respectively (Anonymous, 2016a). In the experiment, each plot was planted in 15 rows, 70 cm row spacing, and 5 m in length. Intervals between plots were 1.5 m and intervals between blocks were 2 m. The seeds were sown on 16 Jun 2014 in the first year and on 20 Jun 2015 in the second year. In the sowing 30 kg ha^{-1} N, 70 kg ha^{-1} P_2O_5, and 100 kg ha^{-1} K_2O were given to the plots fertilizer. Sowing was done with a plot seeder in the amount of 60-80 kg ha^{-1} according to seed size of cultivars. Before planting, soybean seeds

were inoculated with *Bradyrhizobium japonicum* bacteria. The plants were irrigated three times at the beginning of the pre-flowering and during the full flowering and pod formation periods.

2.2. Measurements, chemical and statistical analysis

Harvest was performed in the three maturity stages: full flowering (R2), full pod (R4), and full seed stages (R6) (Cirak and Esendal, 2005). Plant height and leaf/stem ratio (fresh weight) were determined with 20 plants measuring. In the harvest stage, for each plot, the materials in three rows, except for the edge effect, were harvested and the dry and green herbage yield and crude protein value were determined. Dry matter yield was obtained by drying at 70 ^{0}C in oven. The total N of soybean in different harvesting stages was determined, using Kjeldahl's method, and crude protein was calculated by multiplying the N content by 6.25 (AOAC, 1990). ADF, NDF % Van Soest et al., (1991) and DDM %, DMI%, and RFV were calculated by the method indicated by Mayouf and Arbounche (2014) as followed:

$$DDM = 88.9 - (0.779xADF) \quad (1)$$

$$DMI = 120/NDF \quad (2)$$

$$RFV = (DDMxDMI)/1.29. \quad (3)$$

In a study with 4 replications (R) using a split plot experimental design (with main plots as cultivars and sub-plots as harvest periods), the average effects of harvest periods (HS) and cultivars (V) on the investigated traits were determined. All statistical tests were analyzed in the SAS Statistical, Version 9.1 program. Mean comparisons were performed using Duncan's Multiple Range Test. All significant differences were evaluated by $p<0.05$ level tests.

3. Results and Discussions

In both years, the effect of cultivars, harvest stages, and interaction on plant height, green herbage yield, and dry matter ratio was significantly, except for the effect of the variety x harvest stage interaction for dry matter in the second year. The difference between years is also significant (Table 2).

The highest increase in plant height was recorded by the Derry soybean variety in both years (153.7 cm, 188.6 cm, respectively). Adasoy and Yeşilsoy varieties gave shorter plant heights in the same statistical group in the first year. In the second year, Adasoy gave the shortest plant height (119.3 cm). Plant height increased as the maturation stage progressed. However, no significant difference was found at the R4 and R6 harvest stages. In the study, the lowest plant height in both years was measured as 110.5 cm and 127.6 cm, respectively, in the R2 period. It was observed that a higher green herbage yield was obtained from R6 stage of the Derry variety in both years (Figure 1,2). Plant height varies significantly according to ecology and plant growth stages. Acikgoz et al. (2007) reported that the plant heights of forage soybean were 73.6, 100.9, and 105.2 cm at the R2, R4, and R6 harvest stages in Bursa conditions, respectively. Relating to the effect of variety averages, the highest green herbage yields were obtained from the Derry variety as 36513 and 54458 kg ha^{-1} for the first and second years, respectively. On the other hand, Adasoy gave the lowest yields as 278 02 kg ha^{-1} and 390 78 kg ha^{-1} for the first and second years, respectively. As soybean has unlimited growth characteristics, there may be significant increases in plant height and green herbage yields in different harvest stages as the plant growth stage prolongs. Nazlican (2010) who worked on Yeşilsoy and Yemsoy soybean varieties for silage purpose took 400 00-560 00 kg ha^{-1} of green herbage yield. In terms of harvest stage, the highest green herbage yields were obtained as 388 35 and 525 65 kg ha^{-1} at R6 stage in both years, respectively. The lowest green herbage yield was measured at R2 stage in both years. The R6 stage, also known as the green seed stage, is when the pods reach maximum weight . For this reason, the R6 phase is the period when green herbage yield is highest. It was observed that higher green herbage yield was obtained from R6 stage of Derry variety in both years (Figure 1,2). Nevertheless, the dry matter rate ranged between 19.3% and 30.9% in 2014 and between 23.2% and 28.8% in 2015. Similar results were reported by Garcia (2006) who worked on soybean plants and obtained 22-30% dry matter content in soybean. The highest dry matter rates were obtained from Adasoy and Derry varieties as 26.8-26.4% in 2014 respectively, but the

highest dry matter in 2015 (28.0%) was obtained from Derry. Moreover, during both years the highest dry matter rate was obtained at R6 harvest stage (Table 2). Dry matter rate was low in early harvested stages and it increased with the progress of the harvest stage. Hintz et al. (1992) and Munoz et al. (1983) reported that the increase of soybean yield continued until the R7 harvest stage. Sheaffer et al. (2001) mentioned that the increase in the yield at R4 and R6 harvest stage for all varieties was 20%. The same authors stated that the most significant increase in yields was between R3 and R4 for feed type of soybean varieties and at R6 and R7 for grain soybean varieties. Acikgoz (2001) reported that the good quality of green herbage product was obtained from plants during full flowering and full seed stages, and green herbage yield in soybean varied between 20 and 40 tons ha^{-1}.

Table 2. Means and the standard errors of investigated parameters and probabilities of factors

	Year (Y)	PH (cm)	GHY (kg ha^{-1})	DMR (%)	HY (kg ha^{-1})	CP (%)	CPY (kg ha^{-1})	LR (%)	SR (%)
Years	2014	132.1 ±17.4^{b}	320 93 ±545.6^{b}	25.0 ±3.9^{b}	854 2 ±250.6^{b}	13.5 ±2.9	106 9 ±28.7^{b}	50.5 ±13.8	49.4 ±6.9
	2015	146.9 ±23.6^{a}	453 57 ±790.6^{a}	26.1 ±2.6^{a}	120 84 ±310.9^{a}	13.6 ±2.7	160 3 ±39.7^{a}	49.1 ±8.6	49.9 ±7.5
2014	**Harvest Stage (HS)**								
	R2	110.5 ±12.9^{b}	228 08 ±388^{c}	19.3 ±3.2^{c}	442 9 ±122.3^{c}	16.6 ±4.8^{a}	697.5 ±12.6^{c}	52.2 ±14.1^{a}	47.3 ±8.8^{b}
	R4	141.9 ±19.8^{a}	346 35 ±397^{b}	24.8 ±3.1^{b}	886 9 ±129.3^{b}	11.2 ±3.1^{c}	977.6 ±22.1^{b}	48.5 ±11.0^{b}	49.9 ±10.6^{b}
	R6	143.8 ±18.7^{a}	388 35 ±508^{a}	30.9 ±2.4^{a}	123 28 ±197.7^{a}	12.5 ±1.7^{b}	153 1 ±24.7^{a}	46.6 ±11.4^{b}	53.5 ±7.8^{a}
	Varieties (V)								
	Adasoy	121.5 ±11.7^{b}	278 02 ±702^{c}	26.8 ±6.1^{a}	800 3 ±359.1^{b}	14.4 ±1.3^{a}	1150 ±51.1	53.8 ±12.6^{a}	46.5 ±9.8^{b}
	Derry	153.7 ±22.1^{a}	365 13 ±765^{a}	26.4 ±3.9^{a}	102 35 ±372.8^{a}	10.4 ±2.2^{b}	1045 ±43.1	49.0 ±14.1^{b}	51.2 ±10.4^{a}
	Yeşilsoy	120.9 ±16.9 ^{b}	319 64 ±763^{b}	21.8 ±5.4^{b}	738 8 ±309.7^{b}	15.5 ±5.5^{a}	1010 ±24.7	52.0 ±11.2^{a}	47.6 ±12.6^{b}
	P Value								
	R	0.007**	0.704	0.136	0.864	0.323	0.519	0.580	0.718
	HS	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.015*	0.018*
	V	0.000**	0.000**	0.000**	0.000**	0.000**	0.091	0.002**	0.000**
	RxV	0.138	0.342	0.616	0.415	0.331	0.329	0.164	0.170
	VxHS	0.003**	0.000**	0.039*	0.108	0.000**	0.002**	0.100	0.154
2015	**Harvest Stage (HS)**								
	R2	127.6 ±25.1^{b}	336 08 ±902.1^{c}	23.2 ±2.3^{c}	7946 ±291.6^{c}	13.7 ±3.6^{a}	999.4 ±13.8^{c}	58.1 ±7.6^{a}	41.3 ±7.1^{c}
	R4	155.0 ±36.4^{a}	499 01 ±658.6^{b}	26.0 ±2.2^{b}	130 54 ±214.5^{b}	12.3 ±2.4^{b}	158 0 ±25.8^{b}	50.8 ±7.3^{b}	47.8 ±7.6^{b}
	R6	158.0 ±34.8^{a}	525 65 ±677.3^{a}	28.8 ±3.4^{a}	152 33 ±334.4^{a}	14.7 ±2.5^{a}	222 0 ±44.1^{a}	38.2 ±5.7^{c}	60.4 ±5.3^{a}
	Varieties (V)								
	Adasoy	119.3 ±10.9^{c}	390 78 ±932,4^{c}	26.1 ±3.1^{b}	105 09 ±407.6^{b}	13.5 ±3.2^{b}	132 0 ±37.3^{b}	55.5 ±10.1^{a}	43.5 ±9.5^{c}
	Derry	188.6 ±21.8^{a}	544 58 ±766.1^{a}	28.0 ±3.6^{a}	154 36 ±361.2^{a}	11.5 ±2.3^{c}	183 7 ±71.4^{a}	41.9 ±7.5^{c}	57.0 ±7.7^{a}
	Yeşilsoy	132.8 ±15.5^{b}	422 58 ±817,6^{b}	23.9 ±2.3^{c}	103 08 ±263.1^{b}	15.7 ±2.1^{a}	164 2 ±54.6^{a}	49.7 ±10.1^{b}	48.8 ±9.6^{b}
	P Value								
	R	0.342	0.173	0.299	0.083	0.046*	0.477	0.227	0.217
	HS	0.000**	0.000**	0.000**	0.000**	0.001**	0.000**	0.000**	0.000**
	V	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.056	0.000**
	RxV	0.057	0.386	0.513	0.951	0.417	0.288	0.388	0.782
	VxHS	0.001**	0.002**	0.097	0.010**	0.000**	0.009**	0.000**	0.546

*p<0.05, **p<0.01; full blooming stage (R2), full pod stage (R4), full seed stage (R6). Plant height (PH), green herbage yield (GHY), dry matter rate (DMR), hay yield (HY), crude protein (CP), leaf ratio (LR), stem ratio (SR); Different superscript letters denote significant differences (p < 0.05).

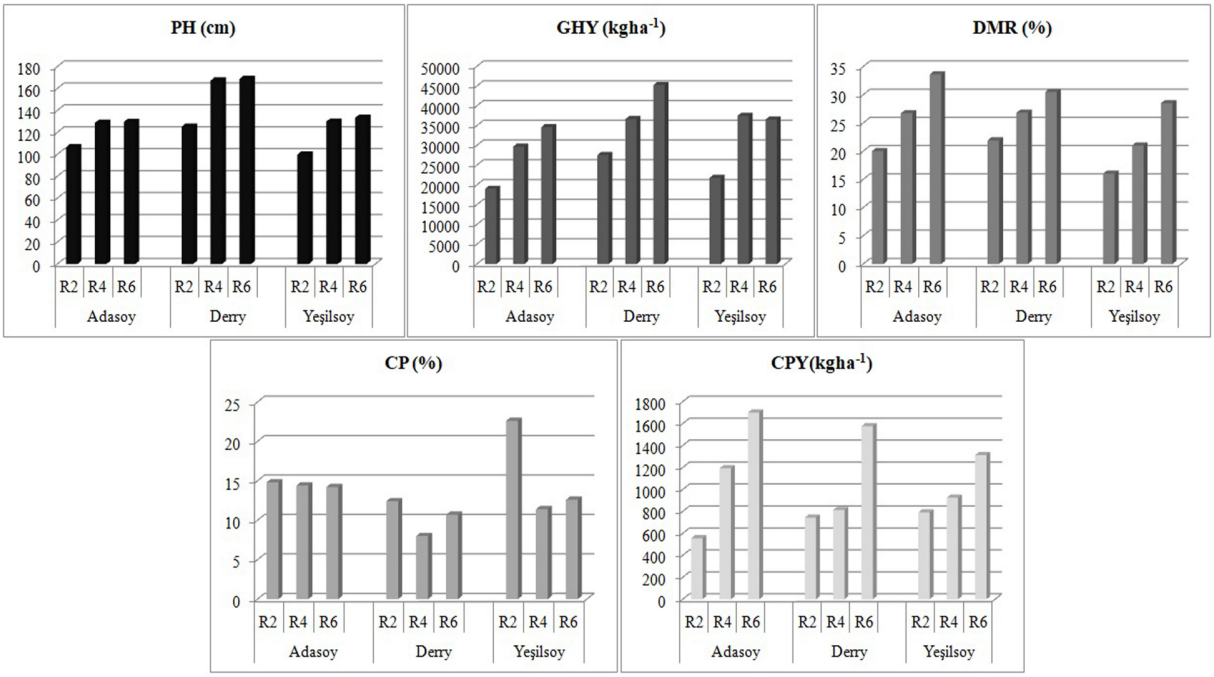

Figure 1. Significant interactions of 2014.

The all harvest stages had a significant impact on hay yield, CP and CPY during both seasons. In the both years, the effect of varieties on hay, CP, and CPY was significant, except for the CPY in the first year. Also, the interaction was effective on the criteria examined except for hay yield in the first year (Table 2; Figure 1,2). In terms of variety average, the highest hay yield was obtained from the Derry variety with 102 35 and 154 36 kg ha^{-1}, respectively during both years, while the lowest value was taken from Yeşilsoy (738 8 and 103 08 kg ha^{-1}, respectively). Sheafer et al. (2001) reported that there were significant differences between varieties of feed and grain types in different maturity stages. These results are in agreement with the results of our study. Derry and Yeşilsoy are forage varieties while Adasoy is a grain type and they have different maturity stages. Derry variety recorded higher yields in all harvest stages than the other two varieties. Derry variety recorded higher yields in all harvest stages than the other two varieties. For CPY, there was no significant difference between the varieties in 2014. In 2015, the highest CPY was obtained from Derry and Yeşilsoy varieties (183 7 and 164 2 kg ha^{-1}, respectively). Adasoy had the lowest yield (132 0 kg ha^{-1}) (Table 2). Different harvesting stages caused significant changes in hay yield. According to the harvest stage averages, the highest hay yield was obtained at the R6 stage and the lowest was obtained at the R2 harvest stage. The highest CP was detected at The R2 stage with 16.6% in the first year whereas in the second year, it was detected at R2 and R6 stages (13.7% and 14.7% respectively). Hintz et al. (1992) pointed out that the crude protein content of soybeans decreased during the transition from the R1 to R3 harvesting stage, while remained constant at R3 and R5, and then increased during the R5 and R7 harvest stages. The ratio of crude protein increases with the progress of the maturity of the soybean (Ocumpaugh et al. 1981.). The nutrient content and forage quality of whole-plant soybeans do not change drastically with advancing maturity because the seed is much higher in protein (Munoz et al. 1983). The highest CPY in both two years was obtained from the R6 harvest stage as 153 1 kg ha^{-1} and 222 0 kg ha^{-1}, while the lowest yield was taken from the R2 harvest stage as 697 kg ha^{-1} and 999 kg ha^{-1}, respectively. The best protein yield was obtained from the R6 harvest period of the Deryy variety in the interaction of the cultivar harvest period (Figure 1, 2). This is related to the high protein content of the R6 harvest period.

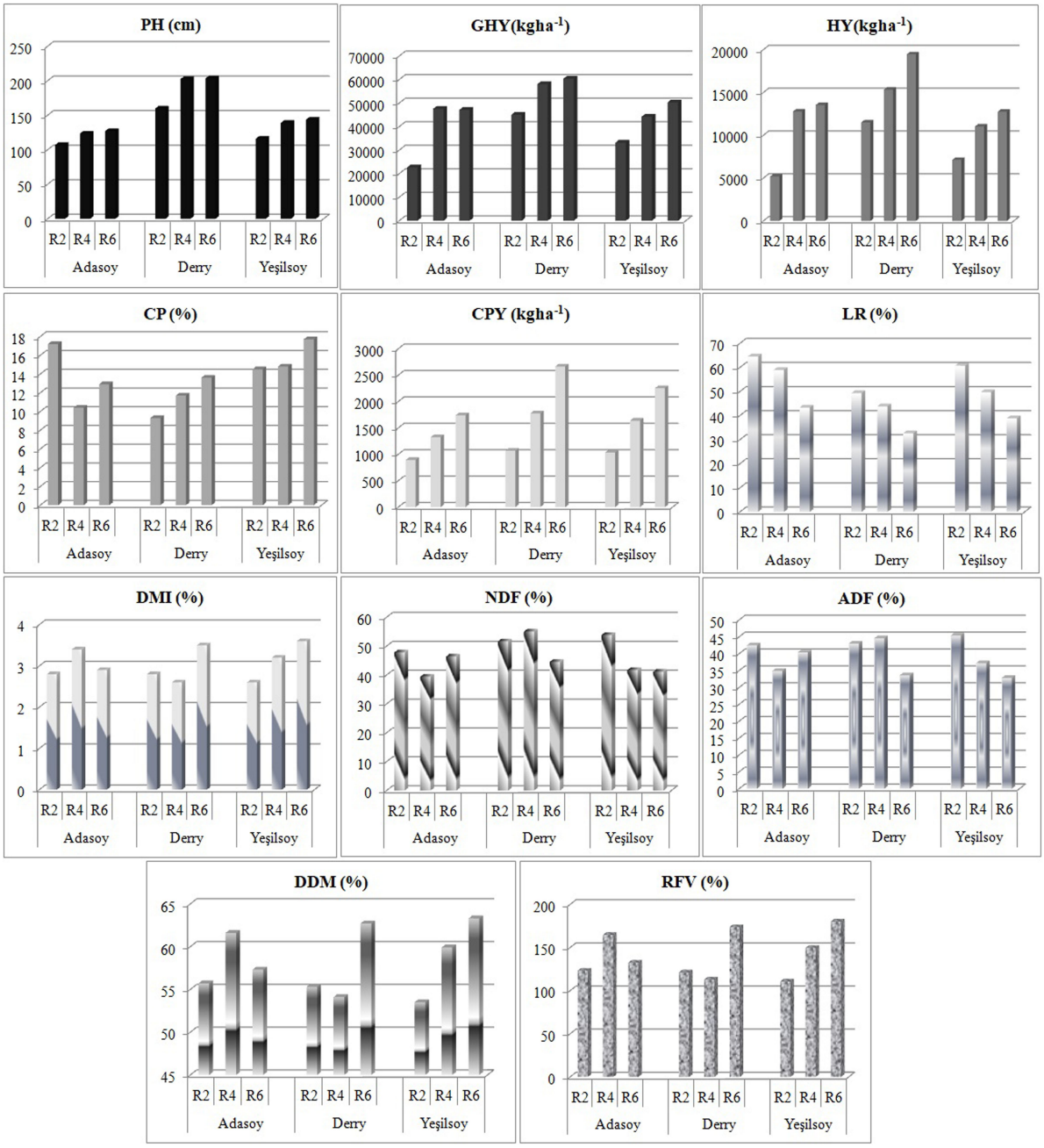

Figure 2. Significant interactions of 2015.

The effect of varieties and harvest stage on the leaf and stem ratio was significant in both the 2014 and 2015 years. Variety x harvest stage interaction was not significant (Table 2). On the other hand, the averages of the varieties of the first year reveal that the highest value of this character was obtained by the Adasoy and Yeşilsoy varieties while the Derry variety was the lowest average. Adasoy variety recorded the highest leaf rate in the second year while Derry variety recorded the lowest rate. In terms of harvest stage averages, in the 2014 year, the highest leaf ratio was 52.2% at the R2 stage and the lowest was 46.6% and 48.5% at the R6 and R4 stages, respectively. According to the harvest stage averages for 2015, the highest leaf rate was obtained from the R2 harvest stage (58.1%), and the lowest leaf ratio was obtained from the R6 harvest stage (38.2%). It was observed that the Derry variety had the highest stem ratio in the first season (51.2%). The lowest stem ratio was obtained from Adasoy and Yeşilsoy varieties with 46.5% and 47.6%. In the second year, the highest stem ratio was obtained from the Derry variety with 57.0%, and the lowest stem ratio was taken from Adasoy (43.5%). The results in Table 2 for the average harvest period of 2014 show that the highest stem rate was determined as 53.5% in the R6 stage and the lowest stem rate was obtained from the R2 and R4 stages as 47.3% and 49.9%.

In 2015, the highest stem ratio was obtained as 60.4% from the R6 stage. The lowest stem ratio was determined at the R2 stage. It is known that the higher leaf rate is better for the quality and taste of the forage. Almost all animals prefer plants. When green herbage or hay is given in a lot amount, it is observed that animals consume the leaves at first, because the leaves are tastier than the stems. Therefore, the quality of forage decreases with decreasing the leaf/stem ratio while in parallel, the rate of crude cellulose increases with increasing the stem ratio. The harvest period is one of the important features affecting quality. In many forage crops, if the harvest delays, dry matter yield, and stem ratio increase but the leaf rate decreases (Acikgoz, 2001). Hintz et al. (1992) reported that, in all soybean types studied, the stems had lower feed quality, because the stem includes lesser crude protein than leaves and pods. Therefore, they emphasized that a high leaf rate is an important feed quality criteria.

In the first year dry matter intake was affected from varieties and harvest stages, while in the second year, it was affected from harvest stage and variety x harvest stage interaction. The highest DMI (2.6 %) was obtained from Yeşilsoy in the first year, whereas DMI rates ranked between 3.0-3.1 % in the second year. According to harvest averages, the highest DMI rate in 2014 was determined at the R2 and R6 stages (2.4%, and 2.3% respectively), while the lowest DMI was obtained from the R4 stage (2.1%). The highest DMI ratio was 3.4% in 2015 at the R6 stage and the lowest was 2.7% at the R2 stage (Table 3). NDF rate was affected from variety and harvest time in 2014, whereas it was affected from variety, harvest time, and variety x harvest stage interaction in 2015. When ADF, DDM, and RFV were examined, it was seen that variety and harvest times affected these three characteristics in the first year, while they were affected by harvest time and cultivar x harvest time interaction in the second year (Table 3). Concerning the variety averages, the highest NDF content in 2014 was detected in the Derry and Adasoy varieties with 56.6% and 53.4%, respectively and the Yeşilsoy variety had the lowest NDF content (46.7%). In the 2015 season, the Derry variety recorded the highest NDF content with 50.4%, while the Adasoy and Yeşilsoy varieties resulted in the lowest NDF contents with 44.5% and 45.5%, respectively. In 2014, ADF rates were 33.6% and 47.6%, in 2015 they varied between 32.8% and 45.4%. In terms of variety averages, the highest ADF ratio in 2014 was obtained from Derry and Adasoy varieties with 43.3% and 41.6%, respectively, and the lowest ADF ratio was obtained from Yeşilsoy with 37.7%. The highest ratio of DDM was found in the Yeşilsoy and Adasoy varieties in the first year (59.5%, and 56.4% respectively) while the lowest ratio was found in the Derry variety with 55.3%. In the second year, the varieties had no effect on DDM. Data presented in Table 3 indicated that for variety averages, the highest RFV value was obtained from the Yeşilsoy variety with 122.6% in 2014. The lowest ones were obtained from Derry and Adasoy varieties as 92.3% and 98.9%, respectively.

The means of harvest stages indicated that R4 and R6 produced the highest NDF values in 2014 (% 55.7, % 51.6 respectively) while the lowest one was taken from R2 (% 49.4). In 2015 the highest NDF content with 51.1% was obtained from the R2 stage while the lowest NDF content was recorded from R6 and R4 with 44.0%, the 45.3%, respectively. In 2014, the highest ADF ratios were determined in the R4 and R6 stages with 43.9% and 40.2%, respectively while the lowest ADF content was determined in the R2 stage as 38.1%. In the second year, the highest ADF rates were achieved at R2 and R6 stages as 43.6, while the lowest ADF content was obtained at R4 with 38.9%. According to the averages of harvest stages for the first year, the highest DDM rate was found in stages R2 and R6 with 59.1% and 57.5%, respectively. The lowest DDM rate was in stage R4 with 54.6 %. In the second season, the highest DDM rate with 61.6% was obtained from the R6 harvest stage while the least value of the DDM rate was 54.8% at the R2 harvest stage. In the first year, the highest RFV value was obtained from the R2 and R6 stages with 113.0% and 108.6%, respectively, while the highest RFV value in the second year was taken in R6 with 162.1% (Table 3).

The forage having low digestibility takes a long time for it to pass through the rumen and it passes slowly through the digestive system, which limits the intake of dry matter. Under these circumstances, the amount of dry matter intake is affected adversely because the rumen, reticulum, and abomasum expand while the feed stimulates receptors on the outer walls of these organs. It should be taken into consideration that when used the low digestibility forage, the most important factor of the digestive system is the neutral detergent fiber ratio (NDF) (Mertens, 1994; Allen, 1996). Generally, dry matter intake is reduced when the percentage of NDF increases (Joachim and Jung, 1997). NDF in general, is closely associated with the feed consumption of an animal while ADF is closely related to the degree of digestion, because the digestion of NDF and ADF by micro-organisms are difficult. NDF contains cellulose, hemicellulose, and lignin. Also, ADF contains cellulose and lignin. When the harvest

is delayed, the amount of lignin in the feed will increase and then the lignin will form a bridge between cellulose and hemicellulose, that will reduce the digestion of feed. In the same connection, Acikgoz et al. (2013) reported that the effect of varieties on ADF and NDF were insignificant whereas the harvest stages had a significantly impact on ADF and NDF. Researchers obtained that ADF rates were 27% and 32.7% for R1 and R5 respectively, and NDF rates were 34.2% and 39.6% for R1 and R5 respectively. In our study, in the both two years, NDF was affected from varieties whereas ADF was only affected from variety in the second year. In the first year, the lowest NDF and ADF were obtained from R2 whereas the lowest NDF in the second year was recorded at R4 and R6 stages, and the lowest ADF at R4 harvest stage. Depending on the feed quality, the feeding behavior of animals, feed consumption, feed digestibility, and its conversion into animal products varies (Van Soest, 1994). Digestible dry matter is calculated from the ADF value. A high level of ADF will cause to decrease digestible of dry matter. In the first season of our study, in terms of DDM value Yeşilsoy had higher values compared to other varieties. The DMI value depends on the NDF value and if the NDF is high, DMI decreases. The quality of forage is usually determined by measuring the chemical, physical, and biological values of forage. Relative feed value (RFV), which was developed for alfalfa plants in the USA and then used in other forage crops, is used to measure the nutritive value of forage (Ball et al., 1996). DMI and DDM values are used to calculate the relative feed value, so ADF and NDF are important in relative feed values (Moore and Undersander, 2002). Alfalfa in the full flowering stage having 41% ADF and 53% NDF is considered to have a feed value of 100%. The feed quality decreases as the relative feed value falls below 100 and increases when it rises (Redfearn et al., 2006). If RFV is less than 75, it is named 5th quality, 4th quality between 75-86, 3rd quality between 87-102, 2nd quality between 103-124, and first quality between 125-150. If the RFV is greater than 150, it is called the highest-quality feed (Rohweder et al., 1978). In our study, Yeşilsoy produced better results than the others in terms of RFV. The effect of harvest stages on RFV varied between years.

Table 3. Means and the standard errors of investigated parameters and probabilities of factors

	Year (Y)	DMI (%)	NDF (%)	ADF(%)	DDM(%)	RFV
Years	2014	2.3 ±0.3^{b}	52.3 ±5.9^{a}	40.8±6.2	57.1±4.8	104.6 ±21.7^{b}
	2015	3.1 ±0.4^{a}	46.9 ±5.6^{a}	39.4±4.9	58.1±3.8	141.0 ±29.6^{a}
2014	**Harvest Stage (HS)**					
	R2	2.4 ±0.3^{a}	49.4 ±5.4^{b}	38.1 ±4.9^{b}	59.1 ±3.8^{a}	113.0 ±17.9^{a}
	R4	2.1 ±0.2^{b}	55.7 ±5.2^{a}	43.9 ±5.6^{a}	54.6 ±4.4^{b}	92.2 ±16.0^{b}
	R6	2.3 ±0.4^{a}	51.6 ±9.1^{a}	40.2 ±7.4^{ab}	57.5 ±5.8^{ab}	108.6 ±32.8^{a}
	Varieties (V)					
	Adasoy	2.2±0.2^{b}	53.4 ±3.8^{a}	41.6 ±4.5^{ab}	56.4 ±3.6^{ab}	98.9 ±13.2^{b}
	Derry	2.1 ±0.2^{b}	56.6 ±5.7^{a}	43.3 ±6.1^{a}	55.3 ±4.8^{b}	92.3 ±17.0^{b}
	Yeşilsoy	2.6 ±0.4^{a}	46.7 ±7.6^{b}	37.7 ±7.4^{b}	59.5 ±5.7^{a}	122.6 ±29.9^{a}
	P Value					
	R	0.753	0.717	0.050*	0.049*	0.288
	HS	0.035*	0.009**	0.003**	0.032*	0.024*
	V	0.000**	0.000**	0.04**	0.043*	0.001**
	RxV	0.185	0.172	0.616	0.626	0.322
	VxHS	0.152	0.153	0.071	0.072	0.106
2015	**Harvest Stage (HS)**					
	R2	2.7 ±0.3^{c}	51.1 ±3.7^{a}	43.6 ±4.1^{a}	54.8 ±3.2^{c}	118.4 ±18.8^{c}
	R4	3.1 ±0.4^{b}	45.3 ±7.5^{b}	38.9 ±4.5^{b}	58.5 ±3.5^{b}	142.5 ±54.3^{b}
	R6	3.4 ±0.4^{a}	44.0 ±4.3^{b}	35.7 ±4.2^{a}	61.1 ±3.3^{a}	162.1 ±19.3^{a}
	Varieties (V)					
	Adasoy	3.0 ±0.3	44.5 ±4.5^{b}	39.3 ±4.3	58.2 ±3.4	140.1 ±23.8
	Derry	3.0 ±0.4	50.4 ±5.4^{a}	40.4 ±5.7	57.3 ±4.5	136.0 ±31.3
	Yeşilsoy	3.1 ±0.5	45.5 ±6.9^{b}	38.5 ±6.1	58.9 ±4.7	146.9 ±32.7
	P Value					
	R	0.356	0.242	0.106	0.226	0.302
	HS	0.000**	0.000**	0.000**	0.000**	0.000**
	V	0.278	0.001**	0.153	0.272	0.236
	RxV	0.176	0.877	0.053	0.326	0.387
	VxHS	0.001**	0.000**	0.000**	0.000**	0.236

*p<0.05, **p<0.01; full blooming stage (R2), full pod stage (R4), full seed stage (R6). Dry matter intake (DMI), neutral detergent fiber (NDF), acid detergent fiber (ADF), digestible dry matter (DDM) and relative feed value (RFV); Different superscript letters denote significant differences ($p < 0.05$).

4. Conclusion

According to the results of this study, the highest plant height and green herbage yield were obtained from the Derry variety, and the lowest was obtained from the Adasoy variety. These results show that forage type soybean varieties have higher yields than grain types which was supported in other studies. In the harvesting stage, the R4 and R6 stages had the highest plant height and green herbage yield values. In the study, the best results were obtained from the Yeşilsoy variety according to the values used in determining feed quality such as DMI, NDF, ADF, and DDM. Although some differences are incurred between the seasons in terms of harvest stages, especially on the basis of the RFV value, the R6 harvesting stage can be considered as the period that produces better quality feed. According to these results, the Derry variety gave the best results in terms of soybean yield as a second crop and the Yeşilsoy variety was prominent in terms of quality as a second crop under Mediterranean conditions where the study was carried out. Generally, from the experimental results, it can be suggested that harvesting soybean at the R6 harvest stage is appropriate. As a result, it can be said that the use of soybeans as feed should be generalized, and adding to the feed rations will contribute positively.

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Research Article

Evaluation of Grain Maize Harvest Residues as Fodder After Partial Torrefaction Under Microwave

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Torrefaction

Abstract: With a project supported by Siirt University (Siirt, Türkiye), we determined the conversion potential of residues of field cropped grain maize into animal feed via torrefaction. Torrefaction application was performed in a microwave device for four different periods of time (0, 4, 8, and 12 minutes) in three replications at three different watts (300, 600, and 900 watts). The dry matter digestion was determined for the ground starter and final materials by the Daisy Incubator Technique (ANKOM). Changes in dry matter, protein, and ADF values were measured by standard feed quality analyses. The effects of applications on dry matter ratio, protein, and in vitro digestibility were found to be statistically significant, whereas the effect on ADF ratio was insignificant. Low-term torrefaction applications significantly increased the dry matter ratio, whereas high power and long application periods significantly increased the protein ratio. In vitro, digestibility was decreased due to the increase in the power application and duration of torrefaction. A material with high dry matter and high protein content but low in vitro digestibility was obtained as a result of the torrefaction applications tested in the study under microwave conditions with grain maize harvest residues. Although the applications tested have decreased the value of maize harvest residues as cattle feed, the obtained material with high dry matter and protein content with this torrefaction method has the potential to be used as a nutrient medium in different living environments (e.g. bacterial or fungal) which may be subjected to further investigations.

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1. Introduction

The amount of grain maize (*Zea mays* L.) harvested in Türkiye in 2021 was 6.5 million tons. For the world, this value was 1.15 billion tons for the same year (FAO STAT, 2021). The harvest index (HI) is the ratio of a harvested crop to the total dry plant weight. The HI for maize is between 0.25–0.58 (Yang and Zhang, 2010). When the harvest index is roughly 0.4, it means that six tons of maize harvest residue are produced for four tons of harvested maize grains. When this calculation was applied to given 2019 values, produced maize residues were equal to 6/4*6=9 million tons for Türkiye and 1.73 billion tons for the world.

Cellulose in maize plants, as the most important component of the plant skeleton, is a polysaccharide formed by connecting D-glucose building blocks in long chains (Klemm et al., 2005).

Cellulose is a renewable polymer source that has been used in a wide range of products and materials for about 150 years (Habibi et al., 2010). Biomass consisting of cellulose, hemicellulose, and lignocellulose contains a large amount of fermentable sugar (Anonymous, 2022). Hemicellulose is a polymer containing mainly the five-carbon sugar C5H10O5 (xylose) (NIST, 2011a). Cellulose is a polymer containing the six-carbon sugar C6H12O6 (glucose) (NIST, 2011b). Due to these properties, it is possible to make a significant contribution to food production by making waste biomass digestible without extra farm field occupation for crop cultivation. Many factors such as lignin content, the crystalline structure of cellulose, and particle size limit the digestibility of hemicellulose and cellulose in cellulosic biomass (Hendriks and Zeeman, 2009).

The use of ligninolytic fungi and their enzymes is a potential alternative to provide a practical and environmentally friendly approach to increasing the nutritional value of straw residues. Today, however, the cost of exogenous enzymes is too high to be preferred by small farms. In addition, the relative scarcity of available data on applications using fungi and their enzymes to improve the digestibility of stem residues limits its applicability (Sarnklong et al., 2010).

As an alternative to enzyme application, torrefaction provides thermal conversion of biomass between the temperature range of 200-300°C (Stelt et al., 2011). Hemicellulose, cellulose, and lignin, which are the main components of biomass, are substrates in the torrefaction treatment. Hemicellulose, cellulose, lignin, xylan, and dextran are components that affect biomass torrefaction (Chen and Kuo, 2011). This treatment improves the properties of biomass through thermochemical processing techniques (Bridgeman et al., 2008). Torrefaction, which is among the potential applications for the production of processed pellets for fuel extraction, has attracted great interest from academics and industry in recent years for the production of high-quality syngas and the replacement of coal in thermal power plants and industrial metallurgical plants (Chen et al., 2015). In the application of torrefaction (Deng et al., 2009), which is a basic pretreatment technology that also reduces the problems of high bulk volume, high moisture content, and poor grindability of agricultural biomass, thermal treatment in the relatively low temperature range decomposes the sensitive hemicellulose part of the material (Prins et al., 2006). During torification, 70% of the initial mass is retained (this amount contains 90% of its initial energy content as a solid product), and the 30% lost mass turns into condensable and non-condensable products. Roasting biomass improves physical properties such as grindability while changing the shape, size, and distribution of particles, pelletability of the material, and intermediate and final content (Tumuluru et al., 2011).

In a different study, it was determined that mild torrection (240°C) significantly destroyed hemicellulose in biomass, but cellulose and lignin were slightly affected, while severe torrection (275°C) significantly affected cellulose (Chen and Kuo, 2011). Deng et al., (2009) reported that temperatures of 200°C, 250°C, and 300°C affect the biomass and the type of raw material which affects the conversion rate due to the difference in volatile content in the raw biomass. Yang et al., (2007) found that at torrefaction, the hemicellulose ratio decreased at 220–315°C, and the cellulose ratio decreased at 315–400°C. They also reported that hemicellulose produced a higher CO_2, cellulose produced a higher CO, and lignin produced higher H_2 and CH_4.

In this research, the potential of converting crop harvest residues from grain-grown maize into highly digestible feed by microwave torrefaction was investigated.

2. Material and Methods

In the experiment, the harvest residues of the maize plants from a farmer's field of main season grain maize crop in the province of Siirt in Türkiye in 2021 year were used as material. Harvest residue materials were collected from farmer fields following harvest, filled in straw sacks, and kept in the warehouse under appropriate storage conditions. Laboratory measurements of the experiment were carried out in the 2022 year. In the study, the torrefaction application was applied in a microwave, during four different periods of time (0 (control group), 4, 8, and 12 minutes), at three different watts (300, 600, and 900 Watt), with three replications. Torrefaction was applied to the maize residues in heat resistant glass containers (Figure 1).

a b c

Figure 1. The microwave device used in torrefaction; b) two pyrex containers of different sizes, resistant to very high temperatures and with a lid, used as a torification vessel; c) the intertwined state of two pyrex containers used as a torrection container. The interspace between the outer and inner container is the breathing volume for the inner container, where a piece of cotton is burned in this intermediate volume at each sample preparation stage before torrection, aiming to let CO_2 and CO but not O_2 exchange during torrefaction.

The microwave device used in the trials was a Beko brand, MD-1505 Model, 230 V, Class I device working at 50 Hz, 1, 200 W, and 2.450 MHz operating frequency. The working range was in the range of 90-900 watts, working time was in the range of 0-60 minutes.

The amount of maize residue to fill the inner chamber of the torrection container was chopped to a size of 2-3 cm with the help of scissors and pliers. The amounts of dry leaves, stems, hollow cobs, and tassels were balanced to be similar for different applications (Figure 2). Two different pyrex cooking pots, which are resistant to high temperatures and can fit inside each other, were used for torrefaction. To consume the oxygen of the inter chamber space between these two vessels to build a partial air-breathing reaction vessel set-, burning cotton was left in between two glass pots, and the outer vessel lid was closed immediately before being placed into the microwave just before the beginning of the torification of each sample. Burning cotton was left in the mid-compartment to ensure oxygen consumption in the "intermediate layer", which was the breathing section of the inner container. After the torrection process, the materials were ground with a laboratory-type spiral blade shredder for 0.5 minutes (Figure 4). In order not to mix the material, the empty turning time, the grinding time of the maize residues, and the cleaning time of the grinder for the new sample were each 0.5-minute intervals. Some samples were reserved for DM, protein, and ADF analyses, the remainder was allocated for in vitro digestibility analyses.

The actual dry matter digestion level was determined by the Daisy Incubator Technique (ANKOM) after the materials were ground for a short period. Changes in dry matter, protein, and ADF values were measured by standard feed analyses. Feed quality analyses such as dry matter (DM), ADF, and protein content (CP) were performed in the "Eastern Mediterranean Agricultural Research Institute Quality Laboratory" (Adana, Türkiye).

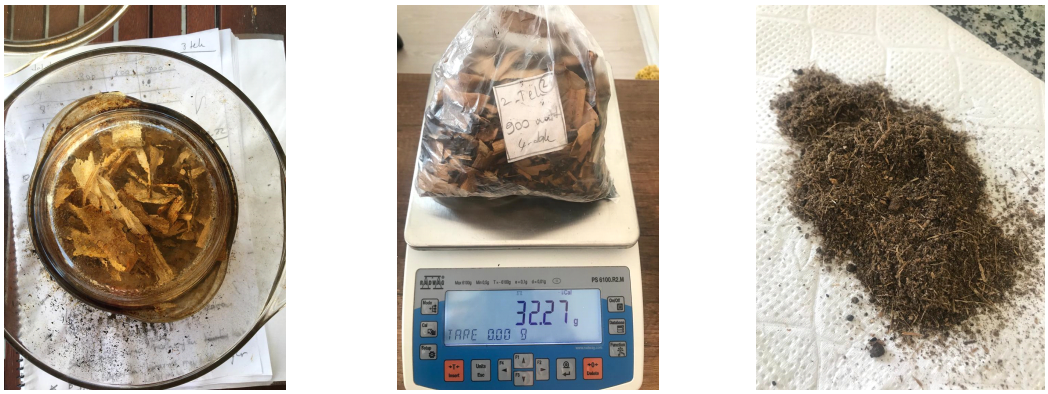
a b c d

Figure 2. Stem part, b) empty cob, c) leaf part, and d) husk (cob cover) of maize harvest residues.

a **b** **c**

Figure 3. Close-up view of the cellulosic stem tissue of the stalk section of maize harvest residues, b) the mixture of harvest residues after the shredding process, and c) sample sample prepared and labeled for applications.

The method of AOAC, (1990) was used for dry matter and nitrogen determination. Goering and Van Soest, (1970) method was used for acid-detergent fiber (ADF) determination. In vitro digestibility analyses were carried out in the laboratory of Uludag University Faculty of Agriculture, Department of Animal Science, Feeds and Animal Nutrition (Bursa, Türkiye). For the in vitro study, rumen content from three healthy rams or cattle that completed their rumen development was obtained to use in Ankom DaisyII Incubator D220. The rumen fluids used in the study were taken from a slaughterhouse, immediately after the animals were slaughtered, accompanied by a carbon dioxide tube. After the sample was taken, it was filtered with the help of two-layer sterile cheesecloth, and two handfuls of rumen solids were added to it and transported to the laboratory without losing time in thermoses at 39°C. In the experiment, nutrient analyses of feeds and residues were determined according to the methods specified in AOAC (1998), and their true dry matter digestibility (TDMd) was applied in the Ankom Daisy Incubator (Ankom, 2002) using the strainer bag technique (Van Soest et al., 1991).



a **b** **c**

Figure 4. A torrefacted sample in a high temperature resistant pyrex glass container set; b) weighing the samples after application; c) one of the ground samples.

The jmppro-13 package program was used for statistical analysis according to the randomized parcels experimental design. According to the results of the F test, the differences between the groups were determined by the LSD (Least Significant Difference) multiple comparison test. In addition, to determine the relationship between applications and features, principal component analysis was performed according to the Scatter plot model in the statistical package program "Genstat 12th" (Copyright 2011, VSN International Ltd).

3. Results and Discussion

The averages of the investigated properties of the material obtained as a result of torrection in the study and the resulting groups are given in Table 1 below.

Table 1. The averages of the examined characteristics and the resulting groups

Applications	DM (%)	Protein (%)	ADF (%)	TDMd*
300 Watt 0 min	88.94 d	4.64 de	44.19	56.85 a
300 Watt 4 min	90.51 c	4.10 de	47.23	52.90 b
300 Watt 8 min	91.30 ab	2.78 e	43.64	50.51 c
300 Watt 12 min	90.90 bc	5.83 de	45.67	47.65 d
600 Watt 0 min	89.61 d	3.42 e	50.40	56.90 a
600 Watt 4 min	91.06 abc	4.38 de	47.67	47.75 d
600 Watt 8 min	90.38 c	11.66 c	43.97	43.90 f
600 Watt 12 min	91.66 a	12.05 bc	46.18	41.32 g
900 Watt 0 min	89.30 d	5.28 de	46.88	56.36 a
900 Watt 4 min	91.28 ab	9.61 cd	45.30	45.62 e
900 Watt 8 min	90.68 bc	17.61 ab	46.08	37.51 h
900 Watt 12 min	90.66 bc	19.54 a	47.31	29.15 ı
Avr.	90.52	8.40	46.21	47.20
LSD	0.35**	2.79**	2.10 öd	0.52**

Note: **: P≤0.01, DM: dry matter, TDMd: True dry matter digestion, ADF: acid detergent fiber. The difference between the means denoted by the same letters is not statistically significant.

Torrection applications were significantly effective on dry matter ratios (Table 2). The lowest DM values (between 88.94-89.61%) were in the control group, whereas the highest DM values (between 91.06-91.66%) were obtained from “300 Watt 8 min”, “600 Watt 4 min”, “600 Watt 12 min” and “900 Watt 4 min” applications. While an application with 300 and 900 watts each produced the highest DM value, two applications at 600 watts of power resulted in the highest DM values.

Torrefaction applications were significantly effective on the amount of protein. The lowest protein values (between 2.78-5.28%) were obtained from all periods at 300 Watt power (Table 2). Higher protein values were recorded at 900 Watt 8 min and 12 min applications, at a rate of 17.61% and 19.54%, respectively. The combination of high power and duration seriously degraded the chemical structure and resulted in significant material structure change through gaseous mass loss. Less period torrefaction applications significantly increased the "dry matter ratio", while high-power and long-term torrefaction increased the "protein ratio", which was associated with the conversion of polysaccharides to volatile form and the remaining protein skeleton. ADF values varied between 43.64-50.40%.

Torrections were significantly effective on in vitro true digestibility (TDMd). The lowest TDMd value (29.15%) was obtained with the most aggressive application (900 watts 12 min). The control group, in which no torrefaction was applied, produced the highest values (56.36-56.90%) in terms of TDMd. The tested torrefaction applications reduced the cattle feed value of maize harvest residues. The feed value decreased almost linearly with increasing duration of torrefaction. In this sense, the greatest amount of decrease was obtained by 900-watt applications.

3.1. Evaluation of the relationship between applications and features according to the scatter plot model

Figures 5, 6, and 7 show the effects of the applications on the examined features by vectors (a), sectors, and mega-periphery (b, c) via the scatter plot model.

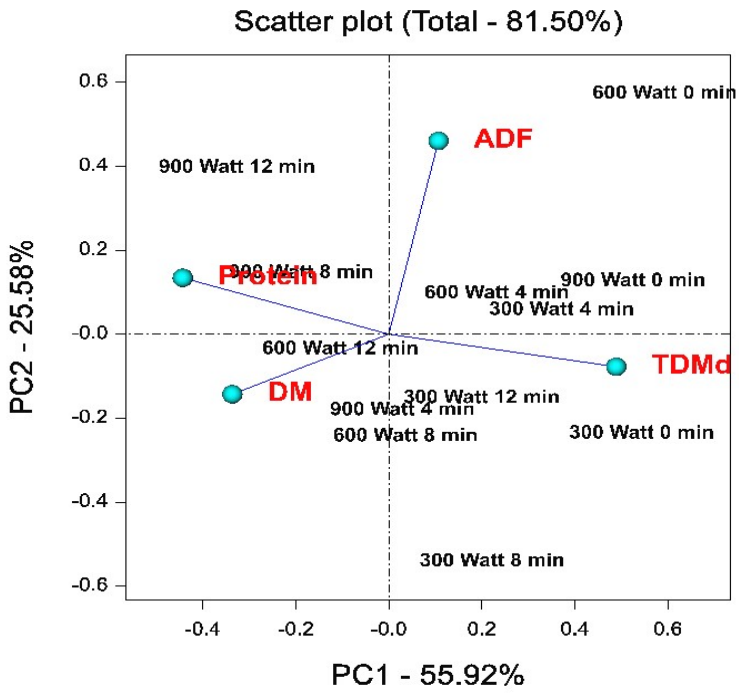


Figure 5. Representation of the effects of the applications on the examined properties with vectors (a) with the scatter plot model.

When evaluating Figure 5, it is visible that the application and features include variation at PC1:55.92, PC2:25.58, PC1+PC2: 81.50. In the scatter plot model, the narrowing angle between the vectors represents the features indicating a high positive correlation, an increase in the angle value indicates decreasing correlation, and the angle value of 90º indicates that there is no correlation (Mohammadi & Amri, 2011).

When Figure 6 is examined, it can be seen that the properties are concentrated in 3 different environments (E1; %TDMd and ADF, E2; DM, E3; Protein). While the "900 Watt 0 minute" application in the first group was the ideal environment for ADF, and %TDMd properties, the "600 Watt 8 minutes" application in the second group was ideal for DM. And the "900 Watt 8 minutes" application in the third group was the most ideal environment for the protein ratio. In addition, it was observed that "300 Watt 8 min, 900 Watt 12 min and 600 Watt 0 min" applications do not stand out in terms of any of the properties examined.

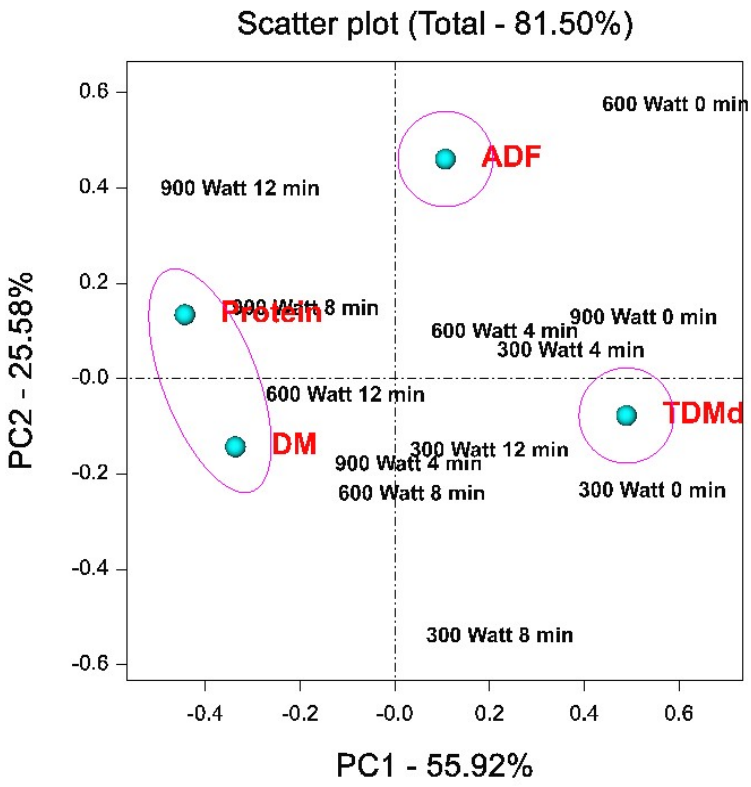


Figure 6. Representation of the effects of the applications on the analyzed features and the resulting averages by sectors (b) via the scatter plot model.

When we look at the relationship between applications and features through polygons and sectors (Figure 7), six different sectors were formed. The distribution of applications and features in different sectors shows that no application stands out in terms of the feature in question, or that no feature stands out in terms of applications. The fact that the application and the feature are in the same sector reveals that there was a positive relationship between the application and the features (Chinipardaz et al., 2016). The applications located on the diagonals of the polygon have the best performance in terms of the features found in the relevant sector (Yan & Tinker, 2006). In Figure 7, it can be seen that 300 Watt 8 min, 900 Watt 12 min, and 600 Watt 0 min applications show the best performance in these features.

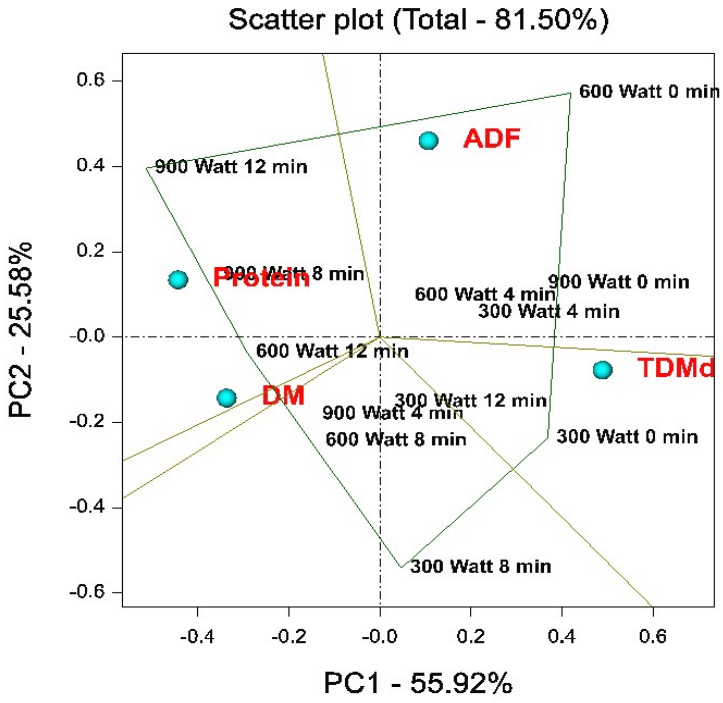


Figure 7. Representation of the effects of the applications on the examined features within the mega environment (c) via the scatter plot model.

3.2. Evaluation of the relationship between the scatter plot matrix and the examined features

The matrix showing the effect of the applications on the examined features through the Scatter Plot Matrix is given in Figure 8 below.

When the scatter plot matrix is examined, it was determined that the relationship between DM and %TDMd was 5% negative and significant (r2= -0.4159). If there exists an uneven distribution on the regression line in the graph that expresses the relationship between the two features examined, it can be concluded that the relationship between these features is weak (Eren & Demirel, 2020). However, if the distribution is regular and agglomerated on the regression curve, it can be said that these features are strongly related to each other. In our study, it was determined that the correlation coefficient between %TDMd and protein was 5% negative and significant (r2= -0.8051).

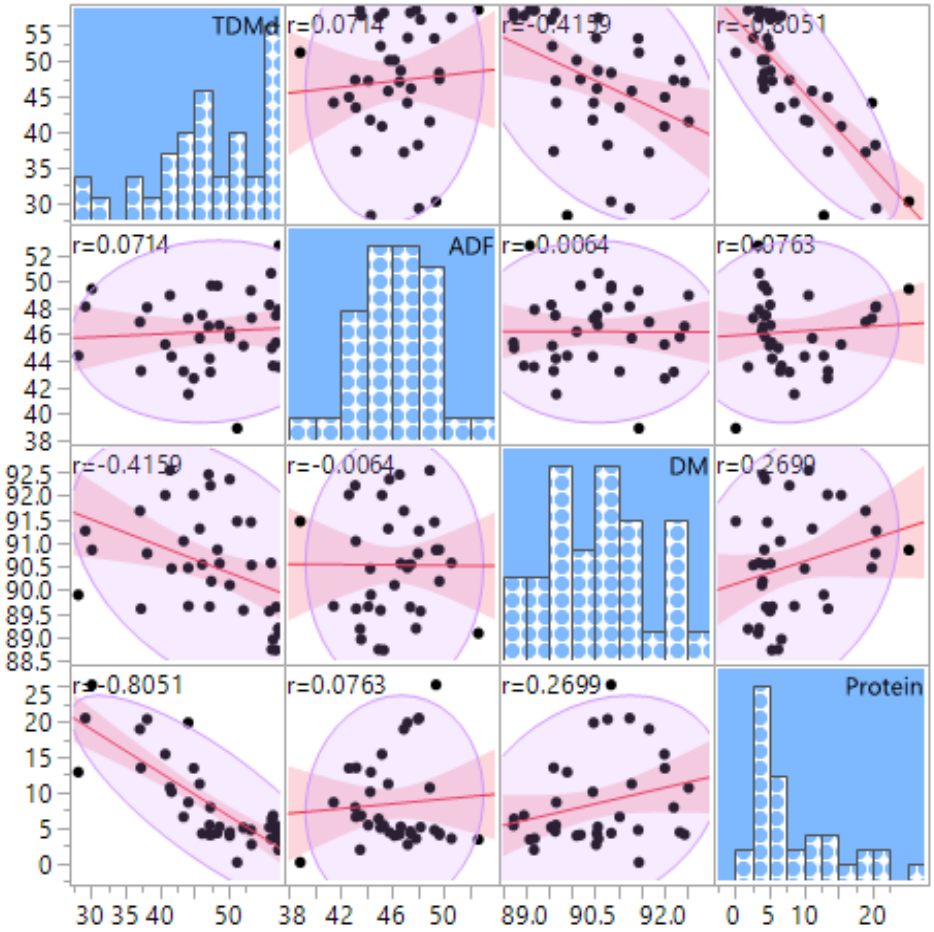


Figure 8. Representation of the effects of the applications on the examined features via scatter plot matrix.

Conclusion

In the study, it was determined that the torrefaction applications were effective on dry matter ratio, protein, and in vitro digestibility, but were not effective on the ADF ratio. Low-term torrefaction applications significantly increased the dry matter ratio, while high-power and long-term torrefaction increased the protein ratio. In vitro, digestibility decreased as the severity and duration of torrefaction increased. As a result, a material with high dry matter and high protein content but low in vitro digestibility was obtained. Although the tested applications reduced the value of grain maize harvest residues as cattle feed, the obtained material with high dry matter and protein content may be a suitable structure for future studies as a substrate for other biological or chemical cultivation aims. Since significant amounts of volatile and flammable gas were discharged during the applications, the potential to produce both energy raw materials and a nutrient medium may contribute to the expansion of the use of these materials.

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Research Article

Screening of Bean Genotypes Against Bean Common Mosaic Virus (BCMV) by Artificial Inoculation and Molecular Confirmation

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Abstract: Bean mosaic virus (BCMV) is a widespread plant pathogen that causes significant bean yield losses in several bean-growing regions worldwide. The use of resistant common bean varieties to BCMV is considered the most efficient and feasible approach to control its effects. Numerous genes and molecular markers associated with resistance to these pathogens have been discovered and used extensively in breeding studies around the world. Screening bean genotypes for resistance to these viruses is a critical step in developing resistant varieties. The goals of the study are to identify virus sources in the region and artificially inoculate Lake Van basin bean genotypes with BCMV. The recovered BCMV strain NL-4 was inoculated with 45 bean cultivars, most of which originated from the Lake Van basin in Turkey. Differentiation between resistant and susceptible was based on visual symptoms, and of the 45 genotypes, 29 were found to be resistant to NL-4, while 16 genotypes were susceptible (8 of them moderately susceptible and 8 of them highly susceptible).

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1. Introduction

The common bean (*Phaseolus vulgaris* L.) is a vital staple crop cultivated in both developed and developing countries. It holds great significance as the most prominent grain legume in the human diet worldwide, primarily intended for human consumption. In terms of calorie contribution, beans rank second after maize, making them a substantial source of nutrition (CGIAR, 2015). Common beans, which are widely distributed in Türkiye, have a broad range of variations, and Türkiye is an important bean-producer country (Erdinç et al., 2017a). Common bean is attacked by several abiotic and biotic stress factors (Erdinç et al., 2017b; Ekincialp and Şensoy, 2018; Bilge et al., 2019; Kıpçak et al., 2019; Tunçtürk et al., 2019). The application of molecular markers in plant breeding programs facilitates the improvement resistant many different viruses (Ibrahim and Erdinç, 2020). Bean common mosaic virus (BCMV), a

member of the genus Potyvirus in the family Potyviridae, is one of the main viruses that damage common beans (Tang and Feng, 2023). A bean seed-borne and aphid-transmitted virus, BCMV causes devastating losses in crop yields and quality. The spread of the virus around the world can occur in nonpersistent ways via the spread of seeds, pollen, and certain aphid species (Morales and Bos, 1988). Genetic resistance is the most successful and long-term management strategy for combating BCMV (Kelly et al., 1995). Bean is also produced in Lake Van Basin which compromises rich bean genotypes which might contain resistance to BCMV. Molecular and morphological (phenotypic evaluation) markers have been increasingly used in plant genetics and breeding research in recent years (Deligöz and Sökmen, 2013; Mangeni et al., 2014; Erdinç et al., 2017b; Ekincialp and Şensoy, 2018; Hatipoğlu and Şensoy, 2022; Uçar and Şensoy, 2022; Usta et al., 2023).

The geographical diversity of bean production, such as the Lake Van Basin in Turkey, presents a unique opportunity to discover natural resistance traits within bean genotypes. In light of these challenges and opportunities, the primary aim of our study is to comprehensively investigate the reactions of the Lake Van Basin's bean genotypes to a selection of BCMV strains. We endeavor to employ a multifaceted approach, combining molecular and classical methods, to assess the responses of these genotypes. Through rigorous artificial inoculation with BCMV, we intend to scrutinize their resistance and susceptibility patterns. By delving into the genetic and phenotypic makeup of these genotypes, we aim to uncover potential resistance mechanisms and markers that could be harnessed to bolster BCMV resistance in common bean cultivation. This study seeks to bridge the gap between theory and practice by elucidating the intricate interactions between BCMV strains and the diverse bean genotypes of the Lake Van Basin. Through an in-depth exploration of their responses, we aspire to contribute valuable insights and tangible solutions to the persistent challenge of BCMV in common bean cultivation, ultimately safeguarding this crucial global food resource.

2. Materials and Methods

A total of 45 bean genotypes were used in the present study (Table 1).

2.1. Culture of BCMV strains and mechanic inoculation of bean genotypes

The Ru-1 strain and NL-4 strain were obtained Directorate of Plant Protection Central Research Institute (Ankara) and artificially inoculated on the 20 plants of the susceptible Stringless Green Refuges (SGR) cultivar in March 2021. The climate chamber room was prepared at a temperature of 26°C with a 16/8 h day/night photoperiod with 60% moisture.

Forty-five bean genotypes were grown in a climate chamber. The seeds of the bean cultivars used in the study were divided into two groups the first group inoculation by NL-4 strain of BCMV included three replications in a randomly replicated experimental design each included 10 seeds per variety and the second group (control) included one replication that included two seeds per variety. Phosphate buffer containing 1% K_2HPO_4 and 0.1% Na_2SO_3 (pH: 7.2) was used (Sengooba et al., 1997). Ten days after planting, the first true leaves were transmitted by mechanical inoculation.

The cultivars were inoculated in 3 replications each replication consisted of 10 plants per genotype and each plant was inoculated with two primary leaves. Four control plants were used for each replication.

Inoculated leaves were washed under tap water and placed in a climate room set at 22 °C ±1 and 12 hours photoperiod against the possibility of the presence of strains that can cause temperature-induced necrosis in the samples.

2.2. Total RNA extraction and cDNA synthesis

Total RNA extractions were conducted using approximately 0.1 g of frozen leaf tissues following the protocol described by Foissac et al. (2001) with minor adjustments. Specific forward and reverse primer sets were adopted from Bhadramurthy and Bhat (2009) which are designed within the coat protein gene (CP), to detect BCMV in the common bean leaf tissues and synthesized to the relevant company (Oligomer/Turkey). In all cDNA processing, a random primer (9mer) was also utilized (Table 2).

Table 1. Passport data of bean genotypes used in the study

Genotype No	Name of Genotype	Provided Place/cultivar name	Growth habit
G.1	Stringless Green Refuges	USDA	Bush
G..2	U1-36 Red mex	USDA	Pole
G.3	Redland green leaf	USDA	Bush
G.4	U1-111	USDA	Pole
G.5	Pinto 114-8	USDA	Pole
G.6	Jubila	USDA	Bush
G.7	Monroe	USDA	Pole
G.8	TR 66342 (Afyon)	Cukurova Univ.	Pole
G.9	TR 68587 (Eskişehir)	Cukurova Univ.	Pole
G.10	Line 10 (USA)	Cukurova Univ.	Pole
G.11	Sarıkız Fasulyesi	Cukurova Univ.	Pole
G.12	Gülnar-II (Barbunya)	Cukurova Univ.	Pole
G.13	Gülnar-VI	Cukurova Univ.	Pole
G.14	France-Gandiyam (Sarı meyve)	Cukurova Univ.	Pole
G.15	F1 103 950	Anadolu Agr. Res.Inst.	Bush
G.16	Van-Merkez	University of Van YYU	Pole
G.17	Van-Merkez	University of Van YYU	Pole
G.18	Van-Merkez	University of Van YYU	Pole
G.19	Bitlis-Tatvan	University of Van YYU	Pole
G.20	Bitlis-Tatvan-Gevar	University of Van YYU	Pole
G.21	Bitlis-Hizan	University of Van YYU	Bush
G.22	Bitlis-Tatvan	University of Van YYU	Pole
G.23	Bitlis-Tatvan	University of Van YYU	Pole
G.24	Van-Erciş-Purmak	University of Van YYU	Pole
G.25	Van-Erciş-Çelebibağı	University of Van YYU	Pole
G.26	Van-Erciş	University of Van YYU	Pole
G.27	Van-Gevaş-G.konak	University of Van YYU	Pole
G.28	Van-Gevaş-G.konak	University of Van YYU	Bush
G.29	Van-Gevaş	University of Van YYU	Pole
G.30	Van-Gevaş	University of Van YYU	Bush
G.31	Bitlis-A.cevaz	University of Van YYU	Pole
G.32	Bitlis-Adilcevaz	University of Van YYU	Pole
G.33	Melisa	University of Van YYU	Pole
G.34	Aysel	University of Van YYU	Bush
G.35	Alman Ayşe	University of Van YYU	Pole
G.36	Karacaşehir-90	University of Van YYU	Pole
G.37	Terzibaba	University of Van YYU	Bush
G.38	Şehirali-90	University of Van YYU	Bush
G.39	Şeker fasulye	University of Van YYU	Pole
G.40	Önceler 98	University of Van YYU	Bush
G.41	Efsane	University of Van YYU	Bush
G.42	Magnum	University of Van YYU	Bush
G.43	Van-Gevaş	University of Van YYU	Pole
G.44	Van-Edremit	University of Van YYU	Pole
G.45	Van-Bahçesaray	University of Van YYU	Bush

Table 2. Primer Information for RT-PCR Detection of BCMV.

Virus		Sequence (5'-3')	Length base	Reference
BCMV	BCMV-F	5'-GGATGCGGAGAATCTGTG-3'	850 bp	Bhadramurthy and Bhat, 2009
	BCMV-R	5'-GATTGACGTCCCTTGCAG-3'		

For the first-strand cDNA synthesis, the total RNAs that were extracted were utilized. To summarize the procedure, the following steps were followed:

1. In a nuclease-free microfuge tube, 1 µl of random primer (20 pmol/µl) was added as a template.

2. 5 µl of total RNA and 1 µl of dNTP (10 mM) were added to the tube.
3. The volume was completed to 12 µl with nuclease-free water.
4. The mixture was incubated at 65 °C for 5 minutes.
5. Subsequently, the mixture was chilled on ice.

To prepare the reaction mixture, 4 µl of 5X RT Reaction buffer, 2 µl of 0.1M DTT, 1 µl of RNAse inhibitor, and 1 µl of Reverse Transcriptase enzyme (Thermo Scientific, USA) were added, bringing the total volume to 20 µl. The reverse transcription (RT) step was carried out at 42 °C for 60 minutes. Subsequently, to deactivate the RT enzyme, the mixture was incubated at 70 °C for 15 minutes. The resulting cDNAs were stored at -20 °C until further processing.

2.3. Detection of BCMV by RT-PCR assays

The cDNA products obtained from total RNAs served as templates for the RT-PCR assay. To detect BCMV, the reagents were adjusted empirically as follows in a total volume of 50 µl: 36.6 µl sterile distilled water, 2 µl cDNA as the template, 3 µl MgCl2 (25 mM), 1 µl dNTPs (10 mM), 1 µl of forward and reverse primers (20 pmol each), 5 µl of 10X Taq buffer, and 0.4 µl Taq DNA polymerase (5 U/µl) (Thermo Scientific, USA).

The temperature cycles for the RT-PCR reaction were optimized as follows: an initial denaturation step at 94 °C for 2 minutes, followed by 37 cycles of denaturation at 94 °C for 30 seconds, annealing at 56 °C for 45 seconds, and elongation at 72 °C for 1 minute. A final elongation step was performed at 72 °C for 5 minutes. (Modified from Bhadramurthy and Bhat, 2009).

After electrophoresis on a 1.5% agarose gel containing Ethidium bromide (EtBr), the amplified fragments (15 µl) were visualized under UV light. A negative control consisting of healthy plants was included in the analysis. Additionally, positive control was incorporated using a confirmed BCMV isolate, verified through sequence analysis.

3. Results and Discussions

BCMV strains have been identified in many industrialized countries where bean varieties are grown, and the development of resistant varieties has significantly reduced the rate of damage from this virus. Bean varieties are selected for disease resistance based on yield factors and their adaptation to the region. Currently, many growers use BCMV-infected seed, resulting in crop losses due to infection. The most prevalent potyvirus affecting legumes is the bean-specific bean common mosaic virus (BCMV), which has been recognized for its impact on the geographic distribution of beans. Beans were introduced to Anatolia approximately 250 years ago and subsequently dispersed throughout the region (Sehirali, 1988). The bean crop is susceptible to various bacterial, fungal, and viral diseases, as well as abiotic factors, posing a significant threat. Multiple genera have been identified to harbor at least 30 virus diseases, all of which contribute to substantial yield losses in bean cultivation areas (Loebenstein and Thottappilly, 2004). Different scientists observed bean symptoms, such as longer, narrower leaves and leaf distortion, along with vein banding and a typical mosaic pattern, in virus-introduced beans (Melgarejo et al., 2007; Deligoz and Sokmen, 2013; Mangeni et al., 2014). In the present study, data were obtained that may be useful for the development of resistant cultivars. Information was obtained on which plants with which gene combination the virus studies can be started.

The NL-4 and RU-1 strains were inoculated on the 20 plants of the susceptible Stringless Green Refuges (SGR) cultivar that were grown in the climate chamber room Plant Protection Department of Van Yuzuncu Yil University in April 2021. Three bands positive for NL-4 and one band positive for RU-1 were obtained for BCMV. However, strain RU-1 was lost during the increase; therefore the genotypes were artificially inoculated with the NL-4 strain.

3.1. Symptoms NL-4 and RU-1

After four days of inoculated Stringless Green Refuges (SGR) cultivar by NL-4 and RU-1, the symptoms of each other of the strains appeared (Figure 1).

Figure 1. Symptoms of RU-1 and NL-4.

3.2. Replicated artificial inoculation trial results with NL-4 strain of BCMV

The 45 bean genotypes were artificially inoculated at 3 replications each consisting of 10 plants per genotype. According to symptoms, BCMV was detected in about 1/3 of the studied bean genotypes in three replications (Table 3-5). The total number of plants infected with BCMV in sampling locations was summarized in Tables 3, 4, and 5.

The results study scored for each plant were inoculated by NL-4 strain for the first replication. 92 plants were susceptible and 358 plants were resistant among 450 plants. According to the number of susceptible plants, the highest susceptible genotypes were G7, G10, and G32 had ten plants that were susceptible after inoculation. Based on the mean of plant susceptibility for the BCMV-NL-4, the highest value (4) was from G1 Stringless Green Refuge, and G29-Van-Gevaş had the lowest value (0.2). According to the scoring, fifteen genotypes (19%) were susceptible and thirty genotypes (79%) were resistant (Table 3).

The second replication inoculation was scored for all bean genotype plants. The result 77 plants were susceptible and 373 plants were resistant among the 450 plants. According to the number of susceptible plants, the highest susceptible genotypes were G1, G8, G10, and G22 had ten plants that were susceptible after inoculation. Based on the mean of plant susceptibility for the BCMV-NL-4, the highest value (4.5) was from G8, and G28 had the lowest value (0.4). According to the scoring, eleven genotypes (24.4%) were susceptible and thirty-four genotypes (75.6%) were resistant (Table 4).

The results study scored for each plant were inoculated by NL-4 strain for the third replication. As a result, 82 plants were susceptible and 368 plants were resistant among 450.

According to the number of susceptible plants, the highest susceptible genotypes were G1, G8, and G22, while ten plants were susceptible after inoculation. Based on the mean of plant susceptibility for the BCMV-NL-4, the highest value (3) was obtained from G8, while G2 had the lowest value (0.1). According to the scoring, twelve genotypes (26.6%) were susceptible and thirty-three genotypes (73.3%) were resistant (Table 5).

Table 3. The first replication group bean genotypes were inoculated by BCMV-NL-4

Genotype	Symptoms BCMV-NL-4										
	R1.P1	R1.P2	R1.P3	R1.P4	R1.P5	R1.P6	R1.P7	R1.P8	R1.P9	R1.P10	Mean
G.1	5	5	4	4	5	3	0	5	5	4	4
G.2	0	1	0	1	0	1	0	0	2	1	0.6
G.3	2	1	5	0	1	1	3	1	0	0	1.4
G.4	0	0	0	0	0	0	0	0	0	0	0
G.5	0	0	0	0	0	0	0	0	0	0	0
G.6	0	0	0	0	0	0	0	0	0	0	0
G.7	2	1	1	2	2	1	3	2	2	2	1.8
G.8	2	4	0	1	5	4	2	1	0	0	1.9
G.9	0	0	0	0	0	0	0	0	0	0	0
G.10	5	4	5	3	4	2	5	4	3	3	3.8
G.11	0	0	0	0	0	0	0	0	0	0	0
G.12	0	0	0	0	0	0	0	0	0	0	0
G.13	0	0	0	0	0	0	0	0	0	0	0
G.14	0	0	0	0	0	0	0	0	0	0	0
G.15	0	0	0	0	0	0	0	0	0	0	0
G.16	0	0	0	0	0	0	0	0	0	0	0
G.17	0	0	0	0	0	0	0	0	0	0	0
G.18	0	0	0	0	0	0	0	0	0	0	0
G.19	0	0	0	0	0	0	0	0	0	0	0
G.20	0	0	0	0	0	0	0	0	0	0	0
G.21	0	0	0	0	0	0	0	0	0	0	0
G.22	3	3	3	2	5	5	0	1	2	1	2.5
G.23	0	0	0	0	5	4	5	4	0	3	2.1
G.24	0	0	0	0	0	0	0	0	0	0	0
G.25	0	0	0	1	2	2	5	4	5	5	2.4
G.26	0	0	0	0	0	0	0	0	0	0	0
G.27	0	0	0	0	0	0	0	0	0	0	0
G.28	3	2	0	0	0	0	0	0	0	0	0.5
G.29	0	0	0	0	0	0	0	0	0	2	0.2
G.30	0	0	0	0	0	0	0	0	0	0	0
G.31	3	3	2	1	0	0	5	2	3	4	2.3
G.32	3	2	2	2	3	3	2	3	5	5	3
G.33	0	0	0	0	0	0	0	0	0	0	0
G.34	0	0	0	0	0	0	0	0	0	0	0
G.35	0	0	0	0	0	0	0	0	0	0	0
G.36	0	0	0	0	0	0	0	0	0	0	0
G.37	0	0	0	0	0	0	0	0	0	0	0
G.38	0	0	0	0	0	0	0	0	0	0	0
G.39	0	0	0	0	0	0	0	0	4	0	0.4
G.40	0	0	0	0	0	0	0	0	0	0	0
G.41	0	0	0	0	0	0	0	0	0	0	0
G.42	0	0	0	0	0	0	0	0	0	0	0
G.43	0	0	0	0	0	0	0	0	0	0	0
G.44	0	0	0	0	0	0	0	0	0	4	0.4
G.45	0	0	0	0	0	0	0	0	0	0	0

R: replication, P: plant, 0: Resistance (non-symptom), 1-5: Susceptible (symptom scoring).

Table 4. The second replication group bean genotypes were inoculated by BCMV-NL-4

	Symptoms BCMV-NL-4										
Genotype	R2.P1	R2.P2	R2.P3	R2.P4	R2.P5	R2.P6	R2.P7	R2.P8	R2.P9	R2.P10	Mean
G.1	4	4	5	3	2	4	3	4	3	5	3.7
G.2	0	0	0	0	0	0	0	0	0	0	0
G.3	1	0	3	0	0	3	0	1	0	0	0.8
G.4	0	0	0	0	0	0	0	0	0	0	0
G.5	0	0	0	0	0	0	0	0	0	0	0
G.6	0	0	0	0	0	0	0	0	0	0	0
G.7	2	0	2	2	4	3	1	1	3	2	2
G.8	5	5	4	5	5	5	5	5	3	3	4.5
G.9	0	0	0	0	0	0	0	0	0	0	0
G.10	5	4	2	2	5	3	3	2	2	2	3
G.11	0	0	0	0	0	0	0	0	0	0	0
G.12	0	0	0	0	0	0	0	0	0	0	0
G.13	0	0	0	0	0	0	0	0	0	0	0
G.14	0	0	0	0	0	0	0	0	0	0	0
G.15	0	0	0	0	0	0	0	0	0	0	0
G.16	0	0	0	0	0	0	0	0	0	0	0
G.17	0	0	0	0	0	0	0	0	0	0	0
G.18	0	0	0	0	0	0	0	0	0	0	0
G.19	0	0	0	0	0	0	0	0	0	0	0
G.20	0	0	0	0	0	0	0	0	0	0	0
G.21	0	0	0	0	0	0	0	0	0	0	0
G.22	4	4	1	1	4	5	5	4	5	4	3.7
G.23	0	0	0	0	0	0	0	0	0	0	0
G.24	0	0	0	0	0	0	0	0	0	0	0
G.25	0	0	0	0	0	0	0	0	4	5	0.9
G.26	0	0	0	0	0	0	0	0	0	0	0
G.27	0	0	0	0	0	0	0	0	0	0	0
G.28	0	0	0	0	0	0	2	2	0	0	0.4
G.29	0	0	0	0	0	0	0	0	0	0	0
G.30	0	0	0	0	0	0	0	0	0	0	0
G.31	0	0	0	0	3	2	1	1	2	3	1.2
G.32	3	2	3	1	2	0	0	0	3	3	1.7
G.33	0	0	0	0	0	0	0	0	0	0	0
G.34	0	0	0	0	0	0	0	0	0	0	0
G.35	0	0	0	0	0	0	0	0	0	0	0
G.36	0	0	0	0	0	0	0	0	0	0	0
G.37	0	0	0	0	0	0	0	0	0	0	0
G.38	0	0	0	0	0	0	0	0	0	0	0
G.39	0	0	3	2	2	0	1	2	2	1	1.3
G.40	0	0	0	0	0	0	0	0	0	0	0
G.41	0	0	0	0	0	0	0	0	0	0	0
G.42	0	0	0	0	0	0	0	0	0	0	0
G.43	0	0	0	0	0	0	0	0	0	0	0
G.44	0	0	0	0	0	0	0	0	0	0	0
G.45	0	0	0	0	0	0	0	0	0	0	0

R: replication, P: plant, 0: Resistance (non-symptom), 1-5: Susceptible (symptom scoring).

Table 5. The third replication group bean genotypes were inoculated by BCMV-NL-4

	Symptoms BCMV-NL-4										
Genotype	R3.P1	R3.P2	R3.P3	R3.P4	R3.P5	R3.P6	R3.P7	R3.P8	R3.P9	R3.P10	Mean
G.1	4	1	1	4	4	4	5	2	5	5	3.5
G.2	1	0	0	0	0	0	0	0	0	0	0.1
G.3	0	1	2	1	0	0	2	1	0	0	0.7
G.4	0	0	0	0	0	0	0	0	0	0	0
G.5	0	0	0	0	0	0	0	0	0	0	0
G.6	0	0	0	0	0	0	0	0	0	0	0
G.7	3	2	4	4	4	5	2	2	2	2	3
G.8	5	5	5	4	3	2	5	5	4	3	4.1
G.9	0	0	0	0	0	0	0	0	0	0	0
G.10	1	2	5	5	4	0	5	5	0	1	2.8
G.11	0	0	0	0	0	0	0	0	0	0	0
G.12	0	0	0	0	0	0	0	0	0	0	0
G.13	0	0	0	0	0	0	0	0	0	0	0
G.14	0	0	0	0	0	0	0	0	0	0	0
G.15	0	0	0	0	0	0	0	0	0	0	0
G.16	0	0	0	0	0	0	0	0	0	0	0
G.17	0	0	0	0	0	0	0	0	0	0	0
G.18	0	0	0	0	0	0	0	0	0	0	0
G.19	0	0	0	0	0	0	0	0	0	0	0
G.20	0	0	0	0	0	0	0	0	0	0	0
G.21	0	0	0	0	0	0	0	0	0	0	0
G.22	2	3	2	1	2	2	1	2	1	3	1.9
G.23	0	0	0	0	4	5	0	0	0	0	0.9
G.24	0	0	0	0	4	4	0	0	4	4	1.6
G.25	0	0	0	0	0	0	0	0	0	0	0
G.26	0	0	0	0	0	0	0	0	0	0	0
G.27	0	0	0	0	0	0	0	0	0	0	0
G.28	0	0	0	0	0	0	0	0	0	0	0
G.29	0	0	0	0	0	0	0	0	0	0	0
G.30	0	0	0	0	0	0	0	0	0	0	0
G.31	0	0	0	0	2	1	1	1	4	4	1.3
G.32	3	2	3	3	1	2	0	0	4	4	2.2
G.33	0	0	0	0	0	0	0	0	0	0	0
G.34	0	0	0	0	0	0	0	0	0	0	0
G.35	0	0	0	0	0	0	0	0	0	0	0
G.36	0	0	0	0	0	0	0	0	0	0	0
G.37	0	0	0	0	0	0	0	0	0	0	0
G.38	0	0	0	0	0	0	0	0	0	0	0
G.39	1	0	2	2	1	0	1	1	3	3	1.4
G.40	0	0	0	0	0	0	0	0	0	0	0
G.41	0	0	0	0	0	0	0	0	0	0	0
G.42	0	0	0	0	0	0	0	0	0	0	0
G.43	0	0	0	0	0	0	0	0	0	0	0
G.44	0	0	0	0	0	0	0	0	0	0	0
G.45	0	0	0	0	0	0	0	0	0	0	0

R: replication, P: plant, 0: Resistance (non-symptom), 1-5: Susceptible (symptom scoring).

3.3. Comparison of three replication symptoms with the control group

Plants in the control group were compared with plants inoculated with the BCMV-NL4 strain based on the symptoms observed on the leaves of the bean plants. The results in Table 6 show that sixteen bean genotypes were susceptible, while twenty-nine bean genotypes out of forty-five inoculated with NL -4 were resistant. According to the symptoms, the leaves of some bean genotypes were highly infected with the virus, while the leaves of some bean genotype leaves were not infected with the virus.

Table 6. Overall evaluation of the bean genotypes/cultivar reaction and symptoms with BCMV-NL-4

Genotype No	Phenotypic Reaction BCMV-NL-4	Symptom
G.1	S	+
G.2	S	-/+
G.3	S	-/+
G.4	R	-
G.5	R	-
G.6	R	-
G.7	S	+
G.8	S	+
G.9	R	-
G.10	S	+
G.11	R	-
G.12	R	-
G.13	R	-
G.14	R	-
G.15	R	-
G.16	R	-
G.17	R	-
G.18	R	-
G.19	R	-
G.20	R	-
G.21	R	-
G.22	S	+
G.23	S	-/+
G.24	S	-/+
G.25	S	-/+
G.26	R	-
G.27	R	-
G.28	S	-/+
G.29	S	-/+
G.30	R	-
G.31	S	+
G.32	S	+
G.33	R	-
G.34	R	-
G.35	R	-
G.36	R	-
G.37	R	-
G.38	R	-
G.39	S	+
G.40	R	-
G.41	R	-
G.42	R	-
G.43	R	-
G.44	S	-/+
G.45	R	-

R: Resistance, S: Susceptible, +: Have symptom, -: No symptom, -/+: Weak symptom.

Depending on the different bean genotypes, leaves showed different shapes (curled, short, and tall) after inoculation and exhibited symptoms of NL -4. As a comparison control group with three replicate groups, four weeks after inoculation, the number of plants of bean genotypes was more resistant than those of susceptible bean genotypes. For further identification, the data numbers susceptible and resistant bean genotypes, each bean genotype plant leaves after 4 weeks after inoculation compared with the control group by two forms the first compared after the triple leaves and the second compared after the single leaves for all plants were inoculated. The prevalence of common bean infection in Türkiye has been estimated to be around 30%, while the transmission rate of BCMV through seeds has been

approximated at 25-40% (Klein et al., 1988; Acikgoz and Citri, 1986; Bashir et al., 2000). In Iran, several researchers have reported the presence of viruses affecting pulse crops, including BYMV, BCMV, and CMV on French beans from the Zanjan province (Kaiser et al., 1967, 1971; Shahraeen, 1993, 2002; Mehraban et al., 2002; Makkouk et al., 2003).

Virus movement within a plant can occur locally, where the virus spreads slowly from cell to cell, or systemically, where it rapidly moves throughout different parts of the infected plant. Host plant resistance to BCMV is conferred by a single dominant *I* gene, which is associated with hypersensitivity (Hegay, 2013).

Although common beans may exhibit similar phenotypes such as seed color, flower color, and molecular markers (e.g., microsatellites), these traits can be utilized to differentiate genotypes within morphologically homogeneous germplasm. These findings are consistent with a previous study by Singh et al. (1991b), which also observed variations in growth habits among common beans (Figure 2).

Figure 2. Plant reactions to the infection of BCMV- NL-4 strain in a climate growth.

3.4. Confirmation of BCMV NL-4 by PCR from molecular methods

According to the gel electrophoreses, the results of susceptible bean genotypes were 31% while due to the score symptoms, the results of 35% of the bean genotypes were recorded to be positive after 21 days from the inoculation. The occurrence of virus infections was investigated by inoculating 2700 leaves of different bean genotypes. It was found that 502 leaves (19% of the total) displayed mixed infections by multiple viruses, highlighting the complex nature of disease resistance in common bean breeding programs. Specifically, the results revealed that 19% of the bean genotypes tested were infected by NL-4 strain viruses. The percentage of BCMV-NL-4 infected samples (19%) was relatively low. After 21 days from inoculated bean genotype plants by BCMV-NL-4 were determined fourteen bean genotypes positive band and thirty-one bean genotypes negative band among forty-five bean genotypes (Figure 3).

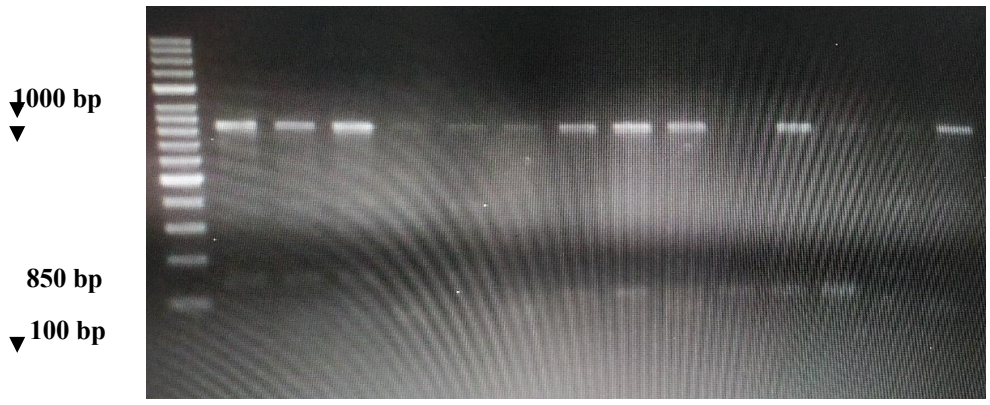

Figure 3. Determination of BCMV-NL-4 virus by the primer BCMV-PCR for G1,3,4,8,10,17,18,19,20, 22, 23, 31, 32, and 34.

The common bean is known to be susceptible to various viruses that have been documented to infect common beans on a global scale. Among them, bean common mosaic virus (BCMV) is recognized as the most prevalent virus worldwide (Hall, 1991). In West Asia, common bean fields in Iraq have reported the presence of BCMV, CMV, and BYMV (Makkouk and Kumari, 1996) (Figure 4). In a study conducted by Deligöz and Sökmen (2013), the resistance of various common bean genotypes against BCMV and BCMNV was assessed using multiple approaches, including symptomatology, enzyme-linked immunosorbent assay (ELISA), and molecular methods. The researchers observed that certain genotypes exhibited a notable level of resistance to both viruses.

In a separate study by Hegay (2013), marker-aided breeding techniques were employed to develop resistance against BCMV and anthracnose in beans cultivated in Kyrgyzstan. The objective of this study was to utilize molecular markers to aid in the breeding process, specifically targeting resistance traits against BCMV and anthracnose. The author used molecular markers linked to the resistance genes and found that marker-aided selection could significantly increase the efficiency of breeding for resistance to BCMV. Mangeni et al. (2014) investigated the distribution and pathogenic characterization of BCMV and BCMNV in western Kenya. They found that both viruses were present in the region and that the prevalence of BCMNV was higher than BCMV. The authors suggested that screening for resistance to both viruses is necessary to develop effective management strategies in the region.

4. Conclusion and Recommendations

In the present study, the responses of 45 bean cultivars to BCMV were determined by artificial inoculation, and the presence of the virus was confirmed at the molecular level. Considering the molecular data obtained, the results of the study were confirmed by measuring the responses of cultivars with resistance genes supported by virus inoculation. The evaluation of the results obtained by inoculation together with the molecular data phenotypically confirmed the success of the markers. The results of the field experiment allow us to conclude: based on the symptoms observed on the leaves of the bean genotypes, the sixteen bean genotypes showed typical mosaic symptoms; however, the remaining twenty-nine bean genotypes had no visible symptoms. Based on the previously mentioned conclusions the following recommendations can be given: The BCMV-NL-4 could be used for the resistance level of the bean genotypes. For the certain determination of bean genotypes other BCMV strains could also be used. The identification of resistant genotypes through these studies holds promise for the development of common bean cultivars with enhanced resistance to BCMV, BCMNV, and anthracnose. These resistant genotypes potentially possess specific genes that confer resistance to these pathogens. By incorporating these resistant genes into breeding programs, it is possible to develop new varieties with improved agronomic traits, ultimately leading to higher yields in field bean production. Screening bean genotypes for BCMV resistance and applying qualitative, quantitative, and molecular methods are critical for developing resistant varieties. Several methods can be used to evaluate the level of resistance in bean genotypes, including symptomatology, ELISA, and molecular methods. Marker-assisted breeding can significantly increase the efficiency of resistance breeding against BCMV. In regions where both viruses are present, screening for resistance to both viruses is necessary to develop effective management strategies.

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Research Article

Different Efficient Responses of Sorghum and Maize Varieties to Different Irrigation Systems

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Abstract: Drought is one of the most common abiotic stressors in the agricultural community. The purpose of this study was to assess the effects of drought on sorghum and maize seedlings. The experiment was conducted as a randomized complete block design (RCBD) in a split plot arrangement with three replicates over two years in Isfahan, Iran. Investigational treatments include three-tier drought stress for two varieties of each plant species. The results demonstrate that the highest energy productivity of fresh forage was obtained in the Pegah variety of sorghum (1.49 kg Mj^{-1}) and the lowest was obtained in the Maxima variety of maize (0.52 kg Mj^{-1}).With 60% irrigation, the lowest water productivity of fresh and dry fodder in maize was found in the Maxima variety (8.32 kg m^{-3}) and the 704 variety (2.15 kg m^{-3}). However, sorghum in the Pegah cultivar at 60% irrigation had the highest water productivity when it came to wet and dry fodder, with an average of 25.45 kg m^{-3} and 7.57 kg m^{-3}, respectively. These results clearly show that in the aspect of energy consumption and production, sorghum was able to optimally convert the consumed energy into more fodder. On the other hand, the Pegah variety in sorghum, having the highest energy efficiency of dry fodder regardless of the amount of water used, was a more suitable plant to choose for planting in areas with water shortage.

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1. Introduction

Water as a limited resource has been affected worldwide by climate change and population growth, which has led to an increase in per capita water consumption in households, industry, and agriculture (Van et al., 1991; Admasu et al., 2019; Dirwai et al., 2021). Agricultural food production represents 70% of global fresh water (Mustafa et al., 2021) and this amount is over 94% in Iran as only 7.7 million hectares (21% of agricultural land) are covered by irrigation (FAO, 2019). The results of

droughts are particularly severe in areas where water scarcity is already limiting plant yield, as is the case in Iran (Nouri et al., 2020) it is therefore more necessary than ever to identify solutions to reduce water use and increase productivity. Water productivity is an index that shows the amount of product obtained from the consumed water. This index is a key basis for studying the production of agricultural products and the efficiency of water resources. In addition to water productivity, the amount of energy needed to produce agricultural products is also very important. Considering the energy crisis and the emission of greenhouse gases, all efforts are aimed at reducing energy consumption as much as possible. Most advanced and even developing countries have tried to optimize their agricultural systems in terms of energy consumption by examining the energy input for the production of various agricultural products and by calculating the energy efficiency index (Tarjuelo et al., 2015).

One way to reduce water consumption is to control irrigation stages and eliminate irrigations that have little effect on crop yield (Karam et al., 2007) and enhance water productivity (Bekele and Tilahun 2007). The water productivity index is a key foundation for the study of agricultural output and water resource efficiency (Passioura, 2006). A number of works on irrigation system performance assessment have been published, presenting the methodology, modeling, and case studies that help to improve the water and energy efficiency of irrigation systems (Khadra and Lamaddalena, 2006; Lamaddalena and Pereira, 2007; Abadia et al., 2008; Calejo et al., 2008). On the other hand, drought-induced stress is defined as the lack of moisture required for normal plant growth and life cycle completion (Mousavi-Avval et al., 2011), which causes 55% of crop losses in the entire of world (Bray et al., 2000) and affects nearly every aspect of the plant such as morphology, physiology and metabolism (Jabereldar et al., 2017; FAO, 2019). Therefore, it is important to choose an alternative crop in place of plants with high water needs and compatible with the stress of drought (Yolci and Tunçtürk, 2022).

Maize (*Zea mays* L.) is the world's third largest cereal crop after wheat and rice, grown mainly for cereal consumption and secondarily for forage (FAO, 2019). It can be cultivated under a wide variety of climatic conditions (Saad-Allah et al., 2021). In a study on maize that was conducted between two treatments of strip drip irrigation system and furrow irrigation system, the superior treatment in strip drip irrigation system (85% irrigation level) compared to the full irrigation treatment in the furrow irrigation system, the seed yield and water productivity was increased by 38% and 171%, respectively (Shahsavari et al., 2018). However, their production and productivity are seriously threatened by the scarcity of water resources (Admasu et al., 2019). Despite its high yield, maize also needs high water content. This has encouraged farmers to seek replacement crops in recent years. In recent years, *Sorghum bicolor* L. was identified as an appropriate option due to its tolerance to drought (Smith and Frederiksen, 2000). Sorghum is a C4 grain that has successfully adapted to semi-arid and arid areas. It can grow in environments with low rainfall and production yield Sorghum can stop its growth in the face of water shortage and continue to grow again with rain or irrigation. The results of Javadi and Esfahani (2023) showed that the input energy of maize is more than its output energy, as a result, it has a negative energy balance, while sorghum has a positive energy balance. Sorghum had a higher output energy (391920 MJ kg^{-1}) and efficiency of energy consumption (2.72) than maize.

Considering the importance of agricultural products such as maize and sorghum under the conditions of lack of water resources in the agricultural sector in Iran, particularly in recent years, it is necessary to be able to use appropriate irrigation methods and also to select suitable crop species and the alternative produced with the highest performance and efficiency of water and energy consumption with a lower volume of water consumption. Hence, the aims of this study were to evaluate the effects of drought on the nutritional compositions of Sorghum and Maize and Comparison of traits of the two plants including water productivity, energy, and drought tolerance indices that were dimensionless.

2. Material and Methods

2.1. Description of where and how to plant

Randomized complete block design (RCBD) in split plot arrangement was employed with three replicates at Isfahan(Latitude 38.5 to 50.5 degrees, Longitude 45 to 32.5 degrees, and height above sea level is 1595 meters), Iran, during the 2017 and 2018 seasons (Table 1). According to the plan, the main plots include three different levels of irrigation (100% (HI), 80% (MI), and 60%(LI) of full irrigation), and the subplots include two varieties of sorghum (Speedfeed (early mature) and Pegah

(late mature)) and two varieties of maize (704 (late mature variety) and Maxima (early mature variety)). Varieties belonging to Iranian forage cultivars were obtained from the Isfahan Seed Improvement Research Centre in Iran.

First of executive operation, a plot of land with an area of approximately 2500 m^2 was deeply plowed. Then ammonium phosphate and potassium sulfate fertilizers were added before planting according to the soil test and at the rate of 250 and 150 kilograms per hectare, respectively, and were placed under the soil using a disc. Other seed bed preparation operations were done in spring when the weather was favorable. In this way, stacks with a distance of 60 cm for sorghum and 75 cm for fodder maize were created as crop rows. When the soil temperature reached 12 degrees Celsius, the plants were planted in the field. The planting date for both was mid-June.

The planting density for sorghum and fodder maize was considered to be 250 and 90 thousand plants per hectare, respectively. Each plot included four planting lines with a length of 12 meters and the distance between the plants on the row was 60 cm (for sorghum) and 75 cm (for maize). Weed control, operations, and harvesting with choppers and machines available in the country for planting, and harvesting sorghum and maize were essential factors for selecting distances. After vegetation and in the stage of four to six leaves, the plants were thinned so that the distance between the plants on the row reached the desired density. The harvest timing of sorghum and corn was set at the beginning of the phase of conversion of seeds from soft pulp to hard pulp, which was the case for sorghum in the first harvest after 90 days for Speedfeed and for Pegah after 112 days after planting. The second harvest occurred 45 days after the first harvest. Varieties 704 and Maxima were also harvested 110 and 95 days after planting, respectively.

To manage irrigation, soil bulk density and the soil moisture content in the field capacity (FC) and permanent wilting point (PWP) were determined by sampling the soil surface in the laboratory. Moisture values around FC, PWP, and bulk density were 25%, 14%, and 1.35 kg m^{-3} respectively. These values were used to control the pure water requirement of two commonly cultivated maize varieties in Iran. Irrigation was done in the form of a drip-strip and the irrigation cycle was determined based on a fixed cycle and according to the pure water requirement of the plant (class A evaporation pan). Irrigation was done by the irrigation cycle. The drip irrigation system consisted of a control unit and distribution lines. Drip laterals of 16mm in diameter had in-line emitters spaced 0.50 m apart, each delivering 4l h^{-1} at the pressure of 100 kPa.

Water requirement was estimated according to daily evapotranspiration values of the reference plant (ET0) and crop-specific coefficient (KC) of the combined Penman-Montes-FAO model (Allen et al., 1998). Crop water use (ET) was estimated based on an onedimensional water balance equation using soil water measured by the neutron and gravimetric sampling methods. Water use was the total of seasonal water depletion (planting to harvest) plus rainfall and irrigations during the same period.

The water balance equation is as follows:

$$ET = I + P \pm DS - D \quad (1)$$

where ET is evapotranspiration (mm), I the irrigation (mm), P the precipitation (mm), D the deep percolation (i.e., drainage, mm), and DS is change of soil water storage in a given time period Dt (days) within plant rooting zone. Water use efficiency was computed as the ratio of crop grain yield to seasonal water use. (Howell et al., 1995).

The irrigation water quality included EC: 1.9 dS/m, pH: 7.2, anion including HCO3: 4.6 meq/L, Cl: 9.2 meq/L, $SO4^{-2}$: 6.5 meq/L, cation including Ca^{2+}: 6.6 meq/L, Mg^{2+}: 3 meq/L, and Na^{+}:10.3 meq/L. Water consumption was also measured by a calibrated meter. The amount of water consumption during the growing season, in 18 to 20 times irrigation in three treatments of 100%, 80, and 60% full irrigation was 5038, 4225, and 3350 m^3 ha^{-1} in 2017 and 4400, 5445, and 3225 m^3 ha^{-1} in 2018 respectively for sorghum and 6449, 5676, and 4550 m^3 ha^{-1} in 2017 and 7100, 5720, and 4710 m^3 ha^{-1} in 2018 respectively for maize.

Table 1. Monthly temperature and precipitation during the growing season in 2017-2018

2017	Temperature (ºC) Min.	Max.	Total precipitation (mm)
May	8	35	13.7
June	15	40.2	0
July	18	41.5	0.1
Aug	15	37	0.2
Sum	56	153.7	14
Average	14	38.5	3.5
2018			
May	10.4	38.6	16.8
June	19.2	41.6	8.4
July	21.3	42.5	0
Aug	20.9	41.5	0
Sum	71.8	164.2	25.2
Average	17.9	41	6.3

2.2. Laboratory analyses

2.2.1. Irrigation water and energy efficiency

Irrigation efficiency refers to the mass of dry matter or the output per unit (m^3) of water consumed by the plant. The total energy required for the production of the two plant species studied in the six major groups, including machine equivalent energy, fuel consumption (Erdal et al., 2007), irrigation (Hatirli et al., 2006), manpower (Ozkan et al., 2004), seed (Mokhtarpour et al., 2000), Pesticides and fertilizer (Lopez-Malvar et al., 2021). For this purpose, all inputs and outputs were converted into equivalent energy using standard conversion coefficients as shown in Table 2. Finally, some energy parameters were calculated according to the following formulas (AOAC, 1995):

$$\text{Energy intensity} = (\text{total consumption energy (Mj ha}^{-1})/\text{plant yield (kg ha}^{-1}) \quad (2)$$

$$\text{Energy productivity} = (\text{plant yield (Kg ha}^{-1)}/\text{input energy (Mj ha}^{-1)}) \quad (3)$$

$$\text{Energy ratio} = (\text{input energy (Mj ha}^{-1})/\text{total input energy (Mj ha}^{-1}) \quad (4)$$

Table 2. Energy equivalents of input and output in maize and sorghum production systems

Energy flow	Unit	Energy coefficients(MJ Unit^{-1})
A. Inputs		
Diesel fuel	L	47.8
Human labor	h	1.96
Machinery	kg	62.7
Nitrogen	kg	47.1
Phosphate(P_2O_5)	kg	15.8
Potassium(K_2O)	kg	9.28
Herbicide	kg	101.2
Insecticide	kg	238
Other fertilizer	kg	0.3
Irrigation water	m3	0.63
Seed	kg	14.7
B. Output		
Maize yield	kg	14.7
Sorghum yield	kg	14.7

2.2.2.Nutritional traits

To evaluate the biochemical characteristics of forage, samples were dried in the oven at 75 °C for 24 h, then passed through a 2 mm sieve and applied for measuring Ash and Crude Protein (CP) (Taize and Zeiger, 1998) Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) (Galeano et al., 1998). In order to determine the total Nitrogen Kjeldahl method was used (Bremner and Mulvaney, 1982)

Traits related to animal nutrition for each plant studied include total digestible nutrient (TDN) and dry matter Intake (DMI) (Lithourgidis et al., 2006), which was calculated according to the following formulas:

$$DMI = 120 \div \% NDF \quad (5)$$

$$TDN = (-1.29 \times ADF) + 101.35 \quad (6)$$

2.2.3.Statistical analysis

The collected data were analyzed through variance analysis using SAS (v. 9.3) to determine the statistical significance of the treatment effects. Additionally, correlation analyses between parameters were carried out using a linear regression model. Treatments were compared using the Duncan test at the 1% and 5% level of probability. The statistical analysis was applied on the data using R software(4.3.19).

3. Results and Discussion

The analysis of variance of related-yield traits indicated that year and plant factors significantly affected all parameters (Table 3) but irrigation and variety did not significantly affect the Water productivity of dried forage. The interaction among traits was not significant for most parameters (Table 3).

Table 3. Analysis of variance of experimental treatments on the measured indices of maize and sorghum forage in 2017-2018

S.O.V	d.f	Energy Ratio	Energy Productivity of Fresh Forage	Energy Productivity of Dried Forage	Energy Intensity	Water Productivity of Fresh Forage	Water Productivity of Dried Forage
Year	1	387^{**}	0.65^{**}	0.05^{**}	1.24^{**}	46^{**}	4.8^{**}
Year*Replication	4	14.36	0.02	0.004	0.06	11.94	1.2
Plant	1	1889^{**}	7.45^{**}	0.54^{**}	13.01^{**}	2653^{**}	200.96^{**}
Plant*Year	1	133.7^{**}	0.1^{**}	0.01^{*}	0.09^{*}	0.22^{ns}	0.01^{ns}
Error a	4	42.4	0.1	0.01	0.19	34.5	3.5
Irrigation	2	45.63^{**}	0.17^{**}	0.01^{**}	0.52^{**}	8.5^{**}	1.17^{ns}
Irrigation*Year	2	4.6^{ns}	0.015^{ns}	0.001^{ns}	0.065^{ns}	5^{ns}	0.5^{ns}
Irrigation*Plant	2	3.47^{ns}	0.006^{ns}	0.004^{ns}	0.35^{**}	31.39^{**}	0.03^{**}
Irrigation*Plant*Year	2	5.84^{ns}	0.18^{ns}	0.002^{ns}	0.83^{ns}	3.2^{ns}	0.41^{ns}
Error b	16	2.2	0.01	0.004	0.34	1.85	1.3
Variety	1	97.16^{**}	0.34^{**}	0.001^{ns}	0.37^{**}	109.1^{**}	0.0004^{ns}
Variety*Year	1	13.5^{*}	0.04^{*}	0.006^{ns}	0.32^{**}	26.42^{*}	1.23^{ns}
Variety*Plant	1	53.3^{**}	0.19^{**}	0.02^{**}	0.008^{ns}	56.18^{**}	8^{**}
Variety*Plant*Year	1	2.88^{ns}	0.004^{ns}	0.0001^{ns}	0.11^{*}	0.1^{ns}	0.06^{ns}
Variety*Irrigation	2	2.06^{ns}	0.002^{ns}	0.007^{*}	0.003^{ns}	1.7^{ns}	1.64^{ns}
Variety*Irrigation*Year	2	2.05^{ns}	0.008^{ns}	0.002^{ns}	0.003^{ns}	2.11^{ns}	0.16^{ns}
Variety*Plant*Irrigation	2	2.21^{ns}	0.008^{ns}	0.006^{ns}	0.02^{ns}	2.7^{ns}	1.72^{ns}
Variety*Plant*Irrigation*Year	2	1.72^{ns}	0.007^{ns}	0.001^{ns}	0.021^{ns}	2.45^{ns}	0.07^{ns}
Error c	24	2	0.01	0.0002	0.01	2.62	0.08

ns, * and ** are no-significant and significant at the five and one percent levels, respectively.

Table 4. Analysis of variance of experimental treatments on forage quality and nutrition of maize in 2017-2018

S.O.V	d.f	Crude Protein	Nitrogen content	Ash Content	Neutral Detergent Fiber	Acid Detergent Fiber	Total Digestible Nutrients	Dry Matter Intake
Year	1	0.65^{ns}	0.001^{ns}	0.56^{**}	81.06^{**}	0.29^{ns}	0.49^{ns}	0.1^{ns}
Year(Replication)	4	0.29	0.007	0.35	16.88	0.19	0.32	0.1
Irrigation	2	3.21^{**}	0.08^{**}	5.08^{**}	663.61^{**}	177.51^{**}	295.4^{**}	107^{**}
Irrigation*Year	2	0.29^{**}	0.007^{**}	0.028^{ns}	5.5^{ns}	0.21^{ns}	0.36^{ns}	0.05^{ns}
Error a	8	0.16	0.004	0.046	1.98	0.58	0.96	0.4
Variety	1	0.77^{**}	0.01^{**}	1.92^{**}	356.83^{**}	81.93^{**}	136.3^{**}	60.4^{**}
Irrigation*Variety	2	0.082^{*}	0.002^{**}	0.35^{**}	57.54^{**}	35.65^{**}	59.3^{**}	23.3^{**}
Variety*Year	1	0.37^{ns}	0.0009^{ns}	0.63^{**}	46.46^{**}	0.14^{ns}	0.23^{ns}	0.05^{ns}
Irrigation*Variety*Year	2	0.012^{ns}	0.0003^{ns}	0.44^{**}	29.36^{**}	0.6^{ns}	0.99^{ns}	0.4^{ns}
Error b	12	0.021	0.0008	0.045	2.92	0.99	0.48	0.1

ns, * and ** are no-significant and significant at the five and one percent levels, respectively.

According to the two-way ANOVA, changes in Irrigation and Variety of maize significantly affect all the nutrition properties. Furthermore, the year factor and the joint effect of the factors significantly changed Ash content and NDF variables (Table 4).

Changes in the factor of the year did not significantly impact all parameters of forage quality and sorghum nutrition. In addition, irrigation and variety had an important effect on all variables except ash content. Nevertheless, the combined effect of the factors has not changed substantially (Table 5).

Table 5. Analysis of variance of experimental treatments on forage quality and nutrition of sorghum in 2017-2018

S.O.V	d.f	Crude Protein	Ash Content	Neutral Detergent Fiber	Acid Detergent Fiber	Total Digestible Nutrients	Dry Matter Intake
Year	1	0.01^{ns}	1.88^{ns}	7.55^{ns}	0.76^{ns}	1.2^{ns}	0.01^{ns}
Year(Replication)	4	0.05	2.67	2.9	8.91	14.8	0.004
Irrigation	2	4.69^{**}	3.73^{ns}	43.21^{**}	21.02^{**}	35^{**}	0.05^{**}
Irrigation*Year	2	0.2^{ns}	1.97^{ns}	0.34^{ns}	0.51^{ns}	0.8^{ns}	0.009^{ns}
Error a	8	0.11	2.25	2.04	1	1.6	0.002
Variety	1	4.89^{**}	3.75^{ns}	498.1^{**}	176.71^{**}	294^{**}	0.67^{**}
Irrigation*Variety	2	0.3^{ns}	3^{ns}	11.87^{*}	3.35^{ns}	5.5^{ns}	0.01^{*}
Variety*Year	1	0.13^{ns}	1.69^{ns}	1.88^{ns}	0.00001^{ns}	0.00001^{ns}	0.004^{ns}
Irrigation*Variety*Year	2	0.28^{ns}	2.59^{ns}	2.09^{ns}	0.22^{ns}	0.3^{ns}	0.003^{ns}
Error b	12	0.1	2.36	1.76	1.91	4	0.002

ns, * and ** are no-significant and significant at the five and one percent levels, respectively.

For all irrigation levels, the energy ratio and energy productivity of fresh and dried forages of both sorghum varieties were significantly higher than those of maize (Table 6). These parameters were significantly higher under Pegah, Speedfed, and Maxima 704, respectively, and were reduced by drought stress (Table 6). Regardless of energy intensity, drought stress has dramatically increased energy intensity for both plant species. Energy intensity was highest in 704 compared to other Maxima, Speedfed, and Pegah plantings, respectively (Table 6). Under drought stress, water productivity for fresh and dried forage was significantly higher under sorghum than under maize. However, the impact of irrigation levels was different among sorghum varieties, maize varieties showed no significant content. There was no difference in water productivity for fresh and dried forage between Pegah and Speedfed when we did not experience drought stress. Pegah, on the other hand, was above Speedfed when drought conditions were high (Table 6).

Table 6. Average comparison of the effects of interaction between varieties of maize and sorghum under drought stress

	Irrigation Levels											
	HI				**MI**				**LI**			
	Sorghum		**Maize**		**Sorghum**		**Maize**		**Sorghum**		**Maize**	
	Speedfed	**Pegah**	**704**	**Maxima**	**Speedfed**	**Pegah**	**704**	**Maxima**	**Speedfed**	**Pegah**	**704**	**Maxima**
Energy Ratio (%)	18.48 ^{c}	23.47^{a}	12.46^{de}	13.23 ^{d}	18.34 ^{c}	21.04^{b}	10.60^{fg}	11.44 ^{e}	17.28 ^{c}	21.05^{b}	9.21^{g}	9.51 ^{g}
Energy Productivity of Fresh Forage (kg Mj^{-1})	1.17 ^{c}	1.49 ^{a}	0.7 ^{de}	0.75 ^{d}	1.16 ^{c}	1.34 ^{b}	0.60 ^{fg}	0.64 ^{ef}	1.1 ^{c}	1.34 ^{b}	0.52^{g}	0.54 ^{g}
Energy Productivity of Dried Forage (kg Mj^{-1})	0.41 ^{a}	0.36 ^{ab}	0.25 ^{c}	0.20 ^{cd}	0.32 ^{b}	0.38 ^{a}	0.21^{cd}	0.16 ^{de}	0.32 ^{b}	0.4 ^{a}	0.17^{de}	0.13 ^{e}
Energy Intensity (Mj kg^{-1})	0.89 ^{e}	0.67 ^{f}	1.44 ^{d}	1.34 ^{d}	0.88 ^{e}	0.76 ^{f}	1.78 ^{b}	1.57 ^{c}	0.92 ^{e}	0.75 ^{f}	1.96 ^{a}	1.89 ^{ab}
Water Productivity of Fresh Forage (kg m^{-3})	17.26 ^{e}	22.41^{b}	9.35 ^{fg}	10.14 ^{ef}	19.21 ^{d}	17.26^{e}	8.48 ^{fg}	9.39 ^{fg}	20.81 ^{c}	25.45^{a}	8.32 ^{g}	8.72 ^{fg}
Water Productivity of Dried Forage (kg m^{-3})	5.97 ^{bc}	5.42 ^{bc}	3.41 ^{d}	2.77 ^{de}	5.22 ^{c}	6.32 ^{b}	3 ^{de}	2.40 ^{de}	6.13 ^{bc}	7.57 ^{a}	2.84^{de}	2.15 ^{e}

The numbers in each column that have at least one letter in common are in a statistical group. LI: light Irrigation (60% full irrigation), MI: Moderate Irrigation (80% full irrigation) and HI: High Irrigation (100%).

To evaluate the relationships between traits under drought stress treatments, principal components analysis was conducted (Figure 1). As illustrated in the figure, the first and second components accounted for approximately 59% and 25.1% respectively. Approximately, all associations between characters have been affected by irrigation levels. Furthermore, Ash, WPFF, WPDF, EPFF, and EPDF were integrally occupied with high correlation with the Low irrigation in Pegah and Speedfed variety while ADF, NDF, and DMI were associated with the application high irrigation. Moreover, the abundance of TDN and EI can be more attributed to the moderate and low irrigation in Maxima and 704 varieties (Figure 1).

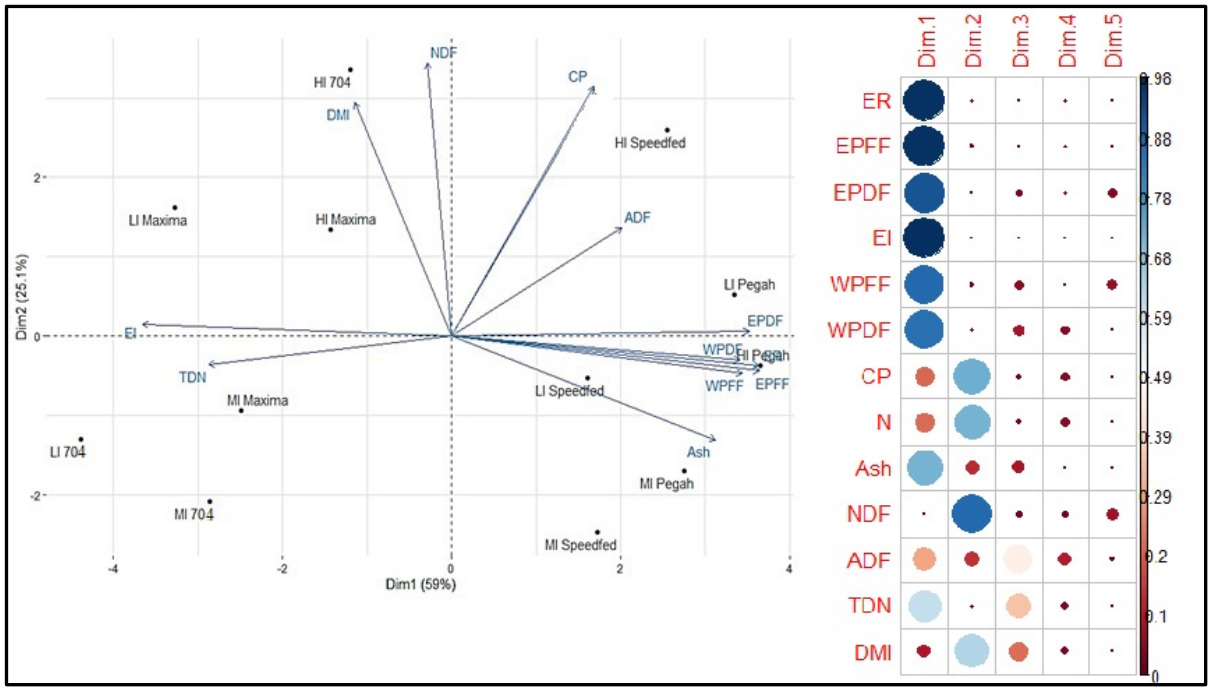

Figure1. Principal Component Analysis (PCA) of data for all characteristics of plants under the different Irrigation. ER: Energy Ratio, EPFF: Energy Productivity of Fresh Forage, EPDF: Energy Productivity of Dried Forage, EI: Energy Intensity, WPFF: Water Productivity of Fresh Forage, WPDF: Water Productivity of Dried Forage, CP: Crude Protein, Ash: Ash Content, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, TDN: Total Digestible Nutrients, DMI: Dry Matter Intake. LI: light Irrigation (60% full irrigation), MI: Moderate Irrigation (80% full irrigation,) and HI: High Irrigation (100%).

In all three irrigation regimes, the EI of Maze varieties was higher than the Sorghum varieties significantly. While this value was quite the opposite for the other parameters (Figure 2). The highest ER, energy productivity, and water productivity were obtained in MI of Speedfeed variety. In the Maxima variety similar to the 704 variety, with decreasing water availability, energy and water parameters were decreased (Figure 2).

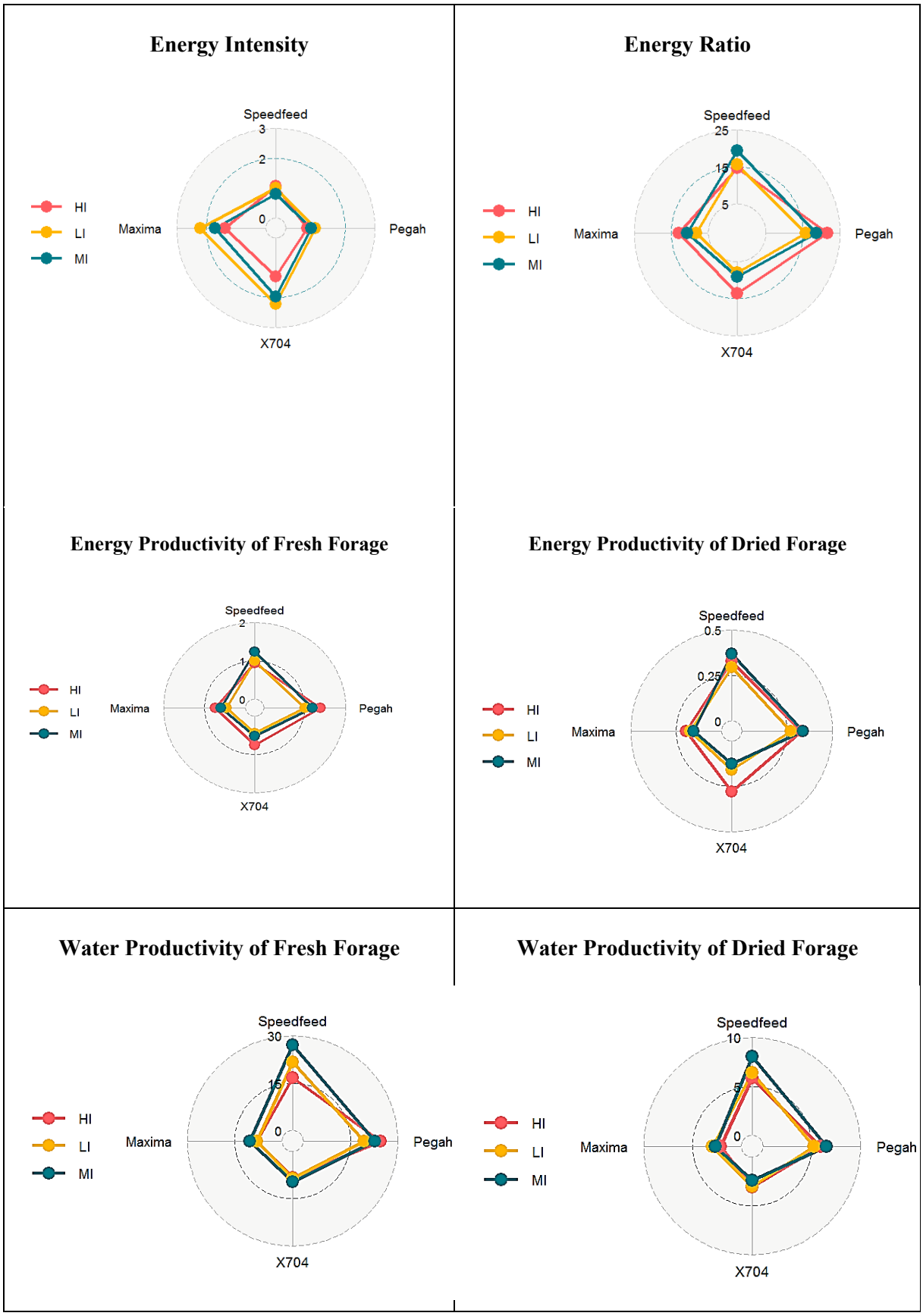

Figure 2. Radar plot comparing energy and water parameters of two Maize and Sorghum varieties under different irrigation levels (LI: light Irrigation (60% full irrigation), MI: Moderate Irrigation (80% full irrigation) and HI: High Irrigation (100%).

4. Discussion

As indicated in the results, most of the measured factors were modified considerably depending on irrigation and the year. Our finding indicated that energy ratio, fresh and dry forage energy productivity significantly increased up to 34% while the rate of energy intensity, fresh and dry forage water productivity reduced up to 25% during 2 years. The assumption is that the primary objective of agricultural production should be to reduce inputs and increase energy production. It seems that by increasing water stress during the year, growth and water-related indicators, especially productivity reduced that this result was also supported by Gomaa et al., (2021).

In general, we observed that the lowest energy intensity was associated with sorghum varieties, which did not differ significantly depending on irrigation levels. These results clearly show that in terms of energy consumption and production of two plants sorghum was able to optimally convert the energy consumption into a larger amount of forage than Maize. On the other hand, the Pegah variety, with the highest energy efficiency of dry forage, regardless of the amount of water consumed, was a more suitable plant to be selected for planting in areas with water shortage. Because under water stress conditions can play a significant role in converting energy use to production. Speedfed hybrid variety has a shorter growth period due to its multiplicity and, except for the amount of soluble sugar, has a preference over the Pegah variety in relation to some qualitative parameters. Its dry matter, protein, and minerals (ash) are higher than the Pegah variety. Although soluble fibre and lignin, in other words, it has lower digestibility than Pegah (Mokhtarpour et al., 2000).

The findings showed that the sorghum variety Pegah in low irrigation has the highest freshwater productivity. This product is well-suited for drought, elevated temperatures, and soil salinity (Bazaluket al., 2021). Farre and Faci (2006) reported that by reducing irrigation water to 50% of full irrigation, Maize yield is reduced by 20%. While grain sorghum yield decreased by only 8% in the 72% reduction of irrigation water (Klocke and Currie, 2009) that we observed the same trend in our study. Unlike other plants, sorghum requires less water and fertilizer per unit of biomass, which is a significant factor leading to a positive energy balance (Bonin et al., 2016) In Italy, the energy efficiency of sorghum is between 1.4-3.9 and 7.5-15 (kg Mj^{-1}) (Garofalo et al., 2016) In Asia (north-eastern China), the energy efficiency of their forages is between 9.3 and 12.4 (kg Mj^{-1}) (Renet al., 2012).

In maize and sorghum plants, the amount of crude protein decreased by about 11% and 7.8%, respectively, by reducing the irrigation level. Crude protein content is one of the most important factors in fodder quality (Wang, 2010). Some studies have reported an increase in the crude protein of fodder plants in the face of drought stress (Jensen et al., 2007; Allahdadi and Bahraini Nejad, 2019; Hatipoğlu et al., 2022), which is probably due to an increase in nitrogen concentration in dry soil. (Jensen et al., 2007). While other researchers have stated the reduction of protein amount in drought stress (Khalil et al., 2015). One of the reasons for protein reduction is their destruction during stress. In such a way that oxygen free radicals cause oxidative degradation of proteins, which occurs in a specific position of amino acids (Wang et al., 2013).

Increasing the water level led to a decrease in the percentage of maize fodder ash, but it did not have a significant effect on sorghum. Among the quality characteristics of fodder, the percentage of ash is a positive indicator of the content of mineral elements in fodder (Daddersan et al., 2016). The increase of ash, or in other words, mineral salts in fodder with the increase of stress, indicates the physiological reaction that the plant shows in dealing with the phenomenon of drought stress. So that the accumulation of mineral salts in the cell sap causes the concentration of the cell sap, and as a result, the negative slope from the root side to the aerial part provides the continuation of water absorption and prevention of the destructive effect of water stress (Abbas et al., 2021), which is in accordance with the findings of the present study.

In maize and sorghum, facing drought stress led to a decrease in the reducing traits of fodder quality, including insoluble fibers in neutral and acidic detergents. Insoluble fibers in acidic detergent and neutral detergent are considered two important features in fodder quality, and high-quality fodder has a low concentration of these two (Caballero et al., 1995).

A good fodder plant should have high dry matter and energy performance (high digestibility) and low fiber for optimal fermentation in silos and storage. These features, except for the amount of protein in maize, are more than other fodder plants (Curran and Posch, 2000). Many studies have shown that the grain and fodder yield of maize decreases under drought stress conditions (Hajibabaei and Azizi, 2014; Farahmandfar et al., 2018). Cakir (2004) evaluated the effect of water stress in maize and reported that water deficiency during the rapid vegetative growth stage reduces dry matter yield by 28-32%. Jama and Otman (1993) investigated the effect of moisture stress in the early stage of maize growth and found that delay in irrigation in this stage reduces the dry weight of the plant. Today, it is well known that the dry matter yield of forage decreases with drought stress (Marsalis et al., 2009; Rostamza et al., 2011; Jahanzad et al., 2013). While no significance was observed in the amount of dry matter in water stress levels with the control treatment in sorghum, it has been reported that sorghum can drain more moisture from the soil in water-deficient conditions (Jose et al., 1990).

Conclusion

The lowest energy intensity was associated with the sorghum Pegah variety, which did not differ significantly in the different irrigation levels. These results clearly demonstrate that in terms of energy consumption and production of maize and sorghum, sorghum has been able to optimally convert energy consumption into fodder. On the other side of the coin, the Pegah variety with the highest energy efficiency of dry forage, regardless of the amount of water consumed, was a more suitable plant to be selected for planting in areas with water shortage. Because under water stress conditions can play an important role in the conversion of energy use to production. Sorghum is more economically preferable to maize in the event of drought. As a result of its morphological characteristics, sorghum has a higher tolerance to drought.

Conflict of interest

The authors declare that they have no conflict of interest.

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Research Article

Investigation on Basil (*Ocimum basilicum* L.) and Lavender (*Lavandula angustifolia*) Cultivation in Aquaponic Aquaculture (Carp, *Cyprinus carpio* L.)

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Abstract: Aquaponic production system, which creates a sustainable production ecosystem by bringing water, fish, and plants together in a closed system, is a reflection of sustainable economic activities in aquaculture. In this context, the aim of the study was to investigate the cultivation possibilities of carp, lavender, and basil in two different grow beds (water and mollusc shell) created in the aquaponic production system. Basil and lavender as plant material and carp as fish material were used in the study. The experimental design was formed from 12 pots of 90 L volume, and the experiment was continued for 60 days with 3 replications. In the study, reasonably high values for specific growth rate (1.96±0.01 g), condition factor (1.5), and survival rate (97.05%) were obtained in carp. While basil (23.5 cm, 23.5 cm, and 16.5 cm) and lavender (16.57 cm, 15.14 cm end 9.73 cm) grown in water media performed better in terms of plant height, root length, and dry herb yield, the green herb yields of basil (49 cm) and Levander (19 cm) were found to be high for both plants in the mollusc shell media. The maximum NH_4, NO_3+NO_2, and PO4 values were determined as 1.30 mg L^{-1} (in 3. quarter and fish tank), 40.07 mg/L (in 3. quarter and Levander shell group), and 0.37 mg L^{-1} (in 1. quarter and basil shell group) respectively.

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1. Introduction

Increasing world population has also increased the demand for food needs (Struik and Kuyper, 2017; van Dijk et al., 2021). This increasing need for food has led to an increase in the development of techniques that can get the most efficiency from the unit area (Kara Öztürk et al.2021) in both vegetable and animal production due to the limited agricultural areas and resources (Struik and Kuyper, 2017; Oliveira et al., 2022). Some of these developed techniques have started to raise problems such as the use of more fertilizers and pesticides with the increase in yield (Oliveira et al., 2022).

Various soilless farming techniques such as Hydroponics, Aquaponics, and Aeroponics have been started to be used in recent years and these techniques allow crop farming to be carried out on balconies, roofs, areas not suitable for agriculture, and in greenhouses (El-Kazzaz and El-Kazzaz, 2017).

Aquaculture, on the other hand, is the cultivation of aquatic animal organisms, usually fish, in a controlled or semi-controlled aquatic environment. The main need to grow healthy fish in aquaculture is clean water providing optimal conditions. However, waste products generated during aquaculture activities also create a pollution load that cannot be ignored in the aquatic environment. Non-consumable feeds, undigested feed compounds, and dissolved metabolic discharge products are important wastes in the aquaculture systems (Tekinay et al., 2006; Munguti et al., 2021). The water used in the aquaculture is contaminated under these three effects over time and the amount of phosphorus, ammonia, and suspended solids increases, and the amount of dissolved oxygen decreases. Aquaponic systems have been developed to reduce these effects (Somerville et al., 2014; Eltez and Taşkavak, 2016). Aquaponic is an application based on the use of wastewater coming from aquaculture in hydroponic systems (Sfetcu et al., 2008; Atique et al., 2022). The main purpose of this system is to reduce the pollution load of water used in aquaculture or to eliminate it completely (Atique et al., 2022). The water used in fish farming is very rich in nutritious elements and plants using this water in hydroponic systems benefit from them (Nguyen et al., 2016). Thus, the water is filtered through the use of these nutrients by the plants (Atique et al., 2022). The pollution load of the water treated by plants is reduced and water that provides optimal conditions is returned to the aquaculture system.

The selection of fish and plants to be used in the aquaponic farming system is very important (Yavuzcanet al. 2017). In the literature, the most commonly used fish species for this purpose are *Maccullochella peelii peelii* (Cod Fish), *Bidyanus bidyanus* (Silver Perch), *Macquaria ambigua* (Golden Perch), *Salmo trutta fario* (Stream trout), *Salmo salar* (Atlantic salmon), *Perca fluviatilis* (Freshwater perch), *Oncorhynchus mykiss* (Rainbow trout), *Ctenopharyngodon idella* (Grass carp), *Hypophthalmichthys molitrix* (Silver carp), *Cyprinus carpio* (Koi) and *Oreochromis* sp. (Tilapia) (Rakocy et al., 2006; Kerim and Tırıl, 2009; Türker, 2018). Carp is a preferred species for breeding because it can live in almost all fresh waters except Antarctica (Rahman, 2015; Fadiloglu and Çoban, 2019)), can easily adapt to sudden temperature changes, is suitable for intensive aquaculture, maintains product quality during the production process, reproduces quickly, has a high survival rate, low aquaculture costs and has a high economic value (Yılmaz, 2004). Plants, on the other hand, are widely used in aquaponic systems in the literature; Lettuce-salad, tomato, cucumber, pepper, spinach, zucchini, parsley, basil, many cultural vegetables, and various ornamental plants (Kargın and Bilgüven, 2018; Türker, 2018). In the study, a medicinal and aromatic plant with high economic value (basil, which is widely used in the aquaponic system, and lavender, which is not used much in the literature) was preferred. Lavender is an important medicinal and aromatic plant that produces essential oils with the highest commercial value in the world (estimated $38 million in 2020) (BAKA, 2020; Crişan et al., 2023). Lavender is used as an input in various production processes such as perfumery, food, cosmetics, health, aromatherapy, and landscaping (BAKA, 2020). With these features, lavender creates a good income opportunity for producers, and its cultivation is preferred (Crişan et al. 2023).

Chemical fertilizers and pesticides, which are considered to be harmful to plants and fish, are not used in aquaponic systems. Thus, it is possible to produce organic products in these systems. The water change is avoided unless it is necessary for the aquaponic system, hence, water, fertilizer, and chemicals are saved, and environmental pollution is prevented (Diver, 2000; Somerville et al., 2014; Olanrewaju et al., 2022).

However, for the elimination of the wastes released by the fish and recirculation of the water, it is very important for the sustainability of the system to determine and optimization of the fish species, fish size, stocking density, plant bed, plant variety, and amount.

This study was conducted to investigate the aquaponics culture potential of common carp, lavender, and basil plants using two different (water and shell) plant mediums. In the study, it was aimed to determine the realization possibilities of aquaponic production, which is a different type of cultivation than traditional production, which may be an alternative to agricultural production in the Mediterranean region of Turkey. Waste, consisting of feed leftovers, fish faeces, and algae from fish tanks rich in nutrients has been used to feed plants in the aquaponic system. These wastes coming from fish tanks, which have a polluting effect above certain levels for the environment are intended to be used by plants that act as biofilters in hydroponic and aquaponic systems, through plant beds, and to help eliminate

ammonia, nitrate, nitrite, and phosphorus. Thus, the possibilities of reuse of the water discharged from the fish tanks have been revealed.

2. Material and Methods

2.1. Fish and plant materials

As fish material, Cyprinus species carp juveniles in the Cyprinidae family from Teleostei (bony fishes) order from the Pisces (fish) class were used. Carp has been preferred because of its characteristics that are mostly grown in hot climatic conditions, can be easily transported to long distances with a minimum loss, can withstand low temperatures, and can be monocultured and polycultured (Váradi, 2022).

As plant material, Lavender (*Lavandula angustifolia*) and Basil (*Ocimum basilicum* L.) from the Lamiaceae family were used. Within the scope of the research, basil seedlings of one (1) month and lavender seedlings of one (1) year were used. Lavender, one of the medicinal and aromatic plants, has been preferred because it is one of the 15 most traded essential oils in the world and because of its high economic value (Georgieva et al. 2021) in terms of the use of lavender in cosmetics, perfume, and pharmaceutical industries (Daneshvar Royandazagh et al., 2022). The other one, Basil, was preferred because of its high economic value in terms of using its essential oils in food flavoring, oral and dental health products, perfumery, and industry as a source of anthocyanins (Günay and Telci, 2017).

2.2. Plant and fish cultivation

This research was carried out at Çukurova University, Adana-Turkey Freshwater Fish Research Station between 01.05.2019 and 30.06.2019. The trial setup and observation of the results were carried out in a greenhouse sheltered against external influences. Day length during the 60-day period in which the experiment was conducted varied between 13:00 and 14:39 h. The 3 pieces of 500 L fish tanks were used in the experiment and air stones in each tank were placed to maintain oxygen level above 5 ppm. The water drained from the fish tanks was transferred to a 1000 L storage tank to capture suspended solids and homogenized water was given to the pots. Twelve plastic pots of 86x40x29 cm were used for plant cultivation. Irrigation water obtained from the fish tanks was used in two different plant types cultivated in two different bedding materials. In the study, the experimental groups were arranged in three replications (Figure 1).

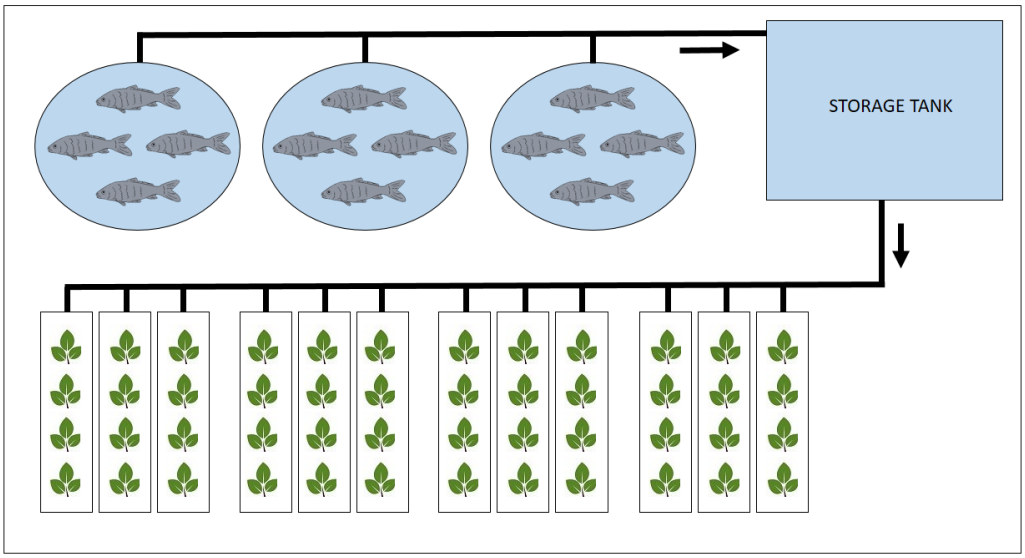

Figure 1. The experimental setup.

Sampling for the water quality parameters and plant growth measurements were made at 15-day intervals in the study. The fish were hand-fed twice a day to apparent satiation from the beginning and a commercial feed with 37% raw protein was used. Two different materials were used as plant growing

beds, water, and marine snail (*Buccinum undatum*) shells. During the experiment, dissolved oxygen, temperature, and pH were measured and recorded in fish tanks. Carp (*Cyprinus carpio* L.) fry which had an initial average weight of 2.1 ± 0.30 g were stocked with a 0.826 mg kg^{-1} stocking ratio in the tanks. The lavender (*L. agustifolis*) and basil (*O.basilicum*) plant species were cultivated as 40 root basil and 15 root lavender in separate pots.

While the basil was arranged to have 30 cm between rows, 20 cm above the rows, and 40 plants per parcel, lavenders were arranged so that there are 90 cm between rows, 60 cm above the rows, and 15 plants per parcel. No additional fertilizer was used for the production of lavender and basil during the experiment.

2.3. Physicochemical parameters

Water temperature (T ºC) and dissolved oxygen (DO) were measured daily with AZ 8402 oxygen meter. The electrical conductivity (EC) and acid or alkaline value (pH) of the water were measured at 15-day intervals by using the WTW Series Cond 720 conductivity meter. Fenat method for ammonium nitrogen (NH_4-N), cadmium reduction method for nitrate + nitrite nitrogen ($NO_3 + NO_2$-N), ascorbic acid method for orthophosphate phosphorus (PO_4-P) were used for the water samples taken (APHA, 2005). Spectrophotometer readings were also carried out with Shimadzu UV at 15-day intervals.

2.4. Plant and fish measurements

In the basil, the plant height (cm) was measured by averaging 10 plants randomly selected from each pot before harvest. The root lengths (cm) of 5 plants at each replication were measured removing the plants without any damage at the end of the harvest.

The plant length (cm) of the Lavender and Basil was determined by measuring the distance of each plant from the water surface to the top point during the harvest period. Flower Stalk Length (cm) was found by measuring the length of the plant flower stalk to the top point, including the flower spike (Sönmez et al., 2018). Flower Spike Length (cm) was measured as the length of the plant flower spike (Sönmez et al., 2018).

The live weights of the fish were made with a 0.1 gram measurement precision scale and total length measurements were made with a 1 mm precision measuring board. The growth parameters of fish were determined based on the daily live weight gain (Wotten, 1990; Rahman and Arifuzzaman, 2021a), the live weight gain (WG) (%) (Heydarnejad, 2012), the specific growth rate (SGR) (De Silva and Anderson, 1995; Hoşsu et al., 2003; Rahman et al., 2022), the condition factor (CF) (Ricker, 1975; Çelik, 2005; Yazıcıoğlu and Yazıcı, 2023), the survival rate (SR) (Çelik, 2005; Opiyo et al., 2017) and the feed conversion rate (FCR) of fish (Santinha et al., 1996).

2.5. Data analysis

SPSS 17.0 (2008) statistics program was used to evaluate the data obtained. The average values and standard errors of the data were calculated, and whether there was a significant difference in the data of the experimental groups was determined by variance analysis (ONE WAY ANOVA) and Duncan test ($P \leq 0.05$) (Düzgüneş et al., 1993).

3. Results

The study was carried out to investigate the aquaponic growing potentials of carp, lavender, and basil plants in two different environments (water and water + mollusk shell). For this purpose, fish population (WG, SGR, CF, SR, and FCR), plant morphological characteristics (plant height, plant root length, plant green herb yield, and plant dry herb yield), and water physicochemical properties (NH_4, $NO_3 + NO_2$, and PO_4) parameters were examined.

3.1. Fish population

Regarding the fish population findings examined within the scope of the study, the daily live weight gain was determined as 0.7609 g while the WG was determined to be 217.40 g carp juveniles at

the end of the experiment in the present study. Eltez and Taşkavak (2016) investigated the possibilities of lettuce cultivation at different doses of fish feeding in aquaponic culture and reported an average fish weight of 49.82 g at 4% feeding (based on live fish weight ratio) over a period of approximately 90 days. Deer et al. (2021) reported the standard average fish weight of 600 g in carp during 6-8 months. The daily live weight gain is a study-specific value due to factors specific to the studies and individuals studied. However, since SGR is a relatively standardizable value, it is considered to be more appropriate to compare it with other studies. In this study, the SGR was found to be 1.96±0.01 g for a 60-day trial. İzci et al. (2020) reported a standard mean SGR of 14.14 g (initial weight: 25-30 g) in carp over a 35-day period. On the other hand, the CF value obtained from the present study is 1.5, and the SR rate of 97.05% was determined. Setiadi et al. (2018) reported a 96% SR of red carp in the aquaponic culture of lettuce cultivation. Yılmaz et al. (2010) reported that the condition factor in carp ranged from 1.34 to 2.29. The average FCR, which is an important tool for calculating the acceptability and feeding efficiency of artificial feed was 1.91 in this study. Hager et al. (2021) reported that the ideal FCR is 1 pound (453.59 g) (1 pound growth of fish fed 1 pound of feed), but in practice, this ratio is 1.4-1.8.

3.2. Plant morphological characteristics

Our findings regarding the findings of Lavender and Basil's plant examined within the scope of the study. The findings related to plant height, root length, green and dry herb productivity, and plant bed of Basil and Lavender are shown in Figure 2.

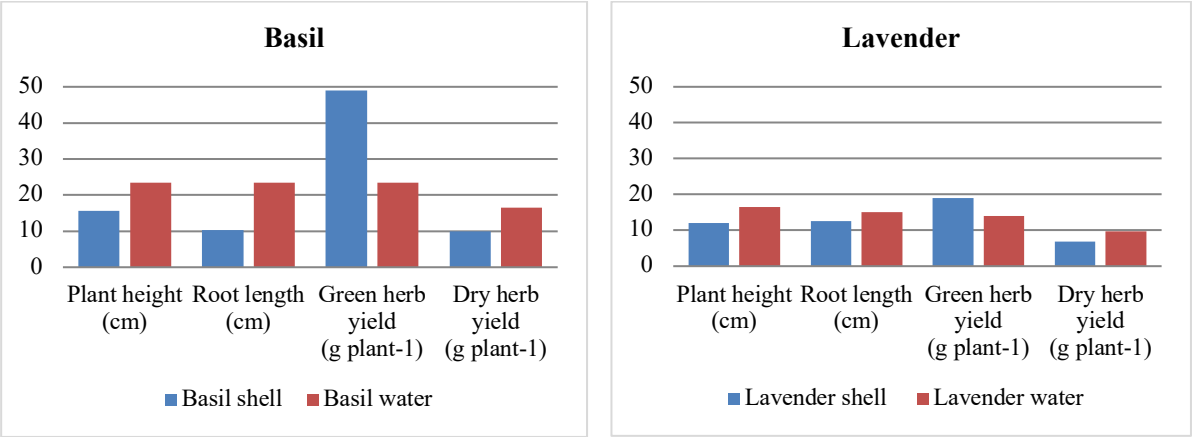

Figure 2. The yields of Basil and Lavender in different plant beds.

The plant height, root length, and dry herb yield values of the basil plant were better in the water plant bed while the basil-shell group was found to have a higher value in green herb fertility (Figure 2). İzci et al. (2020) examined mint cultivation in the aquaponic system and reported a 50% increase in plant roots. In the evaluation made in terms of plant height, the maximum plant height from the basil-water plant bed was measured as 23.50±1.50 cm. Green herb yield was 49 gr for basil and dry herb yield was 16.50 g. İzci et al. (2020) reported that the growth rate of plant leaves increased by 100% and the plant weight growth rate increased by more than 100% in mint cultivation in the aquaponic system. It was determined that the plant height, root length, and dry branched flower productivity of the lavender plant were better in the water plant bed while the lavender group in the shell bed had higher values in fresh branched flower productivity. There were significant differences in the yield of freshly branched flowers in lavender grown in different plant beds. In the experiment, the highest fresh branch flower yield was obtained from the lavender-shell plant bed (19.00±2.00 g plant^{-1}). As a result, it can be speculated that plant beds have a significant effect on plant yield.

3.2. Water physicochemical properties

The physical and chemical parameters (pH, EC, temperature, and DO) measured in the study are shown in Table 1.

Table 1. Physical and chemical parameters measured during the study period

Periods	Cultivation Media	pH	EC (mS/m)	Temperature °C	DO (mg L^{-1})*
15.05.2019	Fish tank	7.20	719	23.80	6.91
	Lavender shell	7.09	761	23.30	-
	Lavender water	7.21	709	23.10	-
	Basil shell	7.12	795	23.00	-
	Basil water	7.10	768	23.10	-
30.05.2019	Fish tank	6.99	775	26.10	6.38
	Lavender shell	7.02	767	26.30	-
	Lavender water	7.11	735	25.90	-
	Basil shell	7.07	783	25.80	-
	Basil water	7.04	779	25.80	-
15.06.2019	Fish tank	7.67	779	25.90	6.35
	Lavender shell	7.69	756	25.10	-
	Lavender water	7.64	761	25.40	-
	Basil shell	7.59	790	25.30	-
	Basil water	7.53	787	25.10	-
30.06.2019	Fish tank	7.91	813	26.80	6.32
	Lavender shell	8.04	808	27.00	-
	Lavender water	8.00	815	27.10	-
	Basil shell	7.94	820	27.20	-
	Basil water	7.93	820	27.00	-

*1 mg L^{-1} (milligram $liter^{-1}$) = 1 ppm (parts per million). pH, Acid or Alkaline Value; EC, Electrical Conductivity, DO, Amount of Dissolved Oxygen in Water.

Standard deviation, mean, and range values of the physical parameters measured on all sampling dates are given in Figure 3.

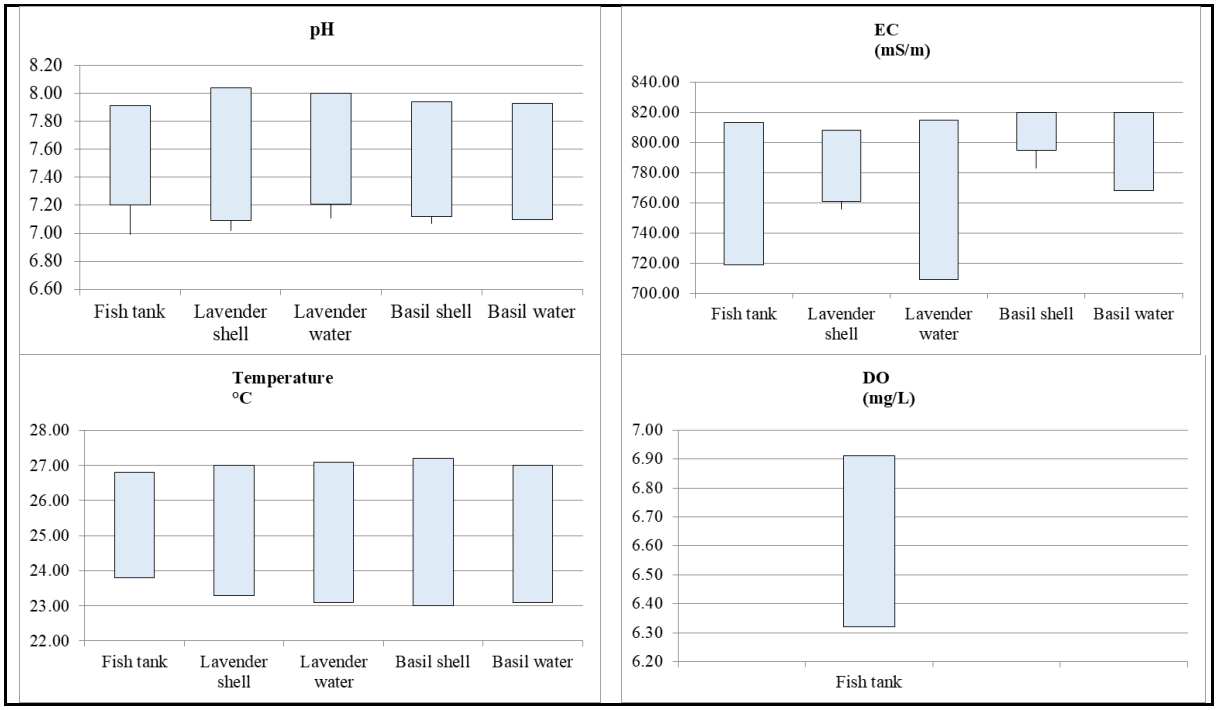

Figure 3. The range of pH, EC, temperature and DO values measured in this study.

According to the study findings, the measured pH values are between 6.99 and 8.00. Deer et al. (2021) investigated the nitrogen cycle in aquaponic culture (with and without fish), and reported that

the pH value in aquaponic culture should be in the range of 6.0-8.50 for fish (cold and hot water fish) and bacterial cycle, and 5.50-7.50 for plants. When the EC values in the applications were examined, the highest EC value was obtained from the basil peel and water application in the 4th quarter in the first 60-day period, and the lowest value was obtained from the lavender shell application in the 3rd quarter. Hager et al. (2021) reported that the ideal EC value in aquaculture should be 0.50-2.0 µS cm^{-1}. The water temperature values measured during the application were between 23-27.2 ºC. Sallenave (2016) reported that the appropriate water temperature required for maximum growth of carp in aquaponic culture should be 27-29 °C. Deer et al. (2021) reported the ideal water temperature in aquaponic culture (in warm water fish) in the range of 22-32 ºC. The dissolved oxygen values measured during the application were between 6.32 and 6.91 mg L^{-1}. Sallenave (2016) reported that the amount of dissolved oxygen should be 5 ppm and above for the healthy development of carp in aquaponic culture. Deer et al. (2021) reported the ideal amount of dissolved oxygen in aquaponic culture (in hot water fish) in the range of 4-6 mg L^{-1}.

During the study, NH4, NO3+NO2, and PO4 measurements were made at the $P \leq 0.05$ significance level in the application environments every 15 days in fish tanks and all plant experimental groups, and the results are presented in Table 2.

Table 2. Effect of applications on the NO3 + NO2, PO4 and NH4

Periods	Cultivation Media	NO3+NO2	PO4	NH4
15.05.2019	Fish tank	35.22±0.71^{ab}	0.06±0.01^{b}	0.25±0.06^{a*}
	Lavender shell	39.30±1.01^{a*}	0.17±0.12^{b}	0.09±0.02^{c}
	Lavender water	37.91±3.32^{ab}	0.04±0.01^{b}	0.14±0.03^{bc}
	Basil shell	24.10±1.79^{c}	0.37±0.14^{a*}	0.12±0.02^{bc}
	Basil water	34.21±3.18^{b}	0.04±0.01^{b}	0.20±0.03^{ab}
30.05.2019	Fish tank	17.44±1.30^{b}	0.11±0.01^{ab}	0.25±0.04^{a}
	Lavender shell	29.24±0.33^{a}	0.31±0.24^{a}	0.18±0.04^{b}
	Lavender water	27.53±1.92^{a}	0.03±0.01^{b}	0.17±0.03^{b}
	Basil shell	28.79±1.00^{a}	0.24±0.13^{ab}	0.17±0.04^{b}
	Basil water	27.29±2.21^{a}	0.05±0.01^{b}	0.12±0.02^{b}
15.06.2019	Fish tank	27.18±1.39^{b}	0.08±0.01^{a}	1.30±0.05^{a}
	Lavender shell	40.07±3.44^{a}	0.06±0.04^{abc}	0.58±0.26^{b}
	Lavender water	34.16±1.51^{ab}	0.04±0.01^{c}	0.71±0.08^{b}
	Basil shell	36.19±8.99^{ab}	0.08±0.03^{ab}	0.70±0.22^{b}
	Basil water	34.14±3.86^{ab}	0.04±0.01^{cb}	0.53±0.05^{b}
30.06.2019	Fish tank	33.82±1.19^{a}	0.06±0.01^{a}	0.15±0.04^{b}
	Lavender shell	30.65±1.60^{a}	0.12±0.06^{a}	0.53±0.21^{ab}
	Lavender water	33.16±0.47^{a}	0.05±0.01^{a}	0.59±0.06^{ab}
	Basil shell	33.51±5.43^{a}	0.08±0.01^{a}	0.79±0.38^{a}
	Basil water	31.99±1.04^{a}	0.23±0.29^{a}	0.51±0.32^{ab}

NO_3, Nitrate; NO_2, Nitrogen Dioxide; PO_4, Phosphate ; NH_4, Ammonium. Differences between applications were statistically evaluated at the significance level of $P \leq 0.05$. The differences between the table values of the applications are symbolized by the letters a, b, and c, the highest value with the letter a(*) and the other values represent the lower values in alphabetical order, and there is no difference between the applications expressed with the same letter.

When the effects of the applications on NH_4 values were examined, the highest NH_4 value was obtained from the fish tank in the first quarter in the first 60-day period, and the lowest value was obtained from the lavender shell application in the second quarter (Figure 4).

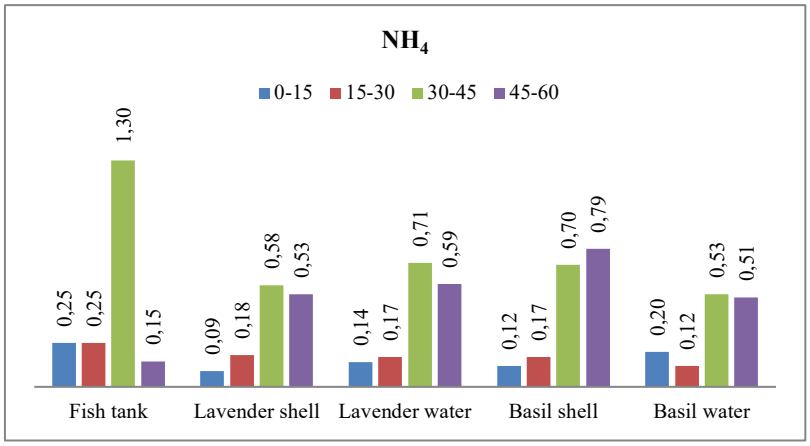

Figure 4. The NH_4 values for all experimental groups.

Effendi et al. (2017) investigated the development of Nile carp in lettuce cultivation in aquaponic culture and reported the amount of NH_4 as 3.10-4.72 mg L^{-1}. Zahidah et al. (2018) investigated the effect of Red Water System (RWS) probiotic applications on water quality in aquaponic culture and reported the highest NH_4 value of 0.77 ppm at 20 days, and the lowest value of 0.28 ppm at 10 days.

When the effects of the applications on NO_3+NO_2 values are examined, the highest NO_3+NO_2 value in the first 60-day period was obtained from the lavender shell application in the first quarter, and the lowest value was obtained from the fish tank in the second quarter (Figure 5).

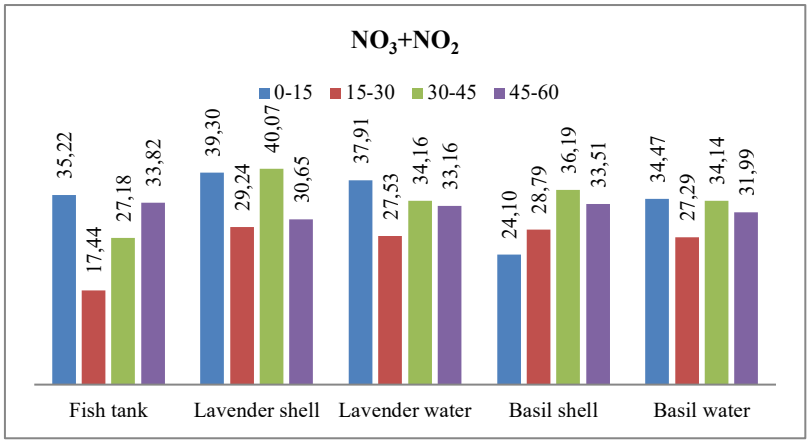

Figure 5. The NO_3 + NO_2 values for all experimental groups.

Effendi et al. (2017) investigated the development of Nile carp in lettuce cultivation in aquaponic culture and reported the NO_3+NO_2 amount in the range of 0.94 (0.91+0.03) - 2.45 (1.52+0.93) mg L^{-1}. While İzci et al. (2020) reported the lowest Ammonium and Nitrite values as 0.061 mg L^{-1} and the highest as 0.226 mg L^{-1} in mint cultivation in the aquaponic system, they reported the lowest Nitrate value as 3.6 and the highest as 110.80.

When the effects of the applications on PO_4 values were examined, the highest PO_4 value was obtained from the basil shell application in the first quarter in the first 60-day period, and the lowest value was obtained from the lavender water application in the third quarter. (Figure 6).

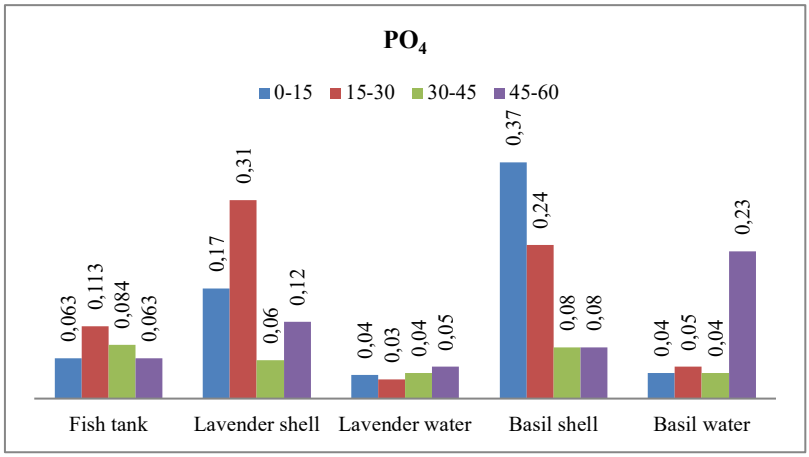

Figure 6. The PO_4 values for all experimental groups.

Türker (2018) investigated the effect of growing different plant species in aquaponic culture on water quality, and in lettuce, he reported the amount of PO_4 as 2.05-2.88 mg L^{-1} for the water entering the system and 0.89-2.25 mg L^{-1} for the water leaving the system. In addition, Türker (2018) reported the amount of PO_4 as 1.78-2.35 mg L^{-1} for the water entering the system and 0.98 mg L^{-1} for the water leaving the system in strawberry. Zahidah et al. (2018) reported that the ideal amount of NO_4 in aquaponic culture should be in the range of 0.20-1.00 ppm.

4. Discussion

In the study, aquaponic cultivation potentials of carp, lavender, and basil plants in two different media (water and water + mollusk shell) were evaluated in terms of fish population (WG, SGR, CF, SR, and FCR), plant morphological characteristics (plant height, plant root length, plant herb yield, and plant dry herb yield) and water physicochemical properties (NH_4, NO_3 + NO_2 and PO_4) parameters.

4.1. Fish population

In the study, fish population characteristics were evaluated in terms of WG, SGR, CF, SR, and FCR parameters.

Regarding WG, the conversion of the nutrients in the feed taken by the fish into live weight and growth is an indicator used to determine the amount of marketable product in fish farming (Gençer and Doğankaya, 2022). Regarding WG, Rahman et al. (2012) tried 4 different protein levels in their feeding study with carp, and they found the growth rate of the WG to be a minimum of 42.7 g and a maximum of 129.2 g. According to Sultana et al. (2001), the WG growth rate of at least 218.91 g in carp where they applied different feeding frequencies, the highest was 334.30 gr during the 45-day trial. Heydarnejad (2012) determined the growth rates of carp at different pH levels between 13.1 and 80. Rahman and Arifuzzaman (2021b) reported the WG value in the range of 36.49±4.09 - 125.19±1.29 g in their study examining the growth performance and survival rate of rohu (Labeo rohita) and tilapia (*Oreochromis niloticus*). As a result, it is seen that quite different data were obtained in the studies conducted. Likely, the genotype of fish, the character and objectives of the studies, and the fish size and environmental factors may be considered to be effective in these differences.

Regarding SGR, which should be known primarily in fish farming, it is used to accurately estimate the growth potential of fish stocked in a certain breeding condition and to calculate the energy or feed amount to be given per day (Korkut et al., 2007). Rahman et al. (2012) found the SGR lowest and the highest values as 0.72 and 1.71 in their seven-week study. Nasır et al. (2016) reported the SGR data for the same species between 0.34 and 0.93 in their 60-day trial. The values obtained in both studies are considerably lower than our values. In contrast, Heydarnejad (2012) found highly variable values between 0.9 and 8.4 partially higher than the values of the present study. However, it can be said that both WG and SGR values obtained from this study are within reasonable limits when compared with previous studies.

Regarding CF, it refers to the best control of morphological structure in assessing nutritional and development criteria in fish, reflecting knowledge of the physiological state of the fish in relation to the welfare of the fish (Korkut et al., 2007). Sac and Okgerman (2016) reached very different CF values varied between 1.33 and 2.66 in their study on carp, which were caught from various lakes. The K value obtained from this study is relatively low compared to other values may be associated with the smaller size of the fish. It is known that one of the most important factors affecting the CF factor is the age of the fish, as well as factors such as nutritional conditions and maturation period (Korkut et al., 2007). Ujjania et al. (2022) reported that the CF value of carp grown in the village pond varied between 1.29 and 1.77 and these values were considered good. Therefore, it can be accepted that the CF factor value (1.5) revealing the health and feeding conditions of fish obtained from this experiment is within the optimum limits.

Regarding SR, it is a parameter that reflects the effect of the suitability of the growing medium on the yield amount in fish farming. In studies on SR, Rahman et al (2012) found a rate between 70% and 93.33%, while Nasir and Hamed (2016) found this rate as 100%. It can be stated that the SR rate of 97.05% determined in our study is satisfactorily high.

Regarding FCR, it is one of the most used indicators to determine the growth performance of fish, both for economical production and providing better conditions (Korkut et al., 2007). While Sultana et al. (2001) determined the FCR as the lowest 1.22. Nasir and Hamed (2016) determined the FCR as 2.21 in their feeding frequency study with carp. A relatively higher FCR value obtained in this study can be considered a more anticipated situation, especially in the fast-growing juvenile fish.

When the fish population characteristics parameters (WG, SGR, K, SR, and FCR) examined in the study are evaluated, the practices are compatible with previous studies in all parameters except WG and provide acceptable aquaponic farming conditions for carp.

4.2. Plant morphological characteristics

In the study, plant morphological characteristics were evaluated in terms of plant height, plant root length, plant green herb yield, and plant herb yield parameters.

Plant growth performance is one of the important criteria in these studies. Serin (1996) recorded the basil plant height values as 52.67-68.37 cm in his study on Adana and Osmaniye basin populations. Simon et al. (1999), on the other hand, determined the plant height values of *Ocimum basilicum* species as 29-49 cm in their studies. According to Nacar (1997), basil plant height values were found to be between 48.39 and 67.17 cm. Telci (2005) reported the height values of basil plants between 32.30-45.70 cm in 2001 and 28.70-40.60 cm in 2002. In a study conducted on basil growth in aquaponic and hydroponic conditions, a remarkable plant height of 89.9 cm was taken from the aquaponic system (Saha et al. 2016). The basil plant height values obtained from this study were lower than the results obtained in previous studies.

Ceylan et al. (1996) stated that the plant height of lavender is 41.3 cm. Arabacı and Bayram (2005) reported that the plant height in the lavender variety used in the study varied between 43.7-69.5 cm, while in another study Karık et al. (2017) reported that the height was determined between 39.50 – 79.25 cm in different lavender cultivars. Akçay et al. (2021) stated that the plant height of lavender is between 49.55 and 54.13 cm. Considering the results obtained from the plant height of lavender in the present study, the lower values were observed compared to the values reported in previous studies. Although plant height is affected by different environmental factors, it is known that the determining factor is the genetic potential of the variety. Plant height also varies according to cultivars and environmental factors (Ceylan et al., 1996; Arabacı and Bayram, 2005). In the study, the dry herp yield value of the lavender plant taken from the lavender water medium was higher than the dry herb value taken from the lavender shell medium. This situation is related to the density of nitrogen forms (NO_3 and NH_4) that can be taken up by plants in the environment and the ease of uptake by the plant. Korkmaz and Alkan reported that when the amount of nitrogen in the environment is low and its uptake by plants is difficult, root development increases in plants, and on the contrary, stem development increases. In the study, The findings of Korkmaz and Alkan support the findings of the study (the root length is shorter in the lavender shell than in lavender water, and the fresh shoot length is longer than in lavender water).

In the study conducted by Saha et al. (2016) on basil grown in aquaponic and hydroponic systems, the green herb yield was 150.2 g and the dry herb yield was 15.9 g in aquaponic conditions.

The values we obtained as a result of the green herb yield were lower than the results obtained in this study. Based on the dry herb yield, the data was parallel to the findings of Saha et al. (2016). This study was carried out in a greenhouse environment. It is thought that one of the reasons for the lower values found in this study may be due to higher temperature and humidity in greenhouse conditions. Also, in this study, no other nutrients were added to the water that the plants can take from the soil in conventional farming practices. Although the wastewater from the fish tank has an important potential for plant nutrition, the conversion of organic matter into inorganic substances that plants can utilize may not have been sufficient. By applying a system where fish, algae, and plants are used together in a controlled environment, or in a system where these three elements are present and optimum environmental conditions are provided, much more effective results can be obtained and waste minimization can be achieved.

4.3. Water physicochemical properties

In the study, water physicochemical properties were evaluated in terms of pH, EC, temperature, DO, NH_4, NO_3 + NO_2 and PO_4 parameters.

In fish farming, the acceptable value in terms of pH ranges between 6.5 and 9.0 (Zweig et al., 1999). Tekelioğlu (2005) reported that DO should not be below 5-6 mg L^{-1} and the T ºC should be between 20-34 ˚C (Abd El-Hack et al., 2022; Panicz et al., 2022) for the best growth. Stone et al. (2013) stated that it is desired to have EC between 60-2,000 µS cm^{-1} for fish farming. In this respect, it has been demonstrated that environmental conditions were suitable for carp farming during the experiment.

Generally in studies, the NH_3 (non-ionized ammonia) and NH_4 (ammonium) are evaluated together and given as TAN (total ammonia nitrogen). NH_3 has a high level of toxicity, however, NH_3 in the water is converted to NH_4 and is present at a certain rate depending on some factors. The presence rate of NH_3 is directly related to water temperature and pH. The fact that a higher level of one or both of these parameters is an important factor in the increase of NH_3 in the water. In the current study, the water temperature did not exceed 27.2 °C, and the pH above 8.04, revealed that the risk of NH_3 toxicity was not high in the fish tanks.

The most suitable value in the water quality chart prepared for freshwater fish farming; is given as <1 mg L^{-1} for NH_4, <50 mg L^{-1} for NO_3, 0.1 mg L^{-1} for NO_2, and <0.1 mg L^{-1} for PO_4 (Anonymous, 2019). In the current study, it was determined that NH_4 does not have a value above 0.79 mg L^{-1} and NO_3 + NO_2 is the highest as 41.3 mg L^{-1}. The values observed are far below the limits. Ten of the total 16 observation values were determined to be lower than the given values in terms of PO_4. In 6 of them, the values increased up to 0.443 mg L^{-1}. On the other hand, 7 of the low values are in the water bed. In the study, significant differences were found in PO_4 values for different plant beds of the same plant species. Therefore, the water bed is thought to be much more effective than the plant in the availability of PO_4.

Graber and Junge (2009) reported the water quality obtained by cultivating tomatoes and cucumber in the aquaponic system where tilapia are used as fish material. The values obtained reveal that the tomato plant's NH_4, NO_3, and NO_2 performance is quite good, but the results of the cucumber plants are lower than the results we obtained. Accordingly, this difference in the availability of chemical ingredients in plant beds can be explained by the extent to which these chemicals are used by different plant species or cultivars.

Boxman et al. (2018) used additional equipment such as biofilters, waterbed aeration systems, and solid separators while conducting their experiments with purslane and black cumin in a marine hydroponic system, therefore they reported that very low values were obtained not only in plant beds but also in fish tanks. As a finding, the researchers stated that plant growth rates are important in sizing plant beds.

Li et al. (2019) tested the performance of the biofilm plant bed using spinach as the plant material, and in this study, they aimed for the plants to absorb high levels of nutrients with the biofilm material. When the results they obtained (TAN, NO_2, and NO_3) are compared with those obtained in this study, it is seen that the values are close, although the results of this study are partially higher.

Cultivations of tilapia fish, bok choy, and green beans in an integrated aquaponic experiment with filter systems were carried out by Estim et al. (2019). When the results obtained by the researchers are compared with our findings, it is seen that the total nitrite and nitrate levels for all groups are lower

than our findings, but the PO_4 levels are higher than those in our study. While it is possible to associate the high PO_4 level in the study of Estim et al. with the nutritional preferences of the plants, the very low NO_2 and NO_3 levels can be explained by the contribution of the filter system used.

Yang and Kim (2019) carried out their studies with a trial setup in which they added a biofilter and ventilation system in their study with Nile tilapia and various leafy vegetables in order to determine the effects of feeding regime on water quality, crop performance, and nitrogen (N) use efficiency. In their study, they experimented with increasing, uniform, and intermediate feeding rates containing the same amount of (120 g) N and feed (1800 g) instead of standard increasing feeding practices (some % of fish body weight) and they found that uniform feeding regime increases quality, yield, N use and photosynthetic performance of the plants and herbs. They stated that simple modification of the feeding regime and subsequent water chemistry changes in aquaponics were effective in improving plant growth and performance. However, in this present study, one of the standard feeding practices used in aquaculture named as ad libitum (to satiation) was applied. It can be speculated that there is a need for fish plant quantity optimization, most likely due to the feeding method in the study.

Calone et al. (2019) reported NH_4 and NO_3 values for their study using catfish and lutus, as lower values than the values obtained in this study. It can be thought that the use of solid matter sedimentation and biofilter methods in their systems is effective in reaching lower values in their studies.

When the findings regarding the physical and chemical properties of the water were evaluated, it was observed that the organic matter wastes in the water, which emerged from the fish and created environmental pollution for the fish, were used by the plants as a food source to improve the water quality for the fish.

5. Conclusion

As a result of the present study, juvenile carp revealed a growth performance similar to previous studies and it was within the optimum limits. These results showed that optimal conditions in which carp could develop were provided in the experiment. In the study, it was determined that a higher amount of basil and lavender which were grown in the water bed were produced and dry herbal yields were higher in the waterbed. However, plant yields obtained in the study were found to be quite low compared to those grown in terrestrial environments. It is thought that this is because the plants could not find some of the nutrient requirements that they could obtain from the soil in the aquatic environment of the experiment. It is thought that it will be beneficial to determine the fish stock ratio and the optimum ratio of fish/plants that can reveal the optimum water usage rates of the plants in question. In addition, according to the literature reports, it can be said that the increase in the waste produced by the fish due to the growth and the change in the consumption amounts of the plants during the production period can be balanced by the use of aquatic plants and algae which can be another by-product in aquponic systems or biofilters. Furthermore, it can be thought that the use of some supports for plant nutrition will have positive effects on plant productivity.

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Research Article

Similarity Appearance of Parents with Progeny of Lombok Local Cantaloupe (*Cucumis melo* var. Cantalupensis) and Melon (*Cucumis melo* L.)

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Abstract: Crosses between local lombok cantaloupe and melon have produced the first progeny (F1). The F1 have a similar appearance to their parents. This study used two similarity assessments based on qualitative and quantitative traits. Qualitative characters are said to have similarities if their phenotypic appearance resembles one or both parents. The similarity of qualitative characters is visually observed using the munsell plant tissue color book and penetrometer. Meanwhile, the quantitative characters are said to have an appearance resembling one or both parents if the standard error line at the histogram between parents and offspring overlaps. In addition, analysis of gene action, heterosis, heterobeltiosis, and the maternal effect was carried out on quantitative character to obtain genetic information for producing superior local lombok cantaloupe varieties. The results of this study showed several changes in the appearance of F1 in qualitative and quantitative characters. The qualitative characters of F1 resemble the female parent, while the quantitative characters do not resemble both parents. Genetic information about potential ratio, heterosis, heterobeltiosis, and maternal effect varied. Gene action is dominated by potency ratio partial dominance and overdominance. Heterosis occurred in all treatments, while heterobeltiosis did not occur in all treatments. The maternal-effect was obtained on fruit weight, fruit diameter, and fruit length characters.

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1. Introduction

Lombok local cantaloupe has the advantages of having soft flesh, being rich in nutrients, and being tolerant of drought and pest diseases (Sholihatin, 2020). The weakness is that the fruit taste is not sweet, so it has low economic value. Plant crosses between local lombok cantaloupe and melon were carried out to combine the genes controlling the superior traits of the two parents. In this crossing, it is hoped that there will be genetic improvements in the lombok local cantaloupe so that the fruit becomes sweet while maintaining its advantages and increasing its economic value. The contribution of this cross is prioritized in its contribution to economic value (Şener and Kaya, 2022). To compete with imported fruit, it is necessary to improve the quality of local plants by developing lombok local cantaloupe (Muhammadi and Daryono, 2022).

Crosses between lombok local cantaloupe and melon produce progeny with high diversity. The diversity of characters is divided into two categories, namely the diversity of qualitative and quantitative traits. Qualitative traits can be clearly distinguished simply by observing the appearance of the plant. It happens because the genes that control the formation of traits appear to be controlled by a single gene with a main effect that is easy to recognize, (Carsono et al., 2022). While quantitative traits cannot be clearly distinguished just by looking at the appearance of the plants, an analytical method is needed to predict the traits that appear in the plants observed. It happens because so many genes are involved that the distribution will be continuous (Ikram and Chardon, 2010).

The appearance of progeny resulting from crosses can be seen by determining the type of gene action, the value of heterosis and heterobeltiosis, and observing the maternal effects of the reciprocal progeny. Gene action describes the relationship between plant genes, which is then expressed into the phenotypic appearance of the plant. An understanding of gene action could provide an overview of the appearance or phenotype of the plant that will be formed. Knowledge of gene action is also important to determine the type of plant variety to be made. Information about the gene action of a character is important to know as early as possible to facilitate the breeding program being carried out (George et al., 2022). This is because it will help accelerate the development of new superior varieties. In addition, information about gene action can also make breeding programs more effective and efficient (Sharma et al., 2013). Another function of gene action information is to determine the appearance of the phenotype in the progeny of plant crosses. The difference in phenotypic appearance in each progeny is caused by the different gene actions resulting from crosses. Analyzing gene action can also determine whether there is heterosis (Jafri et al., 2022). Heterosis is a phenomenon in which the appearance of the first progeny from crosses (F1) is better than the two parents (Kotkar and Giri, 2020). This phenomenon is beneficial for plants in reproduction and adaptation to environmental changes. Generally, plants with high heterosis will be directed to developing new hybrid superior varieties (Liu et al., 2020).

The appearance of characters passed from parents to progeny can occur in two mechanisms, namely chromosomal inheritance (nucleus) and extrachromosomal inheritance. Extrachromosomal inheritance is an inheritance that is controlled by genes that exist outside the cell nucleus. One of the characteristics of this inheritance is that the progeny of the crosses is different from the progeny of the reciprocal crosses, so it can be said that extrachromosomal inheritance is the result of the maternal effect. The maternal-effect is a modification of the appearance of progeny that resembles the appearance or characteristics of female parents (Schwabl and Groothuis, 2019). This happens because the inheritance of plant characters is not only formed due to genetic factors that exist in the cell nucleus and the environment where the plant grows but can be influenced by the maternal effect (Gilsinger et al., 2010; Badiaraja et al., 2021).

The objectives of this study are 1) to determine changes in the appearance of the first progeny from crosses of lombok local cantaloupe (F1) towards their parents and 2) to determine genetic information about pattern inheritance of traits on progeny using gene action, heterosis, heterobeltiosis, and maternal effect.

2. Material and Method

2.1 Plant material and plantation area

The materials used in this study were seeds of lombok local cantaloupe (endes), a pure line of golden melon, the first progeny resulting from crosses of lombok local cantaloupe and melon, and the reciprocal first progeny obtained from the germplasm collection of the Faculty of Agriculture, University of Mataram. The research carried out consisted of 4 treatments, namely P1 (local lombok cantaloupe/endes), P2 (melon), F1, and F1Rs. Each treatment was repeated 6 times to obtain 24 experimental units. Each experiment was planted with 6 plants to obtain a population of 144 plants.

This research was conducted in August - November 2022 in Peresak Village, Narmada District, West lombok Regency, West Nusa Tenggara Province. The height of this place is 136 meters above sea level. The average environmental conditions are rainfall reaching 546mm, temperature 21ºC - 32ºC, and humidity 4% - 96%.

2.2 Methods

2.2.1 Similarity appearance

Observation of similarity in the appearance of the qualitative characters criteria in this study included fruit shape, fruit skin color, fruit flesh color, and fruit texture (Sirojuddin et al., 2015). The qualitative character data was obtained visually for fruit shape character and using a Munsell Plant Tissue Color Book for fruit color and a penetrometer for fruit texture. The quantitative characters are observed, namely plant age, plant height, stem diameter, fruit weight, fruit diameter, fruit length, fruit thickness, and sweetness level (brix%). The similar appearance of the quantitative characters is observed by looking at the standard error line in the histogram. The standard error formula is as follows (Cumming et al., 2007):

$$SD=\sqrt{\frac{\sum (X-M)^2}{n-1}} \quad (1)$$

Where:
SD = Standard error/standard deviation
X = Individual Data
M = Means

When the standard error line at the histogram between progeny and parents that are compared is overlapped, then there is a similar appearance, but if the standard error line of the histogram does not overlap, then there is no similar appearance in that character (Cumming et al., 2007).

2.2.2 Gene action

Gene action is based on the degree of dominance calculated from the value of the potential ratio of Peter and Frey (1966) in Woelan et al. (2015) which is formulated as follows:

$$hp=\frac{mF-MP}{BP-MP} \quad (2)$$

Where:
hp = potency ratio (gene action)
mF = mean value of progeny
MP = the average value of the two parents (mid-parent value)
BP = the best parent mean value

Based on the calculation results above, it could be estimated that gene action on several quantitative characters from crosses based on the value of the potential ratio that reflects the degree of dominance is as follows:
hp = 0, additive gene action (no dominance)
hp = +1 or -1, positive or negative dominant gene action (complete dominance)
hp = -1<hp<0 or = 1>hp>0, negative or positive partial dominance (incomplete dominance)
hp = >+1 or <-1, dominant gene action is more positive or negative (overdominance)

2.2.3 Heterosis and heterobeltiosis

Heterosis and heterobeltiosis were estimated as per the following formula suggested by Lakshman et al., (2018).

$$H\%=\frac{mF-MP}{MP}\times 100\% \quad (3)$$

$$HB\% = \frac{mF\text{-}BP}{BP} \times 100\% \quad (4)$$

Where:
H% = heterosis
HB% = heterobeltiosis
mF = mean value of progeny
MP = the average value of the two parents (mid-parent value)
BP = the best parent mean value

2.2.4 Maternal effect

The genetic information of maternal effect on quantitative characters an analysis using paired t-test. The paired t-test formula is as follows (SAS, 2016):

$$t = \frac{\bar{d}}{\frac{sd}{\sqrt{n}}} \quad (5)$$

Where:
$\bar{d}$: the average difference in paired data
sd: standard deviation

$$sd = \sqrt{\frac{\sum d^2 \frac{(\sum d)^2}{n}}{n\text{-}1}} \quad (6)$$

n: total of sample

3. Result and Discussion

3.1 Similarity appearance

Inheritance of traits to progeny resulting from crosses can be in the form of qualitative traits or quantitative traits. Qualitative traits are generally controlled by a single gene and characterized by a clear phenotype or character appearance. In contrast, quantitative traits or characters are controlled by many genes and display phenotypic variations continuously (Rukundo et al., 2018). This is possible because the quantitative character of the role of the variety of the environment is also relatively large compared to the qualitative character. This is in accordance with the statement (Qi et al., 2015) that quantitative character is controlled by many genes, and the appearance of the character is strongly influenced by environmental factors. Quantitative characters are characters that can be measured using numbers such as flowering time, number of seeds, plant age, and plant resistance (Song et al., 2018). Unlike the case with qualitative characters that cannot be measured with numbers, such as fruit color, leaf color, stem color, fruit shape, and fruit texture (Kiramana and Isutsa, 2017). This happens because the qualitative characteristics of plants are controlled by one gene or major gene (genes that follow Mendel's laws), so the role of environmental variation is relatively smaller (Carsono et al., 2022). Qualitative character observations in this study included fruit shape, fruit color, and fruit texture (Table 1). The appearance of the fruit shape (Figure 1) in the P1 line has an oval shape, while the P2 has a slightly round shape. Crosses between cantaloupe and melon produce F1 with an oval fruit shape. The shape of fruit of the *Cucumis melo* plant generally has a round or oval fruit shape, depending on the sub-species (Mariod et al., 2017). The shape of the cantaloupe bears a resemblance to the gourd, which is oval, but some are round (Zawani et al., 2017).

Table 1. Observation results of qualitative characters

Treatment	Fruit Shape	Fruit Skin Color	Fruit Flesh Color	Fruit Texture
P1	Oval	Green Yellow	Green Yellow	Soft
P2	Slightly Rounded	Yellow	Yellow-Red	Hard
F1	Oval	Green Yellow Yellow	Green Yellow Yellow	Slightly Hard

Note: Soft (<1.5), slightly hard (1,.5-2.75), and hard (2.75<).

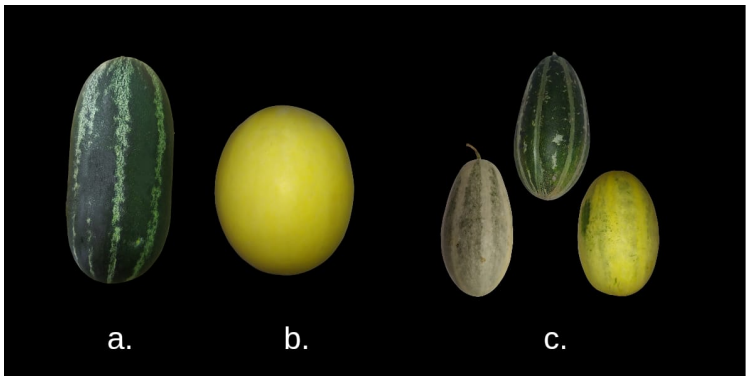

Figure 1. Observation of fruit shape and skin color: a. Female parent (P1), b. Male parent (P2), and first progeny resulting from crosses of lombok local cantaloupe and melon (F1).

Figure 2. Observation of fruit flesh color: a. Female parent (P1), b. Male parent (P2), and first progeny resulting from crosses of lombok local cantaloupe and melon (F1).

The fruit's skin and flesh color appearance is one of the parameters for the diversity of qualitative characters (Table 1). The skin and flesh fruit color characters were measured using the Munsell plant tissue color chart. The female parent (P1) has a green-yellow skin color, while the male parent has a yellow fruit skin color. The cross's progeny have various colors, but most of the fruit has a yellowish-green skin color, and some are yellow. However, the F1 has a stripe pattern that more closely resembles the P1. Similar appearance to the color of the flesh of the fruit (Figure 2), the color of the flesh of the P1 fruit has a green-yellow character, where the color of the flesh is dominated by a yellowish green color. The color of P2 fruit flesh has a yellow-red character, where the color of the flesh is dominated by reddish yellow or orange. The color of F1 fruit flesh has various color characters, namely green-yellow and yellow. The color of the flesh of the first generation is dominated by the hue green-yellow. This means that the progenys' appearance tends to resemble the female parent (P1). Muhammadi and Daryono (2022) obtained the same results, who observed the qualitative characteristics of the progeny from crosses between Kinanti and Sonya. The crosses resulted in a Kinaya line with greenish-yellow fruit skin color. The skin color of the Kinaya fruit is more similar to that of the female parent, namely Kinanti than the male parent of Sonya. The fruit color character shows that the progeny from crosses between Kinanti (female parent) and Sonya (male parent) have the color of the flesh resembling the

female parent, namely RHS 22D or light reddish yellow. Observation of phenotypic characters in the first generation is important for researchers. This information is useful in optimizing genetic resources as materials in plant breeding activities. In addition, observing the appearance can be used as a basis for selection (Akinyosoye, 2022).

The similarity of each quantitative character across the various treatments (Figure 3). In terms of plant age, it is known that F1 were not similar to those of their two parents. In the characters of plant height, stem diameter, and fruit length, F1 had similar characteristics with female parents (P1). The characteristics of fruit weight, fruit diameter, fruit thickness, and sweetness level in F1 were not similar to those of their two parents. This means that the similarity in the appearance of quantitative characters with their parents varies. In addition, it is known that F1 has a dissimilar tendency in quantitative characters to its two parents. The similarity in the appearance of the characters resulting from crosses is inherited from the two parents. Another study by Muhammadi and Daryono (2022) regarding the similarities in the characters of progeny from crosses with their parents showed that crosses between Kinanti and Sonaya produced Kinaya F3 and F4, which have a variety of similar characters. In the stem length or plant height characters, there are similarities in the Kinaya characters F3 and F4 to their parents. As for the diameter of the Kinaya stem, F4 has similarities to the two parents, but F3 does not resemble the two parents. In addition, on fruit characteristics such as fruit weight, fruit length, fruit diameter, and level of sweetness, there are similarities in the characteristics of Kinaya F3 and F4 to the two parents. This occurs due to segregation in the process of meiosis which causes the genes at a locus to separate, and each can form different gametes so that different combinations are possible, which cause different genotypes of the progeny (Zielinski and Scheid, 2012; Datta et al., 2015).

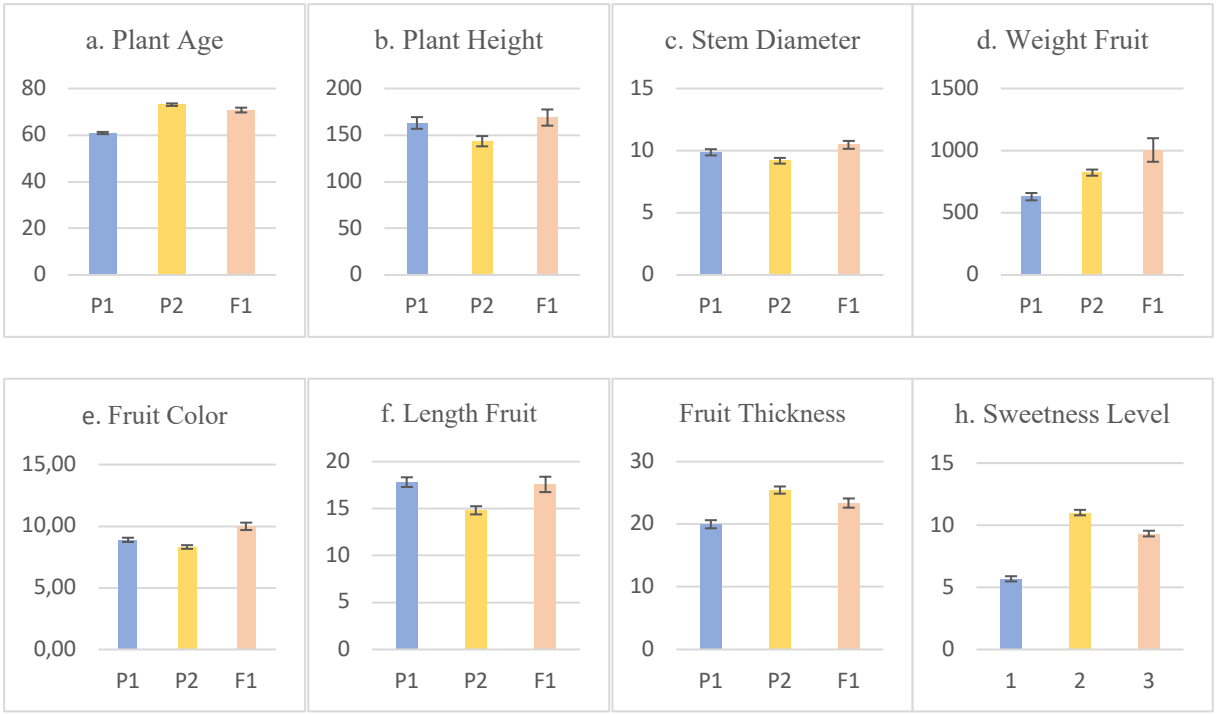

Figure 3. Histogram similar appearance of quantitative character F1 and parents.

3.2 Genetic information

This information is important in planning a plant breeding program. Inheritance information can be known by calculating the value of gene action, heterosis, heterobeltiosis, and maternal effect (Rachman et al., 2022). Gene action provides an overview of the relationship between the genes present in a plant which is then expressed into a phenotypic appearance for the plant. So, differences in gene action in each plant will give a different appearance. According to Hossain (2017), gene action refers to the role of several genes that influence the appearance of plants.

The potency ratio is a value used to determine gene action in progeny resulting from crosses. Research conducted by Rohman et al. (2019) concluded that the value of the potency ratio is important

to know to estimate the level of dominance of genes that play a role in the inheritance of plant traits. Based on the results of the analysis, it is known that there are levels of potency ratio values in all treatments. Most potency ratio values are affected by the action of overdominant and partially dominant genes (Table 5). The characteristics of plant height, stem diameter, fruit weight, and fruit diameter have a more positive dominant potential ratio (Overdominant). Overdominance is an intra-allele interaction in which the presence of multiple alleles results in a performance greater than homozygosity for one of the allelic states (Ghosh et al., 2018). While the treatment of plant age and level of sweetness has a partially negative dominant potential ratio, the treatment of fruit length and fruit thickness has a partially positive potential ratio. According to Frizzell (2013), partial dominance or incomplete dominance results from crosses in which each parent contribution is genetically unique and gives rise to progeny with an intermediate phenotypic appearance or similar between the two. According to Krisnawati and Adie (2022), the overdominance phenomenon in the crossing performance of a population will be different due to selection, and there will be an increase in the homozygosity of alternative alleles in the population to maximize heterozygosity and population crossing performance. Inheritance of character traits with the phenomenon of overdominant gene action is better directed to form hybrid varieties. As opposed to partially dominant, the average dominance degree will decrease, and most of the loci contributing to heterosis will most likely be coupled with a repulsion phase relationship. Therefore, treating plants with partially dominant gene action is better directed at forming synthetic varieties.

Table 2. Calculation results of gene action

Observed Characters	hp	Description
Plant Age	-0.61	Dominance Partial
Plant height	1.52	Over dominant
Stem Diameter	2.37	Over dominant
Fruit Weight	3.02	Over dominant
Fruit Diameter	4.77	Over dominant
Fruit Length	0.76	Dominance Partial
Fruit thickness	0.25	Dominance Partial
Sweetness Level	-0.32	Dominance Partial

Note: potency ratio: additive (0), dominant (+1/-1), dominant partial (0-1/-1-0), overdominant (>1).

Table 3. Calculation results of heterosis and heterobeltiosis

Observed Characters	Heterosis (%)	Heterobeltiosis (%)
Plant Age	5.38	-3.25
Plant height	11.48	3.66
Stem Diameter	6.87	3.85
Fruit Weight	38.53	22.85
Fruit Diameter	16.44	12.56
Fruit Length	7.21	-2.04
fruit thickness	2.9	-7.75
Sweetness Level	10.41	-16.81

Note: + = There is an increase in character than the average parents or better parents, - = There is no increase in character than the average parents or better parents.

Heterosis is a phenomenon that arises when the progeny of various varieties of a species or the result of crosses between species show a superior character or appearance that is greater than the average of the two parents. Meanwhile, heterobeltiosis is a phenomenon that arises when progeny are better than their best parents (Orton, 2020). The heterosis value describes the superiority of the progeny from the cross over the average of the two parents. Meanwhile, the heterobeltiosis value describes the superiority of the progeny from crosses above the average of the best parents (Krisnawati and Adie, 2022). The

results of observations of heterosis and heterobeltiosis values for all treatments varied (Table 3). Heterosis values for all treatments ranged from 2.9% to 38.53%. In all treatments, there is the phenomenon of heterosis, with the lowest heterosis value in the treatment of fruit thickness and the highest in the treatment of fruit weight. Heterobeltiosis values in all treatments ranged from -16.81 to 22.85. This means that several treatments do not experience the phenomenon of heterobeltiosis, namely plant age, fruit length, fruit thickness, and level of sweetness. For the treatment of plant height, stem diameter, fruit weight, and fruit diameter have experienced the phenomenon of heterobeltiosis.

Table 4. Calculation results of T-calc.

Observed Characters	t-calc.	Description
Plant Age	1.04	There is no Maternal Effect
Plant Height	2.08	There is no Maternal Effect
Stem Diameter	0.89	There is no Maternal Effect
Fruit Weight	5.75	Maternal Effect
Fruit Diameter	5.83	Maternal Effect
Fruit Length	3.61	Maternal Effect
Fruit Thickness	0.61	There is no Maternal Effect
Sweetness Level	0.17	There is no Maternal Effect

Note: t-table = 2.57, if t calc. > t table =there is maternal effect if t calc. < t table. = there is no maternal effect

The maternal-effect is a condition in which the appearance of progeny is influenced by the genotype and phenotype of the female parent (Singh et al., 2017). According to Doutrelant et al. (2020), the maternal effect is a non-genetic mechanism in which the progeny phenotype is affected by the environment experienced by the female parent. Based on the results of the calculation of the paired data t-test, it is known that the calculated t-values vary (Table 4). The paired t-test is calculated by comparing the reciprocal F1 value with F1. For the treatment of plant age, plant height, stem diameter, fruit thickness, and sweetness level, the value of t count < t table at 5% level. This means that in this treatment, there was no maternal effect. The treatment of fruit weight, fruit diameter, and length has a value of t count > t table 5%, which means there is a maternal effect in this treatment. Research conducted by Nurhidayah et al. (2021) found a maternal effect on the treatment of the length, width, thickness, and color of the rice grains. This means there is a maternal effect on the value of the treatment. The influence of female parents indicates that the inheritance of traits to their progeny is extrachromosomal or cytoplasmic. Cytoplasmic or extrachromosomal inheritance occurs outside the nucleus, resulting in the increased resemblance between female parents and their progeny compared to male parents (Wolf and Wade, 2009 and 2016).

Conclusion

In conclusion, the qualitative characteristics of F1 were similar to the female parents in fruit shape and fruit color. The fruit texture character has no resemblance to the two parents. Most of the F1 quantitative characters do not resemble the two parents but have a higher value, so there is a heterosis effect on all characters. The value of the potency ratio for the character of plant age, fruit length, fruit thickness, and sweetness level, which is partially dominant, is more suitable for developing synthetic varieties. The potency ratio is overdominant for the characters of plant height, fresh fruit weight, and fruit diameter, so it is more suitable for developing hybrid varieties. In addition, the characteristics of fruit weight, fruit length, and fruit diameter were influenced by female parents. This means that the inheritance of nature is extrachromosomal.

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Research Article

Genetic Diversity and Its Relationship of *Dendrobium* (Orchidaceae) Based on Bioactive Compounds and Their Biological Activities: A Meta-Analysis

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Abstract: Information on genetic diversity and its relationship is fundamental for the preservation and improvement of orchid germplasm. For *Dendrobium*, such information, particularly by a meta-analysis, was limited. The study aimed to assess the genetic diversity and relationships of *Dendrobium* germplasm based on bioactive compounds, their biological activities, and plant organs by a meta-analysis approach. A total of 51 species of *Dendrobium* have been collected and identified as producing bioactive compounds, including their biological activities and plant organs (parts). In this case, the highest genetic diversity was shown by polyphenols (H' index = 0.90) as substances, neuroprotective (H' = 0.80) for activity, and the leaf organ with an H' index of 0.89. The UPGMA analysis showed that *Dendrobium* grouped into seven clusters, where the furthest relationship was presented by *D. moschatum* and *D. catenatum*. However, the closest relation was by *D. scabrilingue* with *D. delacourii*, including *D. snowflake* and *D. ovatum*. Following these parameters, *Dendrobium* shows unique genetic diversity and relationships. Thus, it is valuable for future preservation and improvement programs of *Dendrobium*.

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1. Introduction

Orchid is the second largest flowering plant (Angiospermae), with more than 28 000 species recorded worldwide (Zhang et al., 2022). This genetic resource is essential for edible, ornamental plants, and as a raw material for medicinal and pharmaceutical purposes (Wang et al., 2018). For example, *Dendrobium*, with more than 1 500 species present and spread across the tropics and subtropics (Zheng et al., 2018; Wang, 2021), is one of the orchid genera with the second-highest export value worldwide, with transactions reaching US$ 5.6 million (De et al., 2014). Furthermore, *Dendrobium* has been used for a thousand years by traditional Asian communities, especially in China and India, as a tonic or herbal to treat various diseases, such as inflammation, pyretic, and cancer (Cakova et al., 2017; Zhao et al.,

2019). Consequently, on the one hand, many bioactive compounds have been explored massively from these *Dendrobium* species. On the other hand, breeding efforts to assemble new *Dendrobium* cultivars are also being carried out.

For years, three colorful flowers of *Dendrobium*, i.e., yellow, yellow-white, and purple-white, including sweet smell characteristics, have been modified to make novel hybrids or cultivars (Sawettalake et al., 2017; Li et al., 2021). In this case, over 8 000 *Dendrobium* hybrids or cultivars have been developed by inter-specific hybridization related to flower morphological characteristics (Pongsrila et al., 2014). However, this success is not directly proportional to its existence in the wild. Most wild *Dendrobium* species are susceptible and decrease due to deforestation, natural fragmentation of habitats, and other factors (Hinsley et al., 2018). Further, some are listed as endangered status in the CITES (Convention on International Trade in Endangered Species) Appendix II, e.g., *Dendrobium aberrans* (from Papua New Guinea), *D. acutimentum* (Indonesia), *D. acutilingue* (Philippines), and *D. alabense* (Malaysia) (CITES, 2023). The International Union for Conservation of Nature and Natural Resources also reported some endangered *Dendrobium*, for example, *D. whistleri*, *D. flexicaule*, and *D. leptocladum* (IUCN, 2023).

Thus, the assessment and evaluation of these genetic resources are urgent. Conventionally, comparative morphology and anatomy are familiar in determining or assessing the diversity and relationships of *Dendrobium* (Adams, 2011). However, these two approaches have certain limitations due to the high variability of these orchids. Sometimes, the results are confusing due to environmental factors. Hence, further study needs to be employed by more strong characteristics than morphological and anatomical ones. One of which is biochemical marker application. While this marker is less effective than molecular, the data are beneficial for ensuring quality, efficacy, and safety in the herbal medicine sector (Gahlawat et al., 2017). Besides, analysis of diversity and genetic relationships of *Dendrobium* using biochemical markers is rarely reported or carried out.

In this case, analysis of diversity and genetic relationships of germplasm can be employed by meta-analysis approach. According to Deshmukh (2021), meta-analysis is a statistical combination of results from two or more separate studies extracted from aggregate data of published articles. Then, in addition to being effortless, simple, and low-cost, it can overcome the constraints of formal statistical techniques by aggregating the results of individual experiments to draw general conclusions (van de Wouw et al., 2010). Following Simske (2019), this approach shows high efficiency and is comprehensible to the entire gamut of data science. Several studies using this technique, such as van de Wouw et al. (2010), in evaluating genetic diversity trends in crop cultivars from the twentieth century, and Saputra et al. (2021) for swamp and river buffalo haplotype diversity based on the *cytochrome b* gene. For *Dendrobium*, such studies have never been reported. Thus, this study aimed to assess the genetic diversity and its relationship of *Dendrobium* germplasm based on bioactive compounds, their biological activities, and the type of plant organs by a meta-analysis approach. As a hope, the results of this study provide novelty and good benefits to support future *Dendrobium* breeding and conservation programs.

2. Material and Methods

2.1. Data collection

This study was conducted using a meta-analysis approach, initially by collecting, tabulating, and analyzing various data based on literature searching. A total of 51 species of *Dendrobium* have been collected and identified as producing bioactive compounds, including their biological activities (Table S1, see supplementary file).

2.2. Data analysis

The data obtained were then analyzed in a multivariate manner using a numerical taxonomy approach by the MVSP ver. 3.1 (Kovach, 2007). The genetic diversity of *Dendrobium* was determined using the Shannon-Weaver index (H') by criteria: maximum, H = 1.00; high, H = 0.76–0.99; moderate, H = 0.46–0.75; and low, H = 0.01–0.45 (Mursyidin and Khairullah, 2020). Reconstruction of genetic relationship using cluster analysis with UPGMA (unweighted pair group of arithmetic means) method

and MVSP ver. 3.1 (Kovach, 2007). The relationship was evaluated by principal component analysis (PCA) (Das et al., 2017).

3. Results and Discussion

Bioactive compounds are necessary as raw drug materials for pharmaceutical and medicinal purposes (Atanasov et al., 2021). Most of such materials are obtained from nature today (Atanasov et al., 2021). According to Srivastava et al. (2014), the primary source of drugs comes from medicinal plants and has been explored for the foundation of systematic conventional medicinal products worldwide. In this study, *Dendrobium* was identified as containing dominant compounds, i.e., flavonoid and bibenzyl (Figure 1), as reported similarly by Wang (2021).

According to Hostetler et al. (2017), flavonoids are secondary metabolites belonging to the phenolic class (because they have polyphenolic groups) and are present in various plants, including food sources such as fruits and vegetables. In general, these metabolites are involved in the interaction of plants with their environmental conditions, and hence they are particularly substantial for ecological functions, e.g., allelopathy, animal attractants, seed distribution, and biochemical defense against bacteria, bugs, and herbivores (Srivastava et al., 2014).

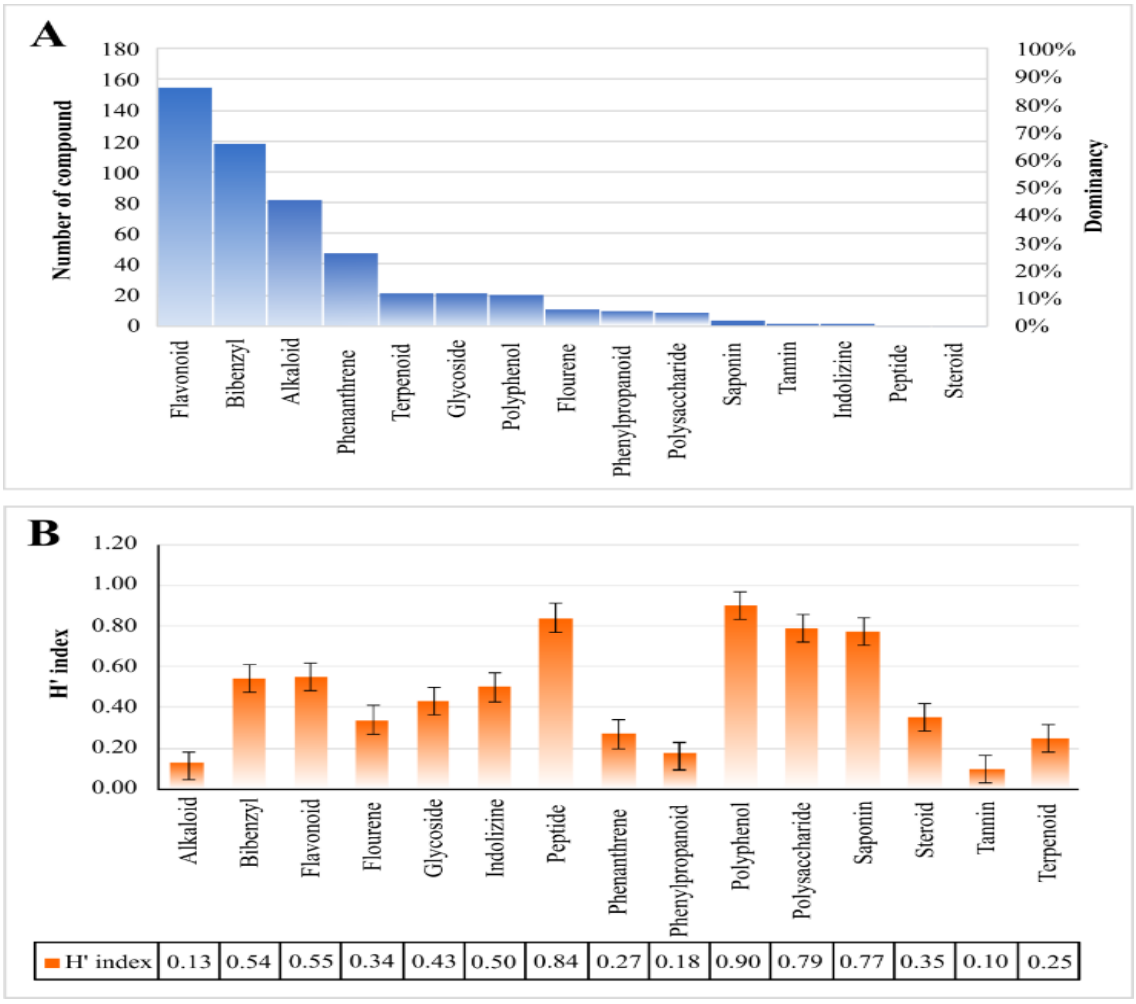

Figure 1. Bioactive compounds found in *Dendrobium*: dominancy (A) and its genetic diversity (B).

In particular, flavonoids have essential biological roles in diverse organisms (Kumar and Pandey, 2013). In plants, for example, this compound is synthesized by the phenylpropanoid pathway and has urgent responsibilities in pigmentation and fragrance in flowers, including attracting pollinators in seed or spore germination and fruit dispersion (Kumar and Pandey, 2013). Furthermore, flavonoids protect plants from many abiotic and biotic stressors, including frost hardiness, drought tolerance

(Panche et al., 2016), and microbial infection (Kumar and Pandey, 2013). Recently, these compounds have been responsible for many pharmacological activities (Kumar and Pandey, 2013).

Meanwhile, bibenzyls are a secondary metabolite distinguished structurally by the absence and presence of two phenyls connected with ethane (C_6-C_2-C_6). Bibenzyls are fragrant chemicals found in plants and bryophytes (Nandy and Dey, 2020). It is generated from the phenylpropanoid biosynthesis pathway and belongs to the polyketide family. This pathway comprises numerous secondary metabolites (Chen et al., 2022). Furthermore, bibenzyls are the synthetic precursors of dihydrophenanthrenes. He et al. (2017) reported twenty-three bibenzyls with flexible structures. For *Dendrobium*, 89 bibenzyl derivatives were present (He et al., 2020).

Interestingly, bibenzyl and flavonoids are interrelated in the biosynthetic pathway (Ahmad et al., 2022). In this case, the difference lies in the enzyme binding to substrates. For example, as a member of the polyketide synthase, chalcone synthase catalyzes the Claisen cyclization to create chalcones and dihydrochalcones (bibenzyl derivates), which are required for flavonoid biosynthesis (Chen et al., 2022). According to Lei et al. (2018), 31 unigenes annotated by the Kyoto Encyclopedia of Genes and Genomes (KEGG) database were engaged in flavonoid pathways by bio-modification, accumulation, transportation, and controlling process.

However, recent interest in the substances of bioactive compounds has shown the potential health benefits. In this study, the bioactive compounds of *Dendrobium* show the highest antioxidant, anticancer, and anti-inflammatory activities (Figure 2). According to Kumar and Pandey (2013), flavonoids' functional hydroxyl groups, for example, can mediate antioxidant effects by neutralizing metal ions and scavenging free radicals. Furthermore, these chemicals are linked to a broad spectrum of health-promoting effects and are essential for nutraceutical, pharmacological, medical, and cosmetic applications (Panche et al., 2016). Beneficial biochemical and antioxidant effects have even been linked to many diseases, such as Alzheimer's, atherosclerosis, cancer, and others (Panche et al., 2016).

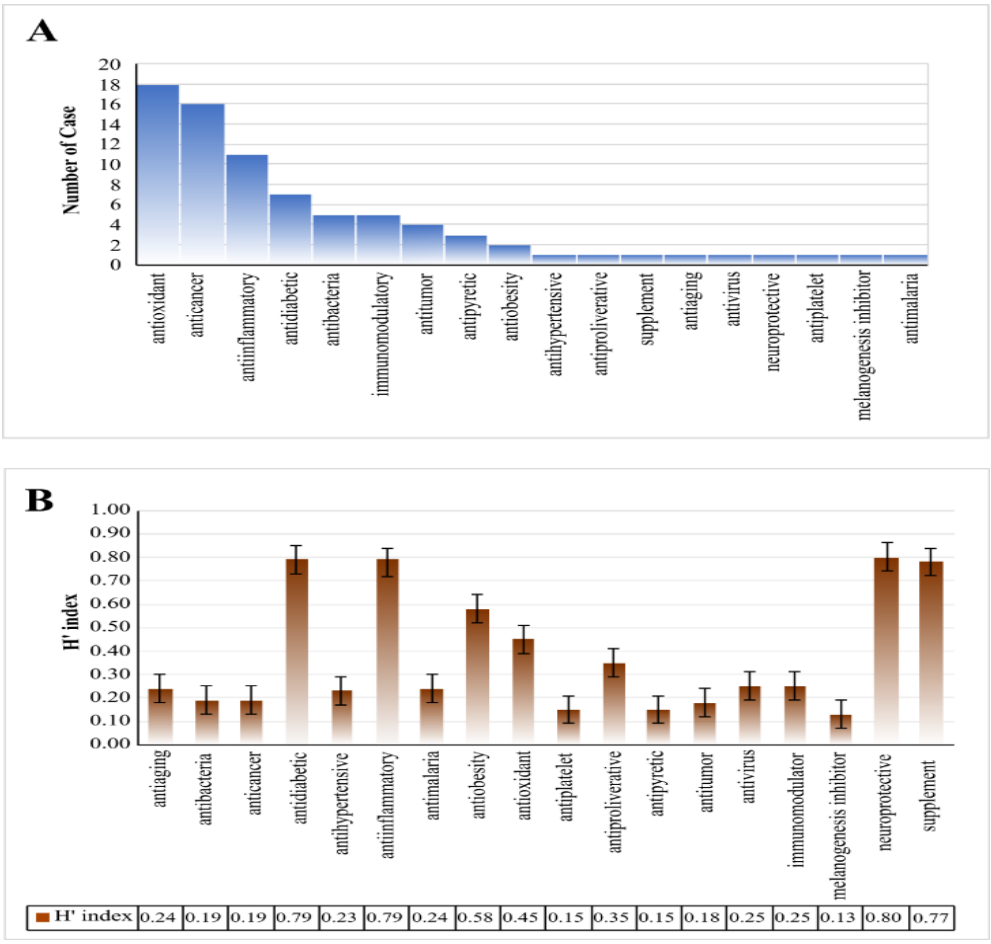

Figure 2. The activity of bioactive compounds found in *Dendrobium*: dominancy (A) and its genetic diversity (B).

Regarding the plant parts used, although the leaf has the highest H index of diversity, i.e., 0.89, the stem is the most widely used with dominance of 90%, followed by the whole part of the plant (Figure 3). In traditional medicine, several parts or plant organs are common, such as roots, stems (barks), leaves, flowers, fruits, or seeds (Srivastava et al., 2014; Khan and Ahmad, 2019). However, as the need for bioactive substances has grown, so has the exploitation of medicinal plants. As a result, alternative technologies for producing metabolites from plants on a big scale without damaging their natural population are urgent (Cragg and Newman, 2013).

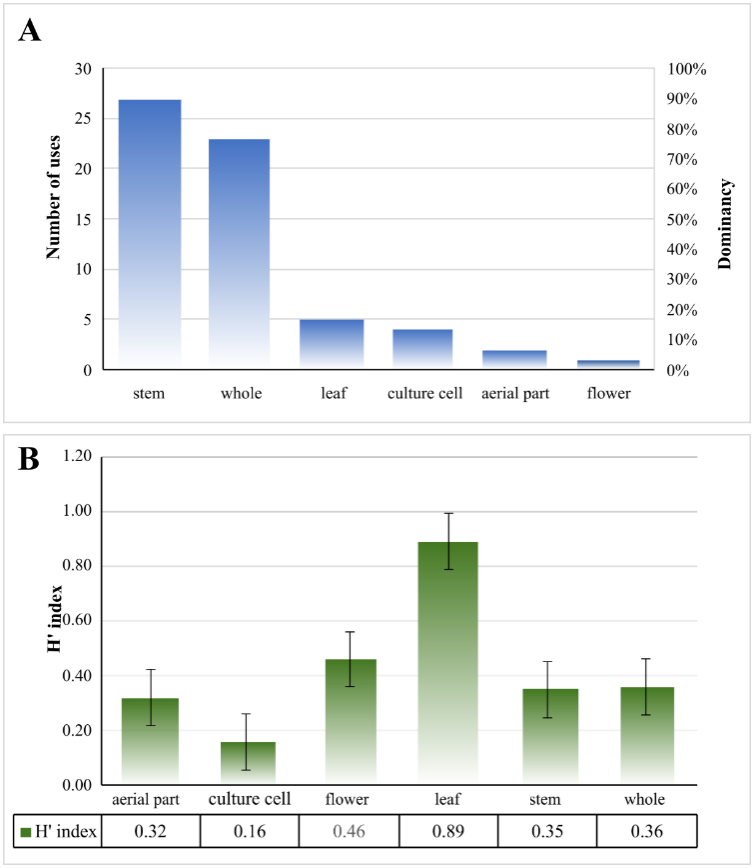

Figure 3. Plant organ and related source of *Dendrobium* which is widely used as a producer of bioactive compounds: dominancy (A) and its genetic diversity (B).

Initially, researchers tried to domesticate or cultivate medicinal plants to support conservation efforts (Phondani et al., 2016). However, due to rapid changes or replacement market demand, the cultivator finds it difficult to choose and decide which plant species to plant (Chen et al., 2016). In addition, most of the medicinal plants come from natural seeds (wild populations) (Hilonga et al., 2019). Generally, these seedlings have low germination rates or specific ecological requirements, making them hard to plant. For example, *Cymbidium* and *Phalaenopsis* are famous orchids that bloom after 2-3 years of vegetative phase (Ahmad et al., 2022).

Another issue is the lack of knowledge about pollination, seed germination, and growth in medicinal plant production (Febjislami et al., 2019). Domestic production of medicinal plants is a potential conservation method that lowers misidentification, genetic and phenotypic diversity, extract variability and instability, hazardous components, and contamination in herbal extracts (Guo et al., 2009; Chen et al., 2016). In this case, biotechnology allows for plant cells, tissues, organs, or entire organisms by cultivating them in vitro to obtain desired substances (Panche et al., 2016; Alamgir, 2018).

Many biotechnological procedures, such as elicitation, embryogenesis, cell line screening, media modification, and organogenesis, have recently been formulated to enhance the secondary metabolites production from medicinal plants (Mohaddab et al., 2022). Cell cultures containing bioactive compounds are collected at a specified period (typically during the stationary phase of their development cycle), dried, extracted, identified, and quantified (Twaij and Hasan, 2022). The core

notion of plant cell suspension is biosynthetic totipotency, in which each cell in the culture preserves the complete genetic information for the range of chemical creation (Fazili et al., 2022).

Then, apart from the essential value of the bioactive and the activities, information on the genetic diversity of *Dendrobium* is also urgent and needed. According to Govindaraj et al. (2015), genetic diversity is necessary to develop a new line population for future evolutionary processes and adaptive environmental changes. Hence, this parameter is critical for future preservation and improvement programs for endangered plants (Lloyd et al., 2016; Mursyidin, 2022). For preservation, assessing genetic diversity has a beneficial impact on increasing the effectiveness and efficiency of that program (Salgotra and Chauhan, 2023).

Similarly, this parameter becomes more valuable in the context of climate change for plant breeding efforts (Govindaraj et al., 2015). In this case, high levels of genetic diversity are beneficial in promoting population survival and adaptive potential guarantee in the face of rapidly changing environmental factors (Teixeira and Huber, 2021). In other words, preserving genetic diversity is urgent in retaining their capability for the current and future crop breeding programs (Swarup et al., 2021).

Besides genetic diversity, analysis of genetic relationships is also a valuable parameter for plant conservation and breeding programs. In this study, the UPGMA analysis showed that *Dendrobium* grouped into seven clusters (Figure 4), where the furthest relationship was presented by *D. moschatum* and *D. catenatum*. Meanwhile, the closest relation was by *D. scabrilingue* with *D. delacourii*, including *D. snowflake* and *D. ovatum* (Figure 5). The PCA shows a different grouping from the UPGMA, where *Dendrobium* was separated into six clusters (Figure 6).

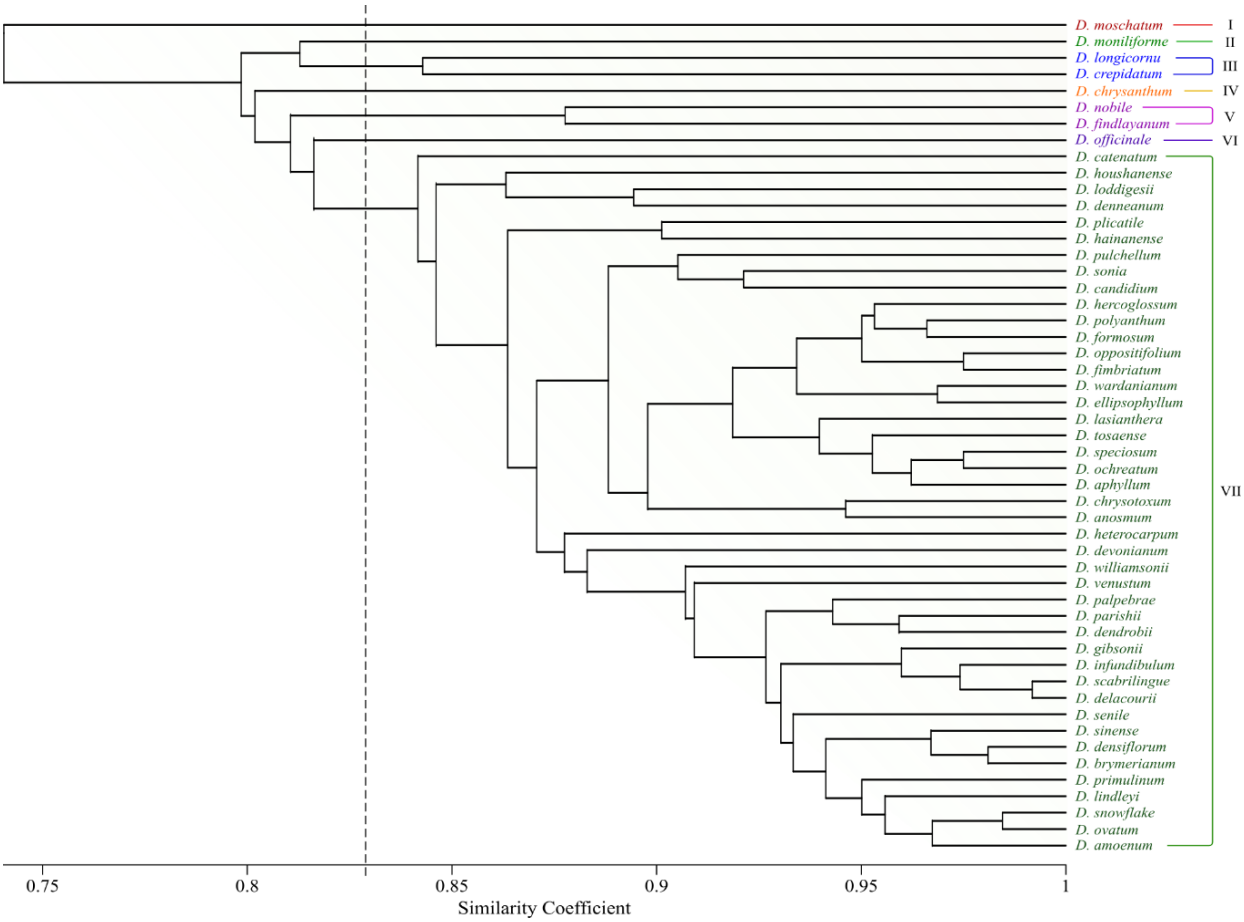

Figure 4. Dendrogram shows the genetic relationship of *Dendrobium* based on bioactive compounds and its bioactivities.

Based on a morphological marker, namely internode number (flowering shoot), *D. moschatum* had a close relationship with *D. secundum, D. chrysanthum*, and *D. aphyllum* (De et al., 2015). *Dendrobium aphyllum* alone had a close relationship with *D. loddigesii* following the ISSR marker

(Wang et al., 2009). Using four chloroplast markers, i.e., *mat*K, *rbc*L, *trn*L intron, and *trn*H-*psb*A, including the nuclear ribosomal DNA or the internal transcribed spacer (ITS), (Xiang et al., 2013) also reported the closed relationship between *D. loddigesii* and *D. aphyllum*. However, by the ITS and the SSR (simple sequence repeat), *D. moschatum* had to be closed with *D. denneanum* and *D. fimbriatum* (Yuan et al., 2009), including *D. heterocarpum* (Zhao et al., 2019). Finally, *D. moschatum* had a close relationship with *D. crumenatum*, *D. anosmum*, *D. macrophyllum*, and *D. spectabile* by chromosome number (2n = 38) (Zheng et al., 2018).

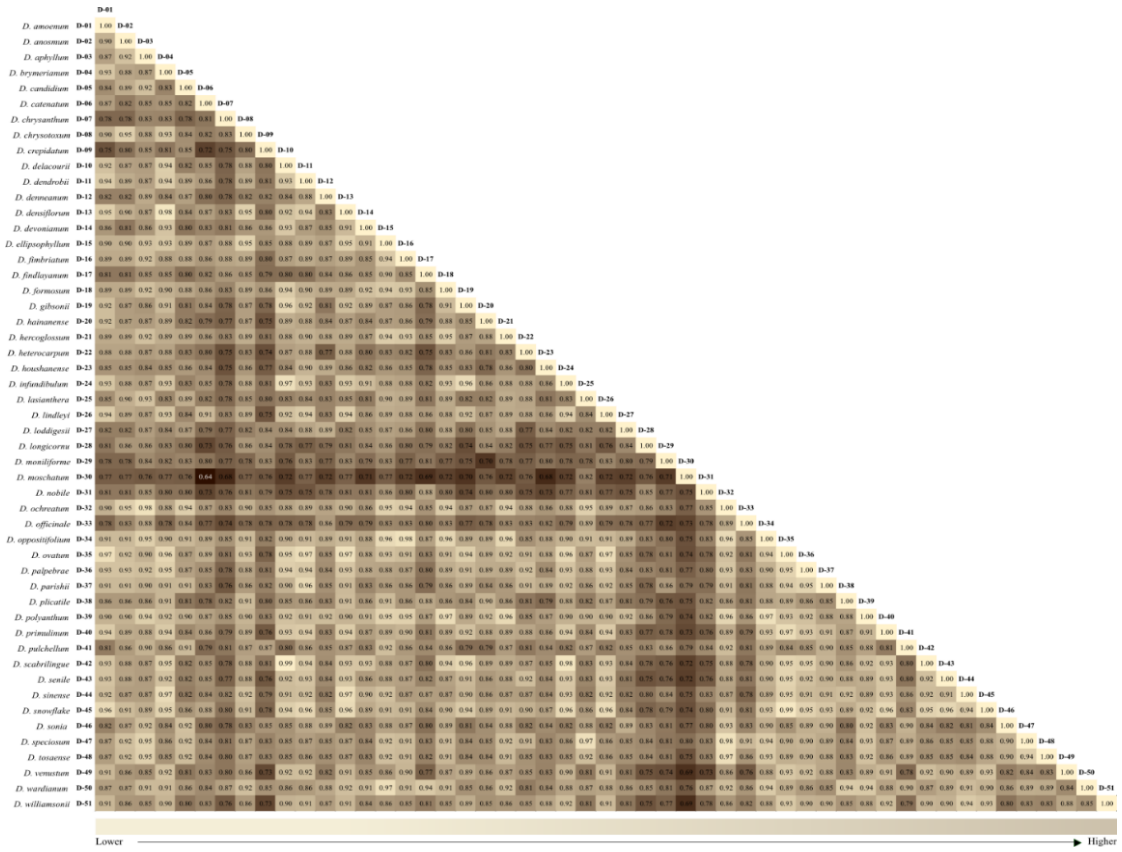

Figure 5. Genetic distance between *Dendrobium* species based on bioactive compounds and their bioactivities, revealed by maximum likelihood (ML).

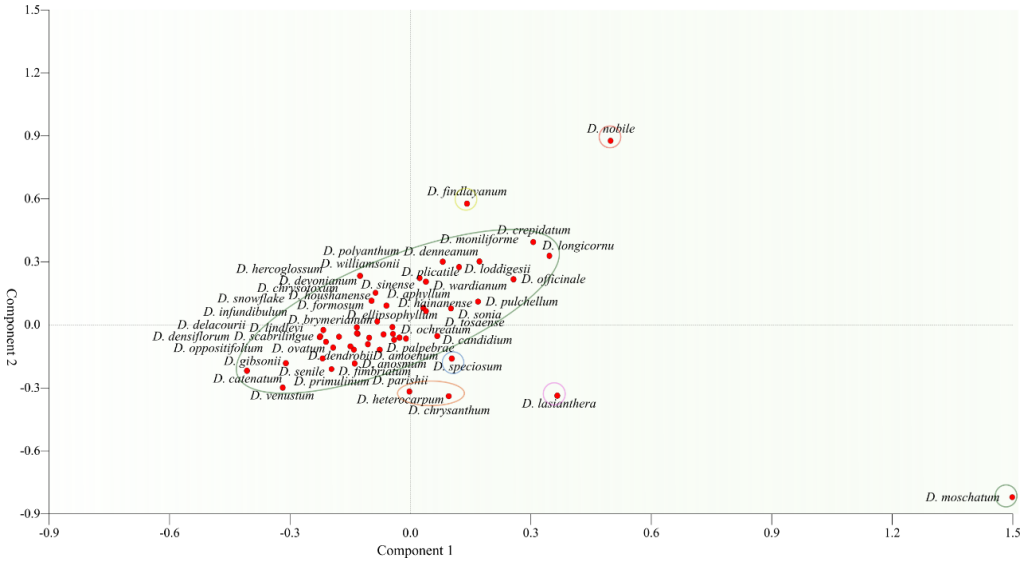

Figure 6. Grouping of *Dendrobium* based on bioactive compounds and their bioactivities, revealed by principle component analysis (PCA).

4. Conclusion

Based on bioactive compounds, their biological activities, and plant organs, *Dendrobium* shows unique genetic diversity and relationships. In this case, the highest genetic diversity was shown by polyphenols (H' index = 0.90) as substances, neuroprotective (H' = 0.80) for activity, and the leaf organ with an H' index of 0.89. The UPGMA analysis showed that *Dendrobium* grouped into seven clusters, where the furthest relationship was presented by *D. moschatum* and *D. catenatum*. Meanwhile, the closest relation was by *D. scabrilingue* with *D. delacourii*, including *D. snowflake* and *D. ovatum*. This information is valuable for future *Dendrobium* conservation and breeding programs.

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Research Article

Effect of Vermicompost Treatment on Oil Quality and Fatty Acid Composition of Peanut (*Arachis hypogaea* L.)

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Abstract: This study examined the impact of vermicompost treatment on the oil quality and fatty acid contents of peanut (*Arachis hypogaea* L.) in 2020-2021 under the ecological conditions of Osmaniye. The research was designed in a randomized complete block design with three replications. Peanut variety NC 7 was used in the study. Vermicompost was applied in nine different doses. In the research oil content, linoleic acid, oleic acid, stearic acid, palmitic acid, arachidic acid, behenic acid, iodine value, and O/L ratio were examined. According to the results, it has been found that oil content varies between 48.38% (T9) and 50.43% (T5). The ratio of oleic acid was recorded between 56.90% (T9) and 59.42% (T5) while the ratio of linoleic acid was between 21.15% (T9) and 23.59% (T8). The lowest palmitic acid value (8.87%) was recorded for the T8 treatment whereas the highest palmitic acid value (9.21%) was obtained from the T6 treatment. The lowest O/L ratio (2.42) was obtained from the T8 treatment while the highest O/L ratio (2.77) was obtained from the T4 treatment. The iodine value varied between 85.56% and 90.28% for T9 and T5 applications, respectively. The findings indicate that under the ecological conditions of Osmaniye, soil and leave treatments of vermicompost show a significant increase in oil content, oleic acid, linoleic acid, and iodine values of peanut.

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1. Introduction

Peanut (*Arachis hypogea* L.) as a member of the legume family is a summer annual plant (Arioglu, 2014). It is regarded as a crucial source of nutrition for both humans and animals thanks to its greater protein, carbohydrate, vitamin, and oil content (Onat et al., 2017; Yasli et al., 2020; Kumar et al., 2021; Sahin et al., 2022). Peanut seeds are an oil plant and contain 44-56% oil and 22-30% protein (Kumari et al., 2022). Its oil is composed of 80% unsaturated fatty acids while the rest is comprised of saturated fatty acids (Barkley et al., 2013).

Peanut cultivation like other leguminous crops has a positive effect on the soil physico-chemical properties. Like other members of the legume family peanut has the ability to fix the free nitrogen of the air to the soil through Rhizobium bacteria. Rhizobium bacteria have a symbiotic relationship with the roots of leguminous plants in the soil (Asik and Arioglu, 2020; Raza et al., 2020). This bacterial species increases the soil's organic nitrogen pool by fixing the freely available nitrogen of the atmosphere into

the soil. It has the ability to fix 20-30 kg da^{-1} of atmospheric N depending on the environmental conditions and plant species (Kadiroglu, 2022).

In Türkiye, the Mediterranean region ranked 1st in peanut production with 85.11% area followed by Southeastern Anatolia Region with 12.64% area. Osmaniye province is a major contributor in the marketing of peanuts (TUIK, 2022). Agronomy operations such as tillage, seed quality, planting density, fertilization, and irrigation play a vital role in obtaining better yield and quality in peanuts (Reddy et al., 2003) However, in order to fulfill the needs of the ever-increasing world population, the yield and quality of the product obtained from the unit area should be increased (Timsina, 2018). To obtain a higher yield per unit area application of high quality fertilizers should be practiced in addition to other cultural operations. Vermicompost is one of the most widely used fertilizers to achieve higher yields (Garg et al., 2010).

Vermicompost has been produced in most European countries and the United States of America for about 40 years and is actively used in agricultural production (Sorathiya et al., 2010). Among 3 000 worm types, the Red Californian worm has an important role in the production of vermicompost fertilizer (Ahmad et al., 2021). In vermicompost production, approximately 250 000 worms in 1 m^3 of raw material continue their activity for three months (Demir, 2010; Welka, 2016). This activity results in the production of a fertilizer highly enriched with plant nutrients. This obtained fertilizer is known as biohumus or vermicompost (Edwards and Bohlen, 1996).

Vermicompost application not only increases the quality and quantity of produce but also improves soil fertility by increasing the beneficial microorganisms present in the soil (Lim et al., 2015). In addition to plant growth and development, it also improves plant resistance against unfavorable environmental conditions (Yadav and Garg, 2015). In general, organic matter content is insufficient in the soils of the world and Turkey Vermicompost helps in the decomposition of organic wastes and returns them to the soil (Alloway, 2009). Vermicompost is vital for the preservation of the environment. Furthermore, the use of vermicompost will provide considerable benefits in organic agriculture (Ramnarain et al., 2019).

This research was carried out in Osmaniye province, the first biggest market of peanut where 90% of its marketing is made (Asik et al., 2018). This search was designed to determine the impact of vermicompost applied directly to the soil as well as to the leaves at various growth stages on oil and fatty acids components of peanut.

2. Material and Methods

This two-year experiment was conducted at the Oil Seed Research Institute (37°07′42”N; 36°11′47”E, 63 m) in 2020 and 2021. The data in the experiment consists of combined years. In this experiment, the most cultivated Virgina market type of peanut in Türkiye i.e., NC 7 variety was cultivated under the treatment of nitroverm liquid type organic vermicompost. Vermicompost has 20% organic matter and 1.5% total nitrogen with a pH of 4.2-6.2. A hundred percent organic vermicompost was obtained by Red California culture worms. Nine treatments were established including the control (Figure 2).

The details of average temperature, precipitation, and average humidity for both years and long years in which the research was practiced are shown in Figure 1. The average temperature during the crop growth period was 20.8 ºC in 2020 and 25.0 ºC in 2021. The total amount of rainfall was 237.3 mm in the first year and 88.0 mm in the second year of the experiment. Mean relative humidity was 66.3% in 2020 and 63.1% in 2021.

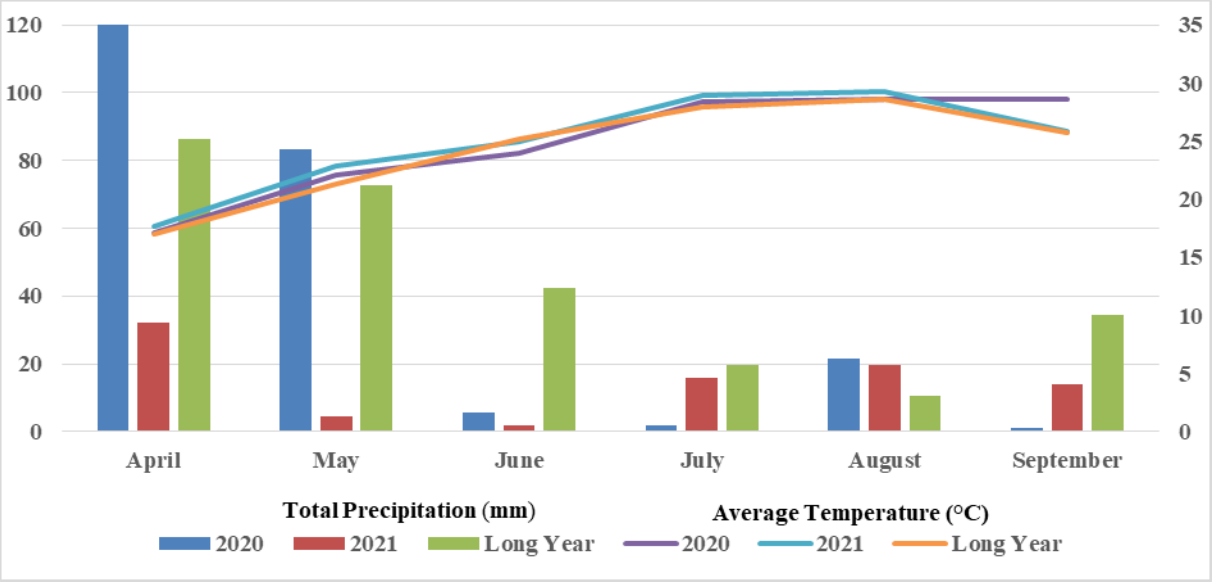

Figure 1. The research field's climate parameters (2020, 2021 and long-year average).

The soil of the experimental field was flat, slightly salty (1.99 dS m^{-1}), extremely calcareous (5.64%), slightly alkaline (pH 8.19), with high water holding capacity (clay), poor in organic matter (1.67%), rich in potassium (59.19 kg da^{-1}) with low phosphorus content (5.21 kg da^{-1}).

The experimental area was deeply plowed with a plow in the fall season. The field was left fallow in the winter season and plowed in the first week of April with a cultivator before sowing. After that, the soil was mixed with a disc harrow and the seedbed was prepared for planting the crop. Before sowing, Diammonium phosphate (18-46) fertilizer at the rate of 25 kg da^{-1} was applied to the soil. A total of 20 kg da^{-1} of urea (46% N) fertilizer was applied in two splits i.e., half before the first irrigation and the other half before the third irrigation. The sprinkler irrigation system was installed and irrigation was applied according to the needs and weather conditions. Each subplot consisted of 2.8 m wide and 5 m long four rows. The area of each subplot was 14 m^2.

The experiment was laid out in a randomized complete block design with three replications. Vermicompost was treated in two different ways (i.e. to the soil and leaves). Soil application was done at once while leave applications were completed in five different splits ((V3 (vegetative stage), R1 (beginning bloom), R2 (beginning peg), R3 (beginning pod), R4 (full pod)) (Figure 2).

Treatment times	Treatments	1. Treatment	2. Treatment	3. Treatment	4. Treatment	5. Treatment	6. Treatment	7. Treatment	8. Treatment	9. Treatment
pre-sowing	Soil treatment (2 L)	X	X	X	X					
V3 stage	Leaf treatment (0.5 L)	X	X	X	X	X	X	X	X	
R1 stage	Leaf treatment (0.5 L)	X	X	X	X	X	X	X	X	
R2 stage	Leaf treatment (0.5 L)		X				X			
R3 stage	Leaf treatment (0.5 L)			X				X		
R4 stage	Leaf treatment (0.5 L)				X				X	

Figure 2. Soil and leave treatments vermicompost time amount and treatments.

The sowing was done manually in the first as well as in the second year on April 25, 2020, and May 2, 2021, respectively. Cultural practices during the crop growth period were carried out properly on time as recommended for the crop. Harvesting of the crop in the 1st and 2nd year was done on September 10, 2020, and September 15, 2021, respectively. All the traits examined in this study were measured by selecting a total of 20 plants representing the middle two rows of each plot from each plot. The oil content of the obtained seeds was measured by Soxhlet. In monounsaturated fatty acids, Oleic acid (C18:1), in polyunsaturated fatty acids, Linoleic acid (C18:2) and Linolenic acid (C18:3) and in Saturated fatty acids; Palmitic acid (C16:0), Stearic acid (C18:0), Arachidic acid (C20:0), Behenic acid (C22:0) and Lignoceric acid (C24:0) were determined as "%" using the GC-FID system (Sahin and İsler, 2022).

Experimental contents were subjected to randomized complete block design analysis of variance using the SPSS 22 application. Duncan's multiple range test was used to combine the means.

3. Results and Discussion

A statistically significant difference was observed between the mean oil content of peanut applied with various vermicompost doses at different growth stages ($p<0.01$) (Table 1).

Vermicompost doses T1 ((pre-sowing (soil treatment 2 l) + V3 (leaf treatment 0.5 l) + R1 (leaf treatment 0.5 l)), T2 ((pre-sowing (soil treatment 2 l) + V3 (leaf treatment 0.5 l) + R1 (leaf treatment 0.5 l) + R2 (leaf treatment 0.5 l), T3 ((pre-sowing (soil treatment 2 l) + V3 (leaf treatment 0.5 l) + R1 (leaf treatment 0.5 l) + R3 (leaf treatment 0.5 l), T4 ((pre-sowing (soil treatment 2 l) + V3 (leaf treatment 0.5 l) + R1 (leaf treatment 0.5 l) + R4 (leaf treatment 0.5 l), T5 ((V3 (leaf treatment 0.5 l) + R1 (leaf treatment 0.5 l)), T6 ((V3 (leaf treatment 0.5 l) + R1 (leaf treatment 0.5 l) + R2 (leaf treatment 0.5 l)), T7 ((V3 (leaf treatment 0.5 l) + R1 (leaf treatment 0.5 l) + R3 (leaf treatment 0.5 l)), T8 ((V3 (leaf treatment 0.5 l) + R1 (leaf treatment 0.5 l) + R4 (leaf treatment 0.5 l)) and T9 (control). The lowest oil content (48.38%) was recorded for control treatment i.e., T9 while the highest oil content (50.43%) was recorded for treatment T5 (Table 2). It was observed that all vermicompost doses increased the oil content compared to the control group (T9). Sahin et al. (2022) in their two-year experiment conducted under Osmaniye conditions determined the average oil content of the NC 7 peanut variety as 49.37%. In this study, it was observed that the treatments of vermicompost caused an increase in the oil content. The 44-56% oil increase in peanuts provides an increase in both the amount of nutrients and the oil content pod yield (Arioglu et al., 2016). Samadzadeh Ghale Joughi et al. (2018), and Feizabadi et al. (2020) found that vermicompost application increased the oil rate in rapeseed; Atteya et al. (2021) *Moringa oleifera* seeds in worm castings improve oil rate and quality; Yururdurmaz (2022) determined that vermicompost applications increased the oil rate in cowpea. Samadzadeh Ghale Joughi et al. (2018), Feizabadi et al. (2020), Atteya et al. (2021) and Yururdurmaz (2022) also obtained results similar to the findings of this study.

Table 1. Results of the analysis of variance for characteristics studied in the experiment

SV	df	OC	OA	LA	PA	SA	BA	AA	LİA	O/L	IV
Block	4	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Year	1	ns	ns	ns	**	ns	ns	ns	ns	ns	ns
Treatment	8	**	**	**	**	**	**	**	**	**	**
Y x T	8	**	ns	ns	ns	**	ns	ns	ns	**	ns

SV: Source of variation, df: degree of freedom, CV: coefficient of variation, OC: Oil content, OA: Oleic acid, LA: linoleic acid, PA: Palmitic acid, SA: Stearic acid, BA: Behenic acid, AA: Arachidic acid, LİA: Lignoceric acid, O/L: Oleic/Linoleic ratio, IV: Iodine value.

Table 2. Average values of oil content, oleic acid, linoleic acid, palmitic acid, stearic acid

Treatments	Oil Content	Oleic acid	Linoleic acid	Palmitic acid	Stearic acid
T1	49.94 ab	58.27 abcd	22.82 b	8.97 bcd	3.65 a
T2	50.11 ab	58.84 abc	22.81 b	8.90 cd	3.66 a
T3	48.94 cd	58.46 abcd	22.03 cd	9.09 abc	3.68 a
T4	50.01 ab	59.15 ab	21.33 e	8.98 bcd	3.60 a
T5	50.43 a	59.42 a	22.62 bc	8.92 cd	3.48 b
T6	50.06 ab	58.78 abc	21.97 d	9.21 a	3.29 d
T7	49.70 abc	57.49 bcd	23.04 ab	9.14 ab	3.42 bc
T8	49.39 bc	57.16 cd	23.59 a	8.87 d	3.35 cd
T9	48.38 d	56.90 d	21.15 e	9.20 a	3.29 d
Average	49.66	58.27	22.37	9.03	3.49
CV (%)	1.0	2.6	2.4	1.8	2.0

CV: Coefficient of variation. Values with different letters in each column mean statistical differences according to Duncan test ($P\leq0.05$).

Different doses of vermicompost applied at various growth stages of peanut had an important effect ($p<0.01$) on mean oleic acid values (Table 1). The ratio of oleic acid (C18:1), one of the monounsaturated fatty acids, and linoleic acid (C18:2), one of the polyunsaturated fatty acids, is close to 80% in peanut (Gursoy, 2019). The control i.e. T9 had the lowest mean oleic acid value (56.90%), while T4 and T5 treatment resulted in the highest mean oleic acid values i.e. 59.15% and 59.42%, respectively (Table 2). The oleic acid value of peanut in the control was found to be lower than all vermicompost doses. An average of 50% increase in oleic acid content of peanut varieties has been recorded however, this amount is found to be 75-80% and above in high oleic varieties (Hassan et al., 2005). Yilmaz (2022) observed that the oleic acid content increased up to 59.42% in the NC 7 variety with the treatment of vermicompost. It has been observed that the vermicompost treatment to peanut through the soil and leaves increased the oleic acid content. Joris and Mensink (2016) reported that consuming peanuts enriched with oleic acid reduces the incidence of cardiovascular diseases. According to Razzaghifard et al. (2017) found that vermicompost applications increased the rate of oleic acid in *Cucurbita pepo* L.; Samadzadeh Ghale Joughi et al (2018), and Feizabadi et al. (2020) found that vermicompost application increased the rate of oleic acid in rapeseed; Atteya et al. (2021). *Moringa oleifera* seeds oleic acid ratio in vermicompost was purified; Yururdurmaz (2022) found that vermicompost applications increased the rate of oleic acid in cowpea; Sánchez Roque et al. (2022) found that vermicompost applications increased the oleic acid ratio in peanuts. In our trial findings, the results were similar to the findings of other researchers.

Vermicompost applied to peanut in different doses at various growth stages was a statistically significant ($p < 0.01$) effect on linoleic acid values (Table 1). The lowest mean value (21.15%) of linoleic acid was obtained from the control application i.e. T9. The highest linoleic acid value (23.59%) was obtained from the T8 treatment (Table 2). It was determined that all vermicompost doses had higher linoleic acid content compared to the control (T9). Vermicompost treatments led to a simultaneous increase in both linoleic and oleic acid content. The increase in the two unsaturated fatty acids i.e., oleic acid and linoleic acid in peanuts is gaining importance in terms of health and nutrition (Arioglu, 2014). Razzaghifard et al. (2017) found that vermicompost applications increased the rate of linoleic acid in *Cucurbita pepo* L.; Samadzadeh Ghale Joughi et al (2018), and Feizabadi et al. (2020) found that vermicompost application increased the rate of linoleic acid in rapeseed; Atteya et al. (2021). *Moringa oleifera* seeds linoleic acid ratio in vermicompost was purified; Yururdurmaz (2022) found that vermicompost applications increased the linoleic ratio in cowpea; Sánchez-Roque et al. (2022) determined that vermicompost applications increased the rate of linoleic acid in peanuts. This study showed an increase in both oleic acid and linoleic acid values of vermicompost applied to peanut.

Saturated fatty acids in peanuts comprised of palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0) and lignoceric acid (C24:0). Vermicompost applications in different doses at various growth stages were found to have significant effect ($p<0.01$) on palmitic acid values in peanuts (Table 1). The minimum average palmitic acid value (8.87%) was recorded for the T8 treatment while maximum average palmitic acid values i.e. 9.21% and 9.20% were recorded for T6 and T9 treatments, respectively (Table 2). Hassan et al., (2005) reported that the low level of saturated fatty acids in peanuts is important in terms of nutrition, and also the palmitic acid value varies between 9.95-10.79%. Arioglu et al. (2020) determined that the palmitic acid value varies between 9.15-10.19%. In this study, it was seen that vermicompost treatment decreased the palmitic acid value and also the palmitic acid ratio was lower than other studies. Razzaghifard et al. (2017) found that vermicompost applications increased the palmitic acid ratio in *Cucurbita pepo* L.; Samadzadeh Ghale Joughi et al (2018), and Feizabadi et al. (2020) found that vermicompost application increased the palmitic acid ratio in rapeseed; Atteya et al. (2021). *Moringa oleifera* seeds palmitic acid ratio in vermicompost was purified; Yururdurmaz (2022) found that vermicompost applications increased the palmitic acid ratio in cowpea; Sánchez-Roque et al. (2022) vermicompost applications were found to reduce the palmitic acid ratio in peanuts. While it was observed that the palmitic acid ratio of vermicompost increased in other plants, it was determined that the palmitic acid ratio in peanut decreased.

Vermicompost treatments in different doses at different growth stages of peanut were found to have a significant effect ($p<0.01$) on stearic acid values in peanuts (Table 1). The control group i.e. T9 showed the lowest average value (3.29%) for stearic acid, while T3 treatment resulted in the highest value (3.68%) (Table 2). Gulluoglu et al. (2017) found that the value of stearic acid varies between 2.28-

4.16%. Asik et al. (2018) determined that the stearic acid value varies between 2.39-4.19% and a study conducted by Yol and Uzun (2018), showed the stearic acid value between 2.40-4.90%. Razzaghifard et al. (2017) found that vermicompost applications increased the stearic acid ratio in *Cucurbita pepo* L.; Yururdurmaz (2022) determined that vermicompost applications increased the rate of stearic acid in cowpea. The stearic acid value recorded in this study was found to be similar to Razzaghifard et al. (2017) and Yururdurmaz (2022).

Table 3. Average values of behenic acid, arachidic acid, lignoceric acid, O/L ratio, Iodine value

Treatments	Behenic acid	Arachidic acid	Lignoceric acid	O/L ratio	Iodine value
T1	2.69 c	1.67 b	1.16 cd	2.55 cd	89.66 ab
T2	2.76 ab	1.72 a	1.17 c	2.58 bcd	90.12 ab
T3	2.73 abc	1.72 a	1.18 bc	2.66 abc	88.41 d
T4	2.59 d	1.63 c	1.22 ab	2.77 a	87.82 d
T5	2.62 d	1.65 bc	1.15 cd	2.62 bc	90.28 a
T6	2.71 bc	1.59 d	1.25 a	2.67 ab	88.62 cd
T7	2.78 a	1.63 c	1.21 ab	2.49 de	89.35 bc
T8	2.79 a	1.63 c	1.22 ab	2.42 e	90.19 ab
T9	2.69 c	1.57 d	1.13 d	2.69 ab	85.56 e
Average	2.71	1.65	1.19	2.61	88.89
CV (%)	1.8	1.2	2.5	3.8	1.6

CV: Coefficient of variation. Values with different letters in each column mean statistical differences according to Duncan test ($P \leq 0.05$).

The behenic acid ratio in peanut was found to be significantly affected by treatments of vermicompost at various stages in different doses ($p<0.01$) (Table 1). The lowest readings came from T4 (2.59%) and T5 (2.62%) treatments, while the highest values were obtained from T7 (2.78%) and T8 (2.79%) treatments (Table 3). Gulluoglu et al. (2017) stated that the percentage of behenic acid varies between 2.11-3.25%; Asik et al. (2018) determined that it varies between 2.26-3.29% whereas Yol and Uzun (2018) documented that behenic acid value varies between 2.56-2.85%. Yururdurmaz (2022) found that vermicompost applications increased the behenic acid ratio in cowpea; Sánchez-Roque et al. (2022) determined that vermicompost applications increased the ratio of behenic acid in peanuts. The behenic ratio found in this study shows similarity with the values found in Yururdurmaz (2022) and Sánchez-Roque et al. (2022).

Different doses of vermicompost treatment at various growth stages significantly ($p<0.01$) affected the arachidic acid ratio in peanut (Table 1). The lowest value was derived from the control (T9) while the highest ratio was obtained from the T2 (1.72%) and T3 (1.72) treatments in the same group (Table 3). The arachidic acid value in the control group was lower than other treatments. Uckun et al. (2019) found that the arachidic acid ratio varies between 1.21-1.74% while Ergun and Zarifikhosroshahi (2020) found that the ratio of arachidic acid varies between 1.43% and 1.62%. Sánchez-Roque et al. (2022) stated that vermicompost applications increased the arachidic acid ratio in peanuts and the amount of vermicompost was important in increasing the arachidic acid ratio.

Lignoceric acid value, which is one of the saturated fatty acids; was significantly affected by vermicompost treatment at various stages in different doses ($p<0.01$) (Table 1). The lowest lignoceric (1.13%) was obtained from the T9 i.e., control while the T6 treatment resulted in the highest (1.25%) lignoceric value. All vermicompost treatments were found higher in lignoceric acid value than the control (T9) group (Table 3).

Vermicompost treatment in different amounts at different growth phases significantly affected the O/L ratio ($p < 0.01$) (Table 1). O/L ratio varied between 2.42 (T8) and 2.77 (T4) (Table 3). In addition, a high oleic/linoleic acid ratio means increased oxidative stability of oils and reduced trans fatty acid formation during the process (Mondal et al., 2018). Vermicompost treatment through both soil and leaves increases the O/L ratio value. Gali et al. (2021) recorded the O/L ratio value between 1.20 and 27.52 while Lopez et al. (2001) reported that the O/L ratio value varies between 0.8 and 2.5. Gali et al. (2021) reported less while Lopez et al. (2001) found a higher value for O/L ratio value. Yururdurmaz (2022) stated that vermicompost applications improve the O/L ratio in cowpea and the ratio of

vermicompost is important in the O/L ratio. In this experiment, the O/L ratio findings in the trial were found to be similar to those of the Yururdurmaz (2022) trial.

Vermicompost treatments in different doses and stages were found to have a significant effect on iodine values ($p<0.01$) (Table 1). The lowest iodine value (85.56) was recorded for control i.e., T9 while T5 treatment resulted in the highest iodine value (90.28) (Table 3). Yilmaz (2022) reported that iodine value varies between 85.99 and 88.28. Yururdurmaz (2022) stated that vermicompost applications improve the iodine value in cowpea and the ratio of vermicompost is important in iodine value. In this study, according to adaptation-like studies, it was determined that vermicompost treatment increases the iodine value.

Conclusion

Vermicompost availability at ease increases production efficiency. Production area also increases with the easy access of vermicompost. An increase was recorded in the Oil content, Oleic acid (C18:1, monounsaturated fatty acid) and Linoleic acid (C18:2, polyunsaturated fatty acids) when the effect of vermicompost treatments on peanut oil quality and fatty acid compositions were examined under Osmaniye conditions. However, an increase in the iodine value, which is important in terms of nutrition was also recorded. According to the results, it can be concluded that the treatment of vermicompost to peanut would be appropriate in order to increase the amount of oil and unsaturated fatty acids.

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Research Article

Morphological Characterization and Selection in Some Summer Squash (*Cucurbita pepo* L.) Genotypes

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Abstract: This study was carried out to determine stage progression and specific plant and fruit characteristics in purposefully created summer squash genotypes. In this study, which was carried out in 2019, 59 squash genotypes were assessed with 24 morphological measurements and observations involving plant and fruit characteristics, and one-generation advancement was achieved. Cluster and PCA were implemented to determine the relationship between genotypes determined at the end of single plant selection. A dendrogram was constructed to assess the morphological similarities between the genotypes. In this respect, four main groups and 12 subgroups were determined in the cluster analysis performed among squash genotypes based on 24 morphological variables, while the 10 PC axis explained 94.3% of the total variation in the PCA. When the variance values of the principal component axis were examined, it was seen that the first principal component axis explained 28.8% of the total variation, the second principal component axis explained 26% of the variation, and the third principal component axis explained 11.2% of the total variation. Principal component analyses revealed that (i) 66% of the qualitative (neck in unripe fruit, curving of the neck in unripe fruit, mottling in unripe fruit, and type of mottling in unripe fruit) variation was explained by the first three components. At the end of the study, the phenotypic diversity that exists in this core collection provides valuable information to improve agronomic traits in the summer squash breeding program.

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1. Introduction

Cucurbita pepo L., which belongs to the *Cucurbitaceae* family, is an important species with a high economic value. The total global production of summer squash is 27 962 481 tons (FAO,2022). In Türkiye, 609 622 tons of summer squash are produced, according to 2021 data (TÜİK, 2022). Summer squash can be grown throughout the world and in our country with no problems due to the suitability of

ecological conditions, its economic value, and its place in the diet. Summer squash is widely grown for different purposes almost in all regions of Türkiye, especially in the coastal areas of the Mediterranean and Aegean regions (Vural et al., 2000). However, productivity and quality in squash farming require good cultivation techniques as well as a high genetic performance. For this purpose, high productivity and quality are crucial in commercial varieties, in addition to resistance to various diseases and pests. Today, hybrid varieties are used almost exclusively in commercially cultivated summer squash in Türkiye (Kesici et al., 2004; Nacar et al., 2011).

F1 hybrid variety breeding is one of today's most commonly practiced breeding methods. The first step of hybrid squash breeding is obtaining well-defined inbred lines and identifying their characteristics. Accordingly, the formation of a seed collection rich in genetic variation at the beginning of breeding, the knowledge of morphological variations within the gene pool, and the distribution of this variation provide significant opportunities to the breeder. Numerous researchers in our country and around the world performed various characterizations on *Cucurbitaceae* family and *C. pepo* species according to the fruit and plant characteristics, and the existing morphological variations were identified in detail (Paris, 2001; Sarı et al., 2008; Méndez-López et al., 2010; Seymen, 2010; Nacar et al., 2011; Nacar, 2014; Türkmen et al., 2016; Aslan et al., 2019; Çetin et al., 2019; Seymen, 2020; Kayak and Türkmen, 2021; Kumar et al., 2022).

Multivariate analysis techniques are used to evaluate the data obtained from qualified gene pools established in breeding programs. Morphological characteristics must be evaluated from multiple aspects to detect variability observed in specific characteristics (Tan, 2005; Alkan, 2011). The data obtained at the end of the characterization studies are used in cluster analysis and principal component analysis, which easily shows similarities and groupings of genotypes (Karaağaç and Balkaya, 2010; Karaağaç, 2013). This study aimed to identify the morphological characteristics of the genetic sources of squash according to the criteria of the UPOV (TG/119/3). Convention, determine the current variation level within the population and explain in detail from which characteristics the factors creating the variation emerge. Another aim was to create a good gene pool by including the selected genotypes in the prospective breeding studies.

2. Material and Methods

The study was carried out in the R&D greenhouse of Beta Tohumculuk company. The plant material consisted of 59 summer squash genotypes selected from the previous year's breeding studies and left for open pollination. During the choosing, the genetic expansion (homogeneous/heterogeneity) within the gene collection was taken into consideration. For the trial, two to three seeds per seedbed were planted directly in the soil on 25 October 2019 with a row spacing and intra-row spacing of 90 × 60 cm in a way that five squash seedlings would be obtained from each squash genotype. When seedlings with two or three actual leaves emerged for genotypes in which seed emergence occurred, the number of plants in each seedbed was reduced to one. Irrigation and fertilization were performed with drip irrigation. Fertilization containing magnesium sulphate, MAP (mono ammonium phosphate), humic acid, and microelements were applied until the fruit formation period. A potassium-based fertilization program was applied to ensure nitrogen-phosphorus-potassium balance after the fruit was formed and towards fruit ripening. The designated morphological parameters were evaluated in each plant with seed emergence, and self-pollination was performed to obtain an advanced stage of breeding in the existing genotypes for the selection breeding of squash (Upov, Document No: TG/119/3). Measurements and observations were performed in squash genotypes. The measurements and observations involve growth habit, branching, the degree of branching, the attitude of petioles (excluding lower external leaves), green color on the stem, tendrils on the stem, incisions on leaf blade, the green color of the upper surface of leaf blade, leaf blade marbling, leaf blade surface area, petiole length, petiole thickness, the shape of the cross-section of petioles, and prickliness of petioles. The observations regarding fruits are related to the neck in unripe fruit, curving of the neck in unripe fruit, the number of major colors in unripe fruit, major colors in unripe fruit, the intensity of major colors in unripe fruit, glossiness in unripe fruit, mottling in unripe fruit, the type of mottling in unripe fruit, the general shape of the fruit, and major color in fully-developed fruit (UPOV, 2006).

In recent years, it has been possible to determine the level of variation existing in populations through taxonomic classification methods called multivariate analyses (Karaağaç, 2006; Balkaya et al.,

2010; Karaağaç and Balkaya, 2010; Kobal Bekar et al., 2019). The study used Minitab (16.00) statistical software for cluster and principal component analysis (PCA), which shows the similarities and differences between squash genotypes (MINITAB, 2016).

3. Results and Discussion

Table 1 shows the plant observations investigated in the study, and Table 2 presents fruit observations. All the plants in the study were observed to be semi-creeping regarding growth habit and showed branching. Their degree of branching was identified as weak in 38 genotypes, medium in 14 genotypes, and strong in seven genotypes. In terms of tendril development on the stem, 39 genotypes had no tendrils or underdeveloped tendrils, whereas 20 genotypes had well-developed tendrils. The stem color was light and dark green in only two plants, while 27 genotypes had only green, and 30 plants had only dark green color.

According to the observations about leaves, the attitude of petioles was identified as erect in three genotypes, semi-erect in 35 genotypes, and prostrate in 21 genotypes. The petiole length was identified as short in 10 genotypes, long in 19 genotypes, and medium in 30 genotypes. The petiole thickness was detected as thin in 24 genotypes, thick in four genotypes, and medium in 31 genotypes. Regarding prickliness in petioles, 16 genotypes showed a medium level, while there were a few prickles in 43 genotypes. The shape of the cross-section of petioles was identified as circular in all the genotypes. According to the observations of leaf blades, marbling in leaf blades was observed in none of the genotypes. Incisions on leaf blade were found in 30 genotypes to be minimum/very slight, 23 genotypes as slight, and six genotypes as medium. The surface areas of leaf blades were identified as small (three genotypes), medium (17 genotypes), large (26 genotypes), and very large (13 genotypes). Finally, the green color of the upper surface of the leaf blade was identified as light in only five genotypes, whereas 33 genotypes had medium, and 21 genotypes had dark green color.

According to the observations about the neck in unripe fruit, six genotypes did not have a neck in the fruit, while the neck existed in 53 genotypes. Curving was detected in the neck in 17 genotypes, but 42 did not have to curve. The number of major colors in unripe fruit was identified as one in all the genotypes, and the major color in unripe fruit was identified as yellow in only one genotype and green in the rest. When the intensity of major colors and the glossiness in unripe fruit were investigated, the color intensity was identified as light in 43 genotypes, medium in 10 genotypes, and dark in six genotypes. On the other hand, the glossiness was slight in 46 genotypes and medium in 13 genotypes. Significant differences were found in the study in terms of the general shape of the fruit, mottling, and major color in fully-developed fruit. When the parameters of the general shape of the fruit were studied, 10 genotypes were ovate, seven genotypes were cylindrical, one genotype was pyriform, and 41 genotypes were elliptic. As for mottling in fruit, mottling was not detected in the fruit of 50 genotypes, whereas mottling existed in nine genotypes. Among these nine fruits, the type of mottling was diffused in five genotypes and diffused in linear bands in four genotypes. Finally, when the major color in the fully-grown fruit was investigated, eight genotypes were identified as cream, one genotype as orange, 10 as green, and 40 as yellow. According to the study results, all the parameters except growth habit, branching, the shape of the cross-section in the petiole, marbling in the leaf blade, and the number of major colors in unripe fruit were effective in determining the level of variation. In this study, which aimed to create a good gene pool by including the selected genotypes in the prospective breeding studies, it is expected that the mean of the population will increase with respect to specific characteristics when selection is implemented in a population. The targeted outcomes were mostly achieved in the characteristics investigated with regard to the wide variation we obtained through selection, which significantly supports the literature (Özbakır et al., 2011; Nacar, 2014; Çetin et al., 2019; Kayak and Türkmen, 2021).

Table 1. Observations about the plant investigated in the study

Observations	G.H	G.B	D.B	T.S	G.C.S	A.P	P.L	P.T	P.P	S.C.S.P	L.B.M	I.L.B	L.B.S.A	G.C.U.L.B
1	2	9	3	1	1	5	5	5	3	1	1	3	5	7
2	2	9	3	1	3	7	5	3	3	1	1	3	3	7
3	2	9	3	1	2	7	5	5	3	1	1	1	7	5
4	2	9	3	1	2	5	5	5	3	1	1	3	5	5
5	2	9	3	1	2	7	5	3	3	1	1	1	5	3
6	2	9	3	1	1	5	3	3	5	1	1	1	7	7
7	2	9	3	1	1	5	5	5	3	1	1	1	7	7
8	2	9	3	1	2	7	5	5	3	1	1	3	7	7
9	2	9	5	2	2	5	5	5	3	1	1	3	5	7
10	2	9	3	1	1	5	3	3	3	1	1	3	7	5
11	2	9	3	1	1	5	3	5	3	1	1	3	7	7
12	2	9	3	1	1	5	7	5	5	1	1	1	7	5
13	2	9	5	2	2	5	5	7	3	1	1	1	9	5
14	2	9	3	1	1	7	5	3	3	1	1	1	5	5
15	2	9	5	2	1	5	5	5	3	1	1	1	7	7
16	2	9	3	1	2	3	5	5	3	1	1	5	7	5
17	2	9	5	2	1	5	7	5	3	1	1	3	7	5
18	2	9	3	1	2	5	5	3	3	1	1	1	7	7
19	2	9	5	2	2	5	3	5	3	1	1	1	5	5
20	2	9	3	1	1	5	5	3	5	1	1	3	5	5
21	2	9	3	1	2	7	5	5	3	1	1	3	7	5
22	2	9	5	2	1	7	7	3	3	1	1	3	5	7
23	2	9	3	1	2	5	7	5	3	1	1	5	7	5
24	2	9	3	1	1	5	7	3	3	1	1	1	5	5
25	2	9	3	1	2	5	5	3	5	1	1	5	7	5
26	2	9	5	2	1	7	5	5	3	1	1	3	5	5
27	2	9	5	2	2	5	5	5	5	1	1	1	7	5
28	2	9	3	1	2	5	7	3	3	1	1	7	7	7
29	2	9	3	1	2	3	5	3	3	1	1	1	7	5
30	2	9	7	2	1	5	7	5	5	1	1	5	9	7
31	2	9	3	1	2	5	3	3	5	1	1	1	3	3
32	2	9	3	1	2	5	7	5	3	1	1	1	7	5
33	2	9	3	1	1	7	7	3	5	1	1	1	5	5
34	2	9	7	2	2	5	7	5	5	1	1	3	7	7
35	2	9	1	1	2	5	3	3	3	1	1	5	5	3
36	2	9	3	1	1	7	5	5	3	1	1	1	7	7
37	2	9	3	1	2	7	7	3	5	1	1	3	5	5
38	2	9	1	1	2	3	3	5	3	1	1	1	5	5
39	2	9	3	1	1	7	5	3	3	1	1	3	5	5
40	2	9	3	1	1	5	5	3	5	1	1	3	7	5
41	2	9	3	1	3	7	7	7	3	1	1	5	9	5
42	2	9	5	2	1	5	7	5	3	1	1	1	9	3
43	2	9	5	2	2	7	7	7	5	1	1	3	9	5
44	2	9	3	1	2	7	7	3	3	1	1	1	5	5
45	2	9	3	1	2	5	5	3	3	1	1	1	9	5
46	2	9	7	2	1	5	5	5	3	1	1	3	9	5
47	2	9	3	1	1	7	5	7	3	1	1	3	9	5
48	2	9	7	2	1	5	7	5	5	1	1	1	9	7
49	2	9	5	2	1	7	5	5	5	1	1	3	9	5
50	2	9	7	2	2	5	3	5	3	1	1	1	7	7
51	2	9	3	1	2	5	3	5	3	1	1	1	7	5
52	2	9	5	2	1	7	7	3	3	1	1	3	7	7
53	2	9	3	1	1	7	3	3	3	1	1	1	3	7
54	2	9	3	1	1	5	7	3	5	1	1	3	9	5
55	2	9	7	2	2	5	5	5	3	1	1	1	9	7
56	2	9	7	2	1	7	5	5	3	1	1	3	9	7
57	2	9	3	1	2	5	5	5	3	1	1	1	7	5
58	2	9	5	2	2	7	5	5	3	1	1	1	7	7
59	2	9	5	2	3	5	7	3	5	1	1	1	5	3

Explanation: G.H: Growth habit (bush(1), semi-trailing(2), trailing(3)), G.B: Growth branching (absent(1), present(9)), D.B: Degree of branching (very weak (1), medium(5), strong(7)), T.S: Tendrils on the stem (absent to rudimentary(1), well developed(2)), G.C.S: Green color on the stem (light(1), dark(2), light and dark(3)), A.P: Attitude of petioles (erect(3), semi erect(5), horizontal(7)), P.L: Petiole length (short(3), medium(5), long(7)), P.T: Petiole thickness (thin(3), medium(5), thick(7)), P.P: Prickliness of petioles (very few(3), medium(5), many(7)), S.C.S.P: Shape of the cross-section of petioles (circular(1), triangular(2)), L.B.M: Leaf blade marbling (absent(1), present(9)), I.L.B: Incisions on leaf blade (wery weak(1), weak(3), medium(5), strong(7), very strong(9)), L.B.S.A: Leaf blade surface area (very small(1), small(3), medium(5), large(7), very large(9)) and G.C.U.L.B: Green color of the upper surface of leaf blade (light(3), medium(5), dark(7)).

Table 2. Characteristics of the fruit investigated in the study

Observations	N.U.F	C.N.U.F	N.M.C.U.F	M.C.U.F	I.M.C.U.F	G.U.F	G.S.F	M.U.F	T.M.U.F	M.C.D.F
1	1	1	1	4	3	5	5	1	0	3
2	1	1	1	4	5	3	5	9	5	5
3	9	9	1	4	3	3	7	1	0	3
4	9	1	1	4	5	3	5	9	7	5
5	9	1	1	4	5	3	5	9	7	5
6	9	9	1	2	3	3	5	1	0	3
7	9	9	1	4	3	3	5	1	0	3
8	9	9	1	4	3	3	7	1	0	3
9	9	1	1	4	5	5	5	1	0	3
10	9	1	1	4	3	3	5	1	0	3
11	9	1	1	4	5	3	7	1	0	2
12	9	1	1	4	3	3	5	1	0	3
13	9	1	1	4	7	5	5	9	5	5
14	9	1	1	4	3	3	5	1	0	3
15	9	1	1	4	3	3	2	1	0	2
16	9	9	1	4	7	5	5	1	0	5
17	9	1	1	4	3	3	2	1	0	3
18	9	9	1	4	5	5	5	9	5	3
19	9	1	1	4	3	3	5	1	0	3
20	9	1	1	4	3	3	5	1	0	3
21	9	9	1	4	7	5	7	1	0	5
22	9	1	1	4	3	3	5	1	0	3
23	9	1	1	4	7	3	5	9	5	5
24	9	1	1	4	3	3	9	1	0	3
25	9	1	1	4	3	3	2	1	0	3
26	9	1	1	4	3	3	5	1	0	3
27	9	9	1	4	3	3	5	1	0	3
28	9	1	1	4	3	5	5	1	0	2
29	1	1	1	4	3	3	2	1	0	3
30	9	1	1	4	3	3	2	1	0	3
31	9	1	1	4	3	3	5	1	0	2
32	9	1	1	4	3	5	2	1	0	3
33	1	1	1	4	3	3	5	1	0	2
34	9	1	1	4	3	3	2	1	0	3
35	9	1	1	4	3	3	5	1	0	2
36	9	1	1	4	5	3	5	1	0	3
37	9	1	1	4	3	5	5	1	0	4
38	9	9	1	4	3	3	5	1	0	2
39	9	1	1	4	3	3	5	1	0	3
40	9	1	1	4	3	3	5	1	0	3
41	9	1	1	4	7	5	5	1	0	5
42	9	1	1	4	3	3	2	1	0	3
43	9	1	1	4	3	3	5	1	0	3
44	9	1	1	4	3	3	5	1	0	3
45	9	9	1	4	3	3	5	1	0	3
46	9	9	1	4	3	3	7	1	0	3
47	9	9	1	4	3	3	5	1	0	3
48	9	9	1	4	3	3	7	1	0	3
49	1	1	1	4	3	3	5	1	0	3
50	9	1	1	4	3	3	5	1	0	3
51	9	9	1	4	7	5	2	9	5	5
52	1	1	1	4	3	3	5	1	0	3
53	9	9	1	4	3	3	5	1	0	2
54	9	1	1	4	3	3	7	1	0	3
55	9	1	1	4	5	5	5	1	0	3
56	9	1	1	4	3	5	2	1	0	3
57	9	1	1	4	5	3	5	9	7	3
58	9	9	1	4	5	3	5	9	7	5
59	9	9	1	4	3	3	5	1	0	3

Explanation: N.U.F: Neck in unripe fruit(absent(1), present(9)), C.N.U.F: Curving of the neck in unripe fruit (absent(1), present(9)), N.M.C.U.F: Number of major colors in unripe fruit (one(1), two(2), three(3)), M.C.U.F: Major colors in unripe fruit (whitish(1), yellow(2), orange(3), green(4), partly white and partly yellow(5), partly green and partly yellow(6)), I.M.C.U.F: Intensity of major colors in unripe fruit (light(3), medium(5), dark(7)), G.U.F: Glossiness in unripe fruit (light(3), medium(5), strong(7)), G.S.F: General shape of the fruit (scallop-shaped(1), ovate(2), globular(3), elliptical(5), cylindrical(7), club shaped(8), pear shaped(9)), M.U.F: Mottling in unripe fruit (absent(1), present(9)), T.M.U.F: Type of mottling in unripe fruit (sparse(3), sparse pieced(5), sparse bands(7), sparse pieced and bands(9)), and M.C.D.F: Major color in fully-developed fruit (whitish(1), cream(2), yellow(3), orange(4), green(5)).

Cluster and principal component analyses (PCA) were conducted to determine the relationships between the populations and obtain information about the usefulness of the relevant plant and fruit

characteristics in identifying the groups. According to the cluster analysis, the genotypes were grouped under four main groups and 12 subgroups. There were five genotypes in Group 1, seven genotypes in Group 2, six genotypes in Group 3, five genotypes in Group 4, five genotypes in Group 5, nine genotypes in Group 6, six genotypes in Group 7, three genotypes in Group 8, six genotypes in Group 9, 10 genotypes in Group 10, two genotypes in Group 11, and two genotypes in Group 12 (Figure 1).

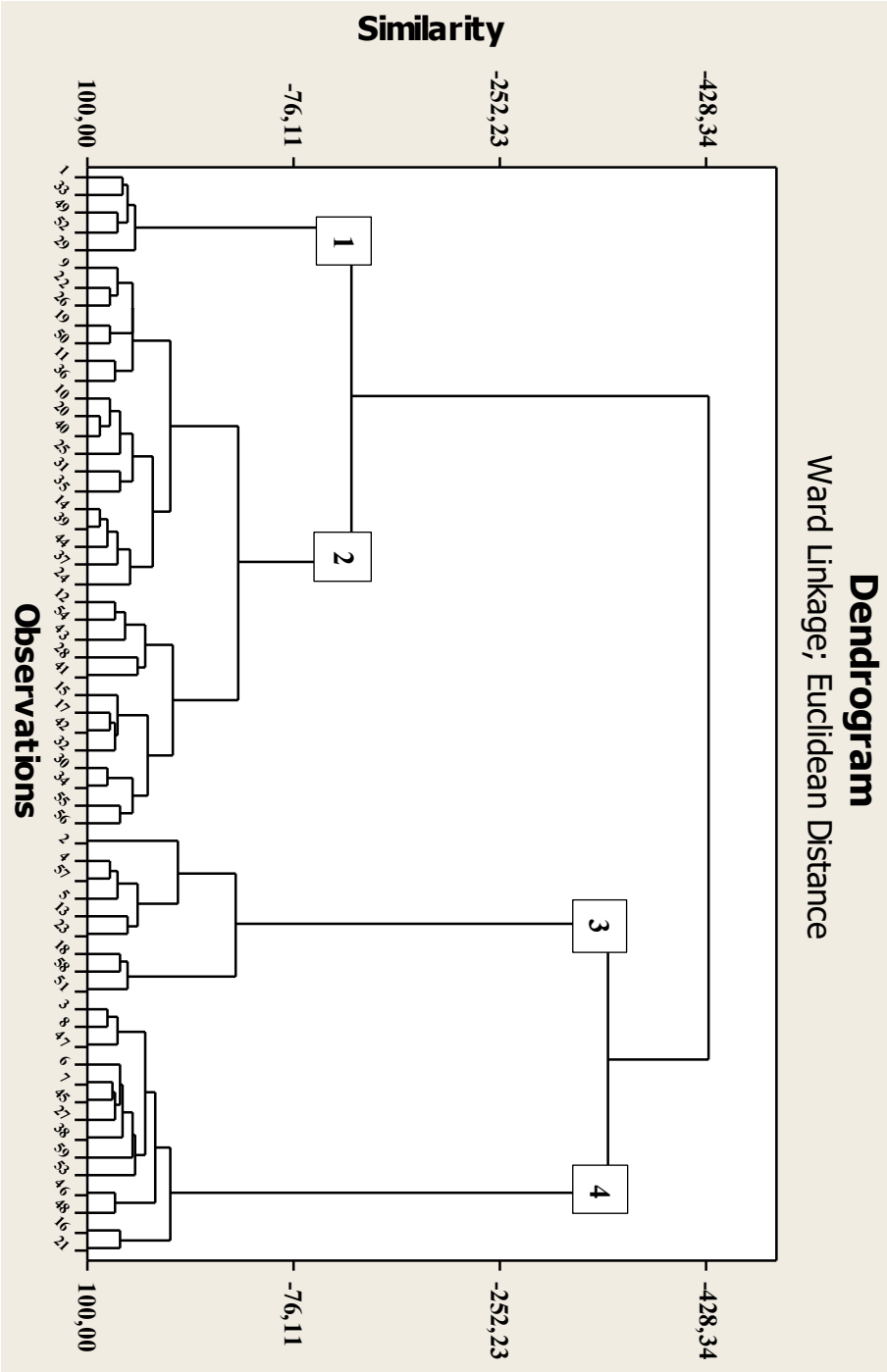

Figure 1: Dendrogram obtained at the end of the cluster analysis performed with morphological data.

At the end of the principal component analysis, 10 independent principal component axes were obtained between the plants (Table 3). According to the principal component analysis results, these 10 principal component axes represent 94.3% of the total variation in the squash population (Table 3). The literature has reported that principal component axes with eigenvalues greater than 1 in PCA are highly reliable (Özdamar, 2004; Balkaya et al., 2010; Kanal and Balkaya, 2021). The study revealed that the

coefficients of the eigenvalues of the first 10 principal components varied between 15.15 and 1.023 (Table 3). When the variance values of the principal component axis were examined, it was seen that the first principal component axis explained 28.8% of the total variation, the second principal component axis explained 26% of the variation, and the third principal component axis explained 11.2% of the total variation. Principal component analyses revealed that (i) 66% of the qualitative (neck in unripe fruit, curving of the neck in unripe fruit, mottling in unripe fruit, and type of mottling in unripe fruit) variation was explained by the first three components. Moreover, the genotypes were grouped under four groups in the principal component analysis (Figure 2). Numerous studies in the literature have been carried out to determine the existing variation levels of populations of *C. Pepo* L.. In their study carried out with 46 C. pepo genotypes selected from 5000 genotypes, Méndez-López et al. (2010) reported that three groups were identified with respect to morphological characteristics. The study by Nacar et al. (2011) on *Cucurbita pepo* L. squash lines established that 14 cluster groups were formed. In our study, 12 different cluster groups were identified. In the cluster analysis performed based on 21 morphological variables in squash, Balkaya et al. (2010) found that 10 different groups were created, and the first five principal component axes explained 65.0% of the total variation between the populations. Mladenoviç et al. (2014), who aimed to determine the morphological characterization in 20 squash genotypes, reported at the end of the PCA that these genotypes were divided into five groups on the PCA axis. Martins et al. (2015) identified 54 *C. pepo* genotypes, 32 *C. maxima* genotypes, and 21 *C. moschata* genotypes collected from the central and northern parts of Portugal based on 20 morphological characteristics. According to their study, three species clearly diverged from each other in the PCA, and the total variation was 52.5%. When the data of the present study and the data from the literature are taken into consideration, it can be understood that the existence of variation was sufficient.

Table 3: Factor groups based on the principal component analysis of the characteristics examined in the study and the corresponding principal component axes

	Principal Component Axes									
Eigenvalue	15.146	13.662	5.859	4.38	2.681	2.088	1.866	1.591	1.23	1.023
Variance value	0.288	0.26	0.112	0.083	0.051	0.04	0.036	0.03	0.023	0.019
Cumulative value	0.288	0.549	0.66	0.744	0.795	0.835	0.87	0.9	0.924	0.943
	Factor Coefficients									
Features	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**PC10**
G.H	0	0	0	0	0	0	0	0	0	0
G.B	0	0	0	0	0	0	0	0	0	0
D.B	-0.059	0.043	0.171	-0.394	-0.366	-0.241	-0.205	-0.255	-0.087	-0.574
T.S	-0.02	0.008	0.047	-0.125	-0.125	-0.087	-0.049	-0.067	0.014	-0.214
G.C.S	0.054	-0.034	0.008	0.011	0.057	0.084	0.047	0.004	0.114	-0.21
A.P	-0.014	-0.029	-0.062	0	0.153	-0.419	-0.064	-0.193	0.485	0.134
P.L	-0.099	-0.045	0.071	-0.242	0.204	-0.369	0.597	-0.226	0.263	0.081
P.T	0.056	0.012	0.2	-0.325	0.047	-0.003	-0.232	0.307	0.251	-0.154
P.P	-0.058	0.04	-0.009	-0.022	-0.055	-0.084	0.308	-0.055	-0.207	-0.162
S.CS.P	0	0	0	0	0	0	0	0	0	0
L.B.M	0	0	0	0	0	0	0	0	0	0
I.L.B	-0.101	-0.046	0.114	-0.12	0.612	0.328	0.031	-0.471	-0.335	-0.197
L.B.S.S	0.018	0.086	0.273	-0.606	0.062	-0.042	0.134	0.363	-0.325	0.397
G.C.U.L.B	0.003	0.03	-0.049	-0.213	-0.026	-0.096	-0.523	-0.487	-0.039	0.471
N.U.F	0.155	0.173	0.872	0.392	-0.045	-0.067	0.002	-0.123	0.027	0.086
C.N.U.F	0.678	0.663	-0.215	-0.094	0.019	0.069	0.121	-0.13	0.037	-0.047
N.M.C.U.F	0	0	0	0	0	0	0	0	0	0
M.C.U.F	-0.007	-0.014	0.008	-0.008	0.015	-0.013	0.008	0.008	0.035	-0.049
I.M.C.U.F	0.186	-0.146	0.104	-0.104	0.338	0.183	-0.21	0.208	0.264	-0.139
G.U.F	0.026	-0.02	0.054	-0.101	0.169	0.191	-0.12	0	0.272	0.022
G.S.F	0.075	0.092	-0.092	0.211	0.451	-0.631	-0.265	0.255	-0.356	-0.152
M.U.F	0.526	-0.549	-0.003	-0.031	-0.084	-0.049	0.053	-0.089	-0.131	0.041
T.M.U.F	0.387	-0.411	0.013	0.007	-0.092	-0.096	0.058	-0.062	-0.123	0.022
M.C.D.F	0.122	-0.105	0.038	-0.09	0.186	0.018	0.047	0.041	0.209	-0.176

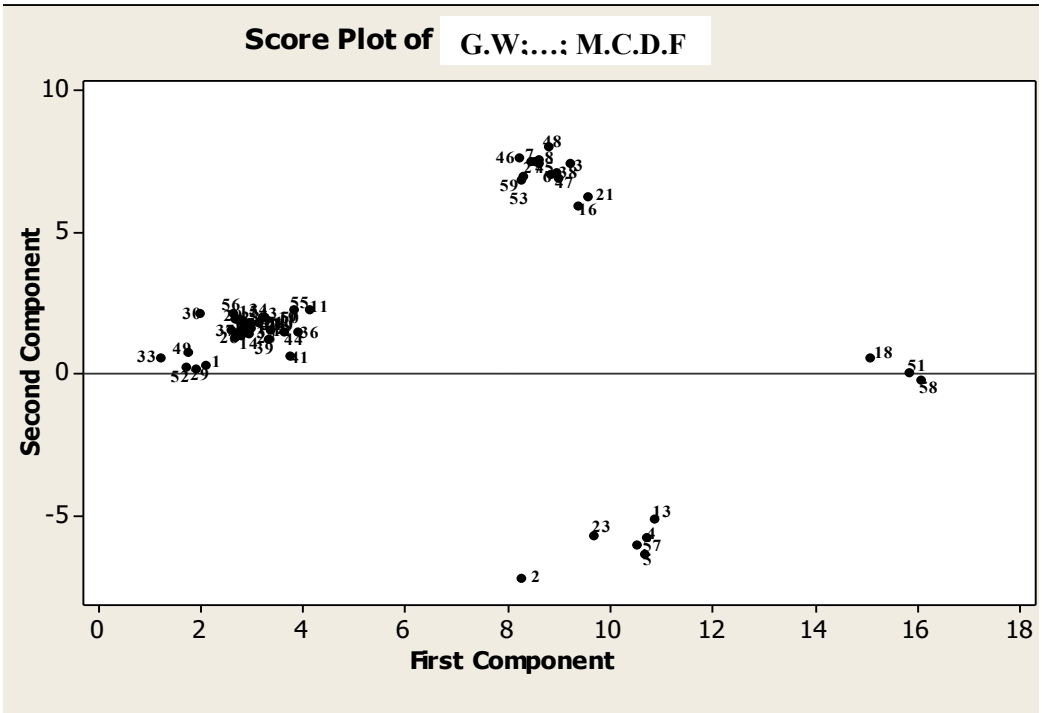

Figure 2: Two-dimensional graphic obtained through the principal component analysis performed with morphological data.

4. Conclusion

The production and consumption of summer squash have gradually increased in recent years. In this study, we identified the accession of Summer squash exhibited the variation in qualitative and quantitative characteristics to develop an F1 hybrid variety of squash, which is also a suitable alternative crop for greenhouse cultivation. Cluster and principal component analyses (PCA) were conducted to determine the relationships between the populations and obtain information about the usefulness of the relevant plant and fruit characteristics in identifying the groups. According to the cluster analysis, the genotypes were grouped under four main groups and 12 subgroups.PCA identified neck in unripe fruit, curving of the neck in unripe fruit, mottling in unripe fruit, and type of mottling in unripe fruit as important traits for variation. Based on agro-morphological traits and cluster analysis, accessions were categorized into four groups. This phenotypic variation observed for agro-morphological traits revealed that agronomic traits can be improved by a selection program. In addition, an assessment of phenotypic variability among the accessions would be useful for germplasm management.

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Research Article

The Level of Fat- and Water-Soluble Antioxidants in Eggs of Free-Range Geese during a Production Season

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Abstract: In this investigation, egg yolk and egg white water and fat-soluble antioxidant concentrations of geese eggs were determined during a production season according to months and weeks. Breeders consumed 100 g commercial layer feed per day during a production season. The farm was located at a semi-open prison of the Ministry of Justice in Van city. Fat-soluble (vitamin A, E, total and individual carotene) of egg yolk and water-soluble (ascorbic acid and GSH) antioxidants of egg white of geese were measured in February, March, April and May per week gazed in pasture conditions. Roche Yolk Color Fun (RYCF) values and Minolta (L* brightness, a* redness, and b* yellowness) values varied according to months and statistically significant differences were observed ($p < 0.05$). RYCF, a * redness, and b *yellowness values were the lowest in February and L* brightness values were the highest in February ($p < 0.001$). According to months (February-May), the egg yolk concentration of vitamin A (retinol) were 3.05±0.11, 1.84±0.10, 1.93±0.09, 2.84±0.19 µg g^{-1}, total vitamin E were 26.87±1.80, 25.07±1.64, 38.16±1.71, 34.30±1.89 µg g^{-1}, and total carotene were 15.49±1.44, 19.50±1.79, 42.39±1.99, 44.30±2.03 µg g^{-1} ($p < 0.05$), respectively. In this study, lutein, cis-lutein, zeaxanthin, apoester, canthaxanthin, and beta-carotene were identified as individual carotene in goose egg yolks. Glutathione (GSH) and Vitamin C or ascorbic acid (AA) were detected in geese eggs white in the last two weeks of February and four weeks of March, April, and May. The results were recorded as: 0.91±0.14, 1.83±0.19, 2.69±0.15, 1.97±0.09 µg g^{-1} for AA and 7.71±1.86, 33.22±2.14, 45.37±2.41, 38.75±1.50 µg g^{-1} for GSH respectively. Both water-soluble GSH and AA data were significantly lower in February and were significantly higher in April ($p < 0.05$) compared to other months.

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1. Introduction

In Türkiye, goose breeding benefits from a favorable climate and geographic structure. Geese are raised in nearly all regions of the country, with significant practice observed in provinces such as Kars, Ardahan, Muş, Yozgat, Şanlıurfa, and Erzurum (Arslan, 2010). The goose population increased from 1 million 374 thousand in 2020 to 1 million 478 thousand in 2021 according to data from TÜİK

(2022). While extensive research has explored antioxidant concentrations in egg yolks, egg quality, antioxidant stability, and nutritional benefits for poultry progeny and human health worldwide (Rezaei et al., 2019; Kljak et al., 2021; Tuță et al., 2023), studies specifically focusing on geese remain limited (Chen et al., 2015; Ma et al., 2020; Fu et al., 2022). Notably, several studies have reported that antioxidant content in eggs positively influences hatching parameters and chick viability post-hatching. Additionally, breeding facilities are closely linked to the feeding practices of commercial breeding poultry (Surai et al., 2019). It has been emphasized that antioxidants are transferred from the plasma of the egg-laying female parent to the yolk during yolk development, therefore, the antioxidant system of the female parent affects the antioxidant level of the egg yolk (Chen et al., 2015). As it has been known that poultry cannot synthesize fat-soluble antioxidants (vitamin E, A, and carotenes) in their organisms, they meet these needs through their feed. It has been suggested that maternal antioxidants prevent chicks from being adversely affected by oxidative stress and have a fundamental role in embryo development (Babacanoğlu et al., 2013).

Egg yolks and day-old chick tissues of farm and wild chukar partridges were compared for different antioxidant (carotenoid, retinol, retinol-ester, vitamin E, and coenzyme Q_{10}) concentrations by Karadas et al., (2017). It was observed that the antioxidant (total carotenoids, retinol, alpha-tocopherol, and vitamin E) concentrations of eggs taken from the wild were significantly higher than only fed by corn-soybean based fed in farm conditions. Tela et al. (2019), investigated the vitamin and antioxidant content of eggs from various avian species using high-performance liquid chromatography (HPLC). They analyzed vitamins A, E, and C; β-carotene; lycopene; ghrelin; oxidized glutathione (GSSG); reduced glutathione (GSH); and malondialdehyde (MDA) in eggs from village chickens (organic), farm chickens, ducks, quails, and geese. They found that the amount of vitamins A and E in farm chicken eggs was higher than that observed in other other avian species. Organic chicken eggs exhibited elevated levels of β-carotene and lycopene. Quail eggs contained higher amounts of vitamin C, ghrelin, GSSG, and MDA. Goose eggs had elevated GSH levels but lower vitamin C, β-carotene, and MDA levels compared to other poultry. In another study conducted by Alataş et al. (2021) with different age and breed) (32, 44 age of tinten breed and 45-48 age of lohman) commercial layer egg of different cities of Türkiye (Muş and Van). In this experiment the total carotene content of the egg yolk changes according to the periods, this difference disappeared after the feed was improved, it had been concluded that each egg for sale on the market shelves may be different in terms of the criteria (pigment and vitamin contents) as a reflection of the feed consumed by the chickens. Although many experimental studies had been conducted on egg yellow pigment score (color pigments added to wheat-based diets (pigment-free) in laying hens and quail's diet) carotene content, fatty acid profile of egg yolk lipids in the world and in Türkiye (Anderson et al., 2011; Altuntaş and Aydın, 2014; Alay and Karadas, 2016; Karadas et al., 2016; Karageçili and Karadaş, 2016; Alataş et al., 2021; Kljak et al., 2021, Panaite et al., 2021) studies on the antioxidant concentration of breeder diet and egg's of geese are very limited (Tela et al., 2019; Zhang et al., 2020; Fu et al., 2022).

Many studies have investigated the antioxidant properties of poultry eggs, but few have focused on geese. Existing studies on geese in our country have mostly focused on their energy and protein needs. Since goose breeding is generally based on pasture feeding in most countries, it was thought that geese eggs could be quite rich in terms of egg yolk pigments (carotenes). However, the type and carotene levels of goose eggs were not known since they had not been studied before. To address this gap, we conducted a study where egg samples were taken from goose breeding based on pasture under free-range conditions between February and May. We determined the antioxidant contents of fat-soluble (vitamins A, E, and carotenes (total and individual)) and water-soluble antioxidants (vitamin C and GSH) in these samples. We also determined the level of change of these data according to months in a production season.

2. Material and Methods

2.1. Animal material

In the study, eggs were taken from 150 females and 50 males total of 200 Chinese geese 12-24 months of age breeders (*Anser cygnoides domesticus*) during the laying period (from the last two weeks of February to the end of May). The goose breeding farm was located at a semi-open prison of the

Ministry of Justice at Erciş town of Van city. Ten eggs were collected randomly every Friday each week and 40 eggs per month, and a total of 140 egg samples were taken for the experiment from a parent flock.

2.2. Feed material

In this study, the breeder flock had access to the pasture area during the day and was kept in barn condition at night. All birds were fed with 100 g commercial layer feed (Table 1) per animal during the night. Crude nutrients (dry matter (DM), crude ash (CA), crude protein (CP), crude oil (CO), crude cellulose (CC)) and vitamin A, vitamin E, total and individual carotene analyzes were made by taking samples from commercial layer feed samples. The ingredients, nutrient composition, and antioxidant concentrations of feed were given in Table 1.

Table 1. Layer breeder feed ingredient, nutrient composition, and antioxidant concentration

Ingredients	**%**
Corn 7.2	52.71
Soybean meal 46	10.59
Full fat soybean 36	8.80
Sunflower seed meal 28	6.38
DDGS 28	7.95
Meat and bone meal 30	2.20
Marble dust	10.60
Salt	0.17
Min-Vit mix	0.30
Dl-methionine	0.10
Sodium bicarbonate	0.10
Toxin bounder	0.10
Nutrient content	
Dry matter (%)*	88.97±3.66
Crude Protein (%)*	16.05±0.22
Crude Cellulose (%)*	2.29±0.8
Crude Ash (%)*	12.25±0.45
Crude Oil (%)*	3.00±0.07
ME (Kcal kg^{-1})	2720
Antioxidant concentration	
Retinol (µg g^{-1})*	0.92±0.203
Gamma-tocotrienol (µg g^{-1})*	7.28±1.337
Alpha-tocotrienol (µg g^{-1})*	0.42±0.085
Delta-tocopherol (µg g^{-1})*	0.38±0.030
Gamma-tocopherol (µg g^{-1})*	16.59±2.448
Alpha-tocopherol (µg g^{-1})*	14.60±3.417
Total Vitamin E (µg g^{-1})*	39.13±3.444
Total Carotene (µg g^{-1})*	9.88±0.595
Lutein (µg g^{-1})*	2.24±0.260
Zeaxanthin (µg g^{-1})*	4.78±0.289
Canthaxanthin (µg g^{-1})*	2.60±0.127
Beta-carotene (µg g^{-1})*	0.26±0.045

*Laboratory analyzed.

2.3. Methods

2.3.1. Determination of egg yolk pigments RCF and Minolta (L, a*, b*) values*

For visual of egg yolk color, DSM Yolk Color Fan commonly known as Roche Yolk Color Fan (RYCF) was used. Konica Minolta CR-400 (CR-400, Minolta, Osaka, Japan) colorimeter instrument was used to measure L* (Brightness), a* (redness), and b* (yellowness) values of egg yolk (Skřivan et al., 2015; Faitarone et al., 2016).

2.3.2. Determination of vitamin E, vitamin A, total and individual carotenoids of egg yolk

Vitamin E, A, total, and individual carotene concentrations of egg yolk were determined by using Shimadzu Prominence (Tokyo, Japan) full-automatic HPLC system. About 200 mg of egg yolk samples was taken for extraction into the samples' tube. 70% NaCl 0.7 mL and 1 mL ethanol had been added to each sample and homogenized with homogenizer about 1-2 second. During first homogenization 2 mL, in the second homogenization 1.5 mL hexane was added to each tube. The upper layer (fat-soluble extract with hexane) was transferred to the evaporation tube and it was evaporated under nitrogen at 65 °C temperature. Dried samples were diluted with 1 mL mixture of dichloromethane (DCM): methanol (50:50, v/v) then transferred to HPLC vials to detect each peak of analyses by HPLC instrument.

For total carotene concentrations, 20 µl of sample were injected by AS 3500 autosampler in the LC 20A pump with a flow rate of 1.5 mL min^{-1} and methanol: water (97:3, v/v) mobile phase accompanied by a Spherisorb type 5 µ NH_2 column (25x4.6 mm; Phase Separation, Clwyd, UK) and the SPD-20A detector at a wavelength of 440-450 nm. For individual carotenes same SPD-20A detector at a wavelength of 440-450 nm was used but mobile phase was designed A phase (methanol: water (97:3, v/v)) and B phase (acetonitrile:DCM: methanol (70:20:10, v/v/v)) accompanied by a Spherisorb type 5 µ ODS2 column (25x4.6 mm; Phase Separation, Clwyd, UK).

For Vitamin E analysis, 20 µl of the same sample was injected into the system, using 3µ C18, reverse-phase column (15 cm x 4.6 mm, Spherisorb ODS2, Phase Separation, Clwyd, UK) with a flow rate of 1.05 per minute with methanol: distilled water (97:3, v/v) mobile phase with excitation at 295 nm and emission at 330 nm in fluorescence detector (Surai et al., 1996).

2.3.3. Determination of ascorbic acid (AA) and GSH analyses in egg white

The amounts of water-soluble vitamin C (ascorbic acid) and GSH in goose egg whites were measured by an HPLC device according to Mitić et al. (2011). Briefly, the extraction method was given flowing; approximately 300-350 mg of egg white sample was taken, 700 mL of 2% metaphosphoric acid was added and centrifuged at 4000 rpm in the centrifuge at +4 °C for 4 minutes. Metaphosphoric acid was taken from the top and transferred to another glass tube. After this process was repeated 2 times, the solution was filtered through a 0.45 µm Millex-syringe filter and taken into HPLC vials. HPLC operating conditions with a DAD detector at 244 nm UV wavelength, mobile phase: at pH 2.54 pure water adjusted with H_2SO_4 was used, and a flow rate of mobile phase was 0.7 ml per minute was provided. GSH and AA levels were determined after the device was calibrated using Hypersil Gold AQ 150x4.6 mm 5µ (Thermo scientific) as a column using GSH and ascorbic acid (L-Ascorbic acid, sigma-aldrich) standard.

2.4. Statistical analysis

The main factors were determined by the monthly variation of the fat and water-soluble vitamin levels in the eggs of the geese fed on the pasture, and the SAS (2017) computer package program was used for the statistical analysis of the obtained data. One-way analysis of variance for detecting differences between months; The Duncan test was applied to control the significance of the differences between each month.

The mathematical model of the experiment is given as Yij:µ+ai+ eij

µ: General average of the investigated feature, ai: Change of the examined feature according to months, eij: error.

3. Results and Discussion

It highlights the increasing demand for free-range and organic eggs due to concerns about synthetic color additives in poultry feed. Synthetic egg yolk colorants such as apo-8-carotenic acid ethyl ester and canthaxanthin are not permitted in organic egg production. Free-range poultry has access to natural sources of carotenoids, primarily lutein, and other antioxidants, through pasture grazing (Kljak et al., 2021). Investigating the effects of months during the laying season on the antioxidant composition of goose egg yolks and whites would be a valuable reference for other free-range and organic egg production systems. The color of the egg yolk is an important quality attribute for consumers, as it is an indicator of freshness and naturalness. Therefore, egg production companies consider yolk color measurement to be a crucial aspect of their egg production. The most efficient and effective methods for measuring yolk color are based on visual measurements of RYCF and, Minolta calorometric devices (Milovanovic et al., 2021).

3.1. RYCF and Minolta L*, a*, b* measurement results of goose eggs

Egg roche yolk color fun (RYCF) scale and Minolta L*, a*, b* measurement values of goose eggs were given in Table 2. It has been seen that a difference was recorded in the RYCF value, which was the color pigment physical measurement scale value, according to months ($p<0.05$). Same way, Minolta L* brightness, a* redness, and b* yellowness values changed according to months and the statistical differences ($p<0.05$) were recorded.

Table 2. RCF and Minolta L*, a*,b* weekl measurement values of free range geese egg yolks during production season (Mean±SEM)

Month	weeks	RYCF	L*	a*	b*
February	3	10.60±0.77	45.76±3.98	-0.28±0.92	32.83±4.20
	4	10.95±0.76	44.53±4.00	0.42±1.38	31.52±2.76
Mean		10.77±0.76^{C}	45.15±3.93^{A}	0.07±1.20^{B}	32.18±3.52^{B}
March	1	10.95±0.54	46.29±3.36	32.15±4.42	90.03±2.34
	2	12.20±0.91	43.53±2.84	31.20±4.06	86.09±3.17
	3	11.85±0.62	41.59±2.86	32.34±3.00	85.55±2.75
	4	13.10±0.65	39.53±3.70	30.10±3.73	82.14±2.64
Mean		12.02±1.02^{B}	42.74±3.99^{B}	31.45±3.80^{A}	85.95±3.97^{A}
April	1	12.65±0.94	46.29±3.36	42.15±21.76	80.83±3.61
	2	13.10±0.51	40.52±3.83	32.07±4.71	79.58±2.83
	3	13.55±0.55	40.41±3.71	30.09±5.36	78.84±5.18
	4	13.05±0.49	37.59±4.19	29.85±4.11	79.81±2.70
Mean		13.08±0.70^{A}	41.20±4.85^{B}	33.54±2.29^{A}	79.76±2.16^{A}
May	1	13.30±0.63	38.59±4.20	31.48±5.33	78.83±3.68
	2	12.80±0.67	39.66±3.85	30.42±4.29	81.43±1.98
	3	13.50±0.66	39.21±4.52	32.55±5.25	79.00±2.55
	4	12.90±0.51	40.61±2.23	32.17±2.38	83.09±2.06
Mean		13.12±0.66^{A}	39.52±3.73^{C}	31.66±4.38^{A}	80.59±3.12^{A}
P		<0.00012	<0.00018	<0.0001	<0.0001
F		21.70	6.62	29.76	357.58

^{A,B,C}Capital letter shows differences among months ($p<0.05$).

The same amount of standard commercial layer feed was given to all breeders, but with the arrival of spring and by growth of grass, the RYCF value improved significantly from the 4th week of March compared to the other weeks, and this pigment level continued until the end of May.

These results were similar to those of Alataş et al. (2021), who investigated the RYCF values of egg yolks from different breeds of commercial layers and found them to be 11-12. These values were slightly higher than the Roche scale value of 10.77 for goose egg yolks in February, which might be due to the use of synthetic egg yolk pigments in commercial layer feed and the insufficient availability of green grass in February, as spring arrived late in Van province. We can compare our findings with other studies on egg yolk color in different poultry species. The RYCF values of goose egg yolk were 13.08 and 13.12 for the samples collected in April and May, respectively. These values were higher by 1-2 units (RCF=11-12) than the values reported by Karageçili and Karadaş (2016), and Alataş et al. (2021) for commercial laying hen's egg yolk from different breeds and ages. A possible explanation for this difference is that the free range goose breeder was exposed to carotenoids from pasture during the day. The RYCF results obtained in this study were parallel to 13.89 RCF value of the 10 ppm canthaxanthin supplemented breeder quail's diet group egg yolk and 2 units higher than the 10.21 RCF value of the 10 ppm marigold powder supplemented diet egg yolk (Alay and Karadaş, 2016).

Egg yolk color is one of the factors that influence consumer's purchase decision, but it varies across different regions. For example, consumers in northern European countries are satisfied with egg yolks that have a Roche Yolk Color Fan (RYCF) score of 9-11, while consumers in southern European countries prefer more intense egg yolks with a RYCF score of 12-14 (Grashorn 2016). Therefore, the results of this study are more suitable for the preference of southern European consumers.

Our results are also parallel to the results of Spasevski et al. (2018a), who used natural carotenoids (marigold, paprika, and carrot) at different concentrations to replace synthetic colorants in the diet of Lohmann Brown hens. The RYCF values in their study ranged between 12.2 and 13.4. Similar results (RYCF: 8.67-14.71) were reported in another report by Spasevski et al., (2018b), who used carrot and paprika as natural colorants in Lohmann Brown layers' diet compared to synthetic pigments (carophyll red 0.05 g/kg and carophyll yellow 0.01 g/kg) (RYCF: 14.63).

Lightness (L*) value of egg yolk showed the highest value in March inversely proportional to color pigments, it showed the lowest value in May and there was a significant decrease in L* lightness value between March, and April ($p<0.05$). Minolta L* brightness value was inversely proportional to a* and b* values, it showed the highest value in February, and the lowest value in May, and there was a significant decrease in L* brightness value among months ($p<0.05$). The L* values were recorded as 39-45 in this study. This result was 10 units lower than the values (45-55) reported by Alataş, et al. (2021), in the egg yolk from different commercial breed's laying hens and Spasevski et al. (2017) reported as L* (45.55-51.91) values using dietary carrot and paprika in the diet of Lohmann Brown layers. But, the results were similar to the values reported by Lokaewmanee et al. (2010).

Minolta a* redness value was significantly lower in February compared to other months, and a* value gets darker significantly (0.07, 31.45, 33.54, and 31.66 respectively) ($p<0.05$). When the findings were compared with commercial laying hens, a* value was between 5.35-and 8.75 (Alataş, et al., 2021), it was observed that this value was almost 4 times higher (32-34) in goose egg yolk.

Yellowness (b*) value of egg yolk was also similar to the a* values. It showed that the lowest values were recorded in February then a significant increase in the following months, and finally, showed a stable value after reaching the saturation scale.

Minolta a*, b* values of goose eggs were compared with commercial laying hens, and quail eggs since they have not been examined before in goose egg yolk.

Yelowness (b*) value was recorded between 33-41 values in commercial laying hens (Alataş et al., 2021). It was observed that the b* value in goose eggs was similar to 31-32 in February as in commercial laying hens, but these values increased to 79-85 values from March to May and did not show any similarity with commercial poultry results (Alataş et al., 2021). However, the b* value was closely similar to the b* (76-77) value of the egg yolk of a breeder fed with Hungarian hybrid corn in a laying hen diet studied by Kljak et al. (2012).

3.2. Vitamin A and E concentrations of goose's egg yolk

In this study, vitamin A (retinol) and Vitamin E contents of goose eggs in February-May in a production season were given in Table 3. As seen in Table 3 all parameters were statistically different according to the months.

Table 3. The Concentration of vitamin A and E (µg g^{-1}) in free range geese egg yolk during production season (Mean±SEM).

Month	Week	Retinol	Alfa-ttn	Del-toc	Gamma-toc	Alpha-toc	Total Vit E
February	3	2.89±0.13	1.00±0.09^{a}	0.49±0.48^{a}	15.81±2.13	8.73±0.95	26.05±3.04
	4	3.22±0.17	0.75±0.04^{b}	0.25±0.04^{b}	16.27±1.13	10.49±0.80	27.77±1.91
Mean		3.05±0.11^{A}	0.89±0.06^{A}	0.38±0.04^{A}	16.03±1.2^{B}	9.57±0.65^{B}	26.87±1.80^{B}
March	1	2.26±0.17^{a}	0.54±0.05^{a}	0.27±0.03^{b}	13.57±1.50^{b}	7.07±0.78^{b}	21.45±2.29
	2	1.64±0.19^{bc}	0.48±0.11^{a}	0.43±0.05^{a}	16.82±1.96^{b}	7.41±0.71^{ba}	25.15±2.65
	3	1.37±0.16^{c}	0.26±0.63^{b}	0.16±0.02^{b}	18.41±2.07^{b}	8.30±0.98^{ba}	27.14±2.84
	4	1.94±0.14^{ba}	0.11±0.01^{b}	0.16±0.02^{b}	16.77±2.55^{a}	9.82±0.89^{a}	26.59±3.47
Mean		1.84±0.10^{B}	0.35±0.04^{B}	0.27±0.27^{A}	18.90±1.27^{A}	8.15±0.44^{AB}	25.07±1.64^{B}
April	1	1.70±0.15	0.14±0.02^{a}	0.05±0.01	21.79±1.84	11.06±0.98	32.97±2.31
	2	2.01±0.23	0.17±0.02^{a}	0.11±.0.03	22.68±1.36	13.62±0.84	36.60±2.23
	3	2.12±0.15	0.15±0.02^{a}	0.12±0.02	27.25±3.14	14.74±1.79	41.99±4.93
	4	1.88±0.17	0.07±0.01^{b}	0.08±0.01	27.11±1.95	13.40±1.00	40.51±2.93
Mean		1.93±0.09^{B}	0.13±0.01^{B}	0.10±0.01^{B}	24.76±1.14^{A}	13.20±0.63^{A}	38.16±1.71^{A}
May	1	1.61±0.12^{b}	0.13±0.01^{b}	0.07±0.01^{b}	26.44±1.10^{a}	11.57±0.46	38.20±1.56
	2	2.22±0.32^{b}	0.25±0.07^{b}	0.48±0.17^{a}	21.13±3.32^{b}	9.30±1.09	30.69±1.66
	3	4.00±0.24^{a}	0.78±0.12^{a}	0.48±0.09^{a}	22.15±0.19^{b}	9.360±1.82	32.16±1.78
	4	3.46±0.25^{a}	0.58±0.11^{a}	0.52±0.07^{a}	21.01±0.13^{bc}	14.21±2.06	36.13±2.23
Mean		2.84±0.19^{A}	0.44±0.06^{B}	0.39±0.05^{A}	22.68±1.89^{A}	11.12±1.19^{A}	34.30±1.89^{A}
P		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
F		17.95	32.53	11.33	18.72	27.38	9.95

Retinol: Vitamin A, Alfa-ttn: Alfa-tocotrienol, Del-toc: Delta-tocopherol, Gamma-toc: Gamma-tocopherol, Alpha-toc: Alpha-tocopherol, Total Vit E: Total vitamin E.
^{A,B}Capital letter shows differences among months ($p<0.05$).
^{a,b,c}Small letter shows differences among weeks in each month ($p<0.05$).

Egg yolk retinol levels were found to be significantly higher in February, and May than the mean of March, and April. The amount of retinol was recorded between 1.37-4.00 µg g^{1}. These values were reported as 4.97 µg g^{-1} for chicken, 8.96 µg g^{-1} for quail, and 2.67 µg g^{-1} for duck eggs by Irie et al. (2010). In a more recent study by Tela et al.(2019), the retinol levels in egg yolks of commercial chicken, organic chicken, duck, quail, and goose were reported as 6.07, 2.33, 2.77, 3.62, and 4.64 µg g^{-1} respectively. However, the retinol level of feed was not reported in this study (Tela et al., 2019). The retinol level of commercial egg feed consumed by the breeding goose was reported as 0.92±0.20 µg g^{-1} (n=4) in our study. The retinol level of breeder partridge's feed was given as 1.11 µg g^{-1} in previous reports (Karadaş et al., 2017), which seems to be quite close to this value.

The values for the retinol content of the egg yolk were similar to the retinol levels of duck, quail, and goose eggs above. However, it was observed that organic laying hens had lower retinol levels in their egg yolks. In another study by Karadaş et al., (2017), farm and wild partridge eggs were compared, and the retinol level of partridge eggs was found to be 2.72 µg g^{-1} when fed in a farm-reared system and 5.01 µg g^{-1} for wild-partridge eggs.

Similarly, free-range, caged hens and quail egg yolk retinol concentrations were reported as 4.76, 4.74, and 6.37 µg g^{-1} respectively by Rmalho et al., (2006). All these results were one or two units higher than goose egg yolk retinol content. There is a wide variation in the retinol concentration of egg yolk reported in the literature, depending on the dietary level of vitamin A, other antioxidant concentrations of feed, age, and breed of poultry. It is also possible to increase retinol concentration by using high concentrations of retinyl acetate (5 000 and 35 000 IU/kg) in diet, which increased egg yolk retinol concentration as 5.8, 8.7 µg g^{-1} (Yuan et al. 2014; Ilhan and Bulbul 2016).

The mean value of alpha-tocotrienol was significantly higher in February than the mean of the other three months, and there was no significant difference between the averages of the other three months ($p>0.05$).

The delta-tocopherol content of eggs was found to be significantly low only in April, and no difference was reported among other months ($p>0.05$). Likewise, while gamma-tocopherol was significantly low only in February, it remained at a similar level for 3 months, statistically insignificant in all other months. Alpha-tocopherol level showed a tendency to increase significantly in April and started to decrease again in May.

The total amount of vitamin E was the sum of other tocopherols and tocotrienols and the highest amount was detected in April. A downward trend was observed again in May. When our egg yolk's total Vitamin E content was compared with the literature reports, our results were very close to the results of 32.70 µg g^{-1} Vitamin E (VE) in the egg yolk of the control treatment (Fu et al. 2022) who supplemented 40, 200, 2000 IU VE kg^{-1} to the diet of breeding geese. They confirmed that when increased concentration of VE in the diet, egg yolk concentration of vitamin E significantly increased to 61.64, 129.88, and 215.52 µg g^{-1} respectively per treatment. Similarly, Marques et al. (2011) examined the effects of quail's diet supplemented with different levels of vitamin E (control, 200, 400, 600 IU Vitamin E kg^{-1}). The egg yolk vitamin E level was reported as 190 µg g^{-1} for the control group and 580, 790, and 1100 µg g^{-1} for the other groups, respectively. Even in the control group, the vitamin E level was about six times higher than that found in this study. The higher level of supplemented treatments was 35 times higher than that found in this study. This difference could be due to geese being a different breed, age, or diet. Tela et al. (2019) compared the vitamin E contents of different poultry, such as commercial and organic chicken, duck, quail, and goose egg yolk. The vitamin E levels were reported as 3.33, 2.88, 0.40, 0.32, and 0.55 µg g^{-1}, respectively. These values were lower than the results of this research. Therefore, it was thought to be due to the use of the separation technique as a method. Free-range chicken eggs contain 55-113 µg g^{-1} without adding vitamin E to their feed, free-range pheasant eggs contain 49-86 µg g^{-1}, and free-range goose egg yolks contain vitamin E at the level of 23-32 µg g^{-1} (Surai et al., 1998; Surai, 2002). Therefore, it was seen that there was a very close similarity between the Vitamin E content of free goose eggs reported in the literature and in this study (25-38 µg g^{-1}). Alpha-tocopherol contents of farm and wild partridge eggs' yolk were reported as 40.80 and 59.58 µg g^{-1}, respectively (Karadaş et al., 2017). Our findings were not similar to this study since our results were lower than partridge eggs. However, when gamma tocopherol values were compared, it was seen that goose eggs had a minimum of 13.57 µg g-^{1} and a maximum of 27.25 µg g^{-1}. The gamma-tocopherol levels of 4.90 µg g^{-1} in partridge eggs and 3.45 µg g^{-1} in wild partridge eggs are three to nine times higher than those observed in goose eggs.

3.3. Total and individual carotene content of goose egg yolk

Total carotene, and individual carotene (lutein, zeaxanthin, apoester, cantaxanthin, and beta-carotene contents of goose egg yolk were given in Table 4.

Table 4 . Total and individual carotenoid content (µg g^{-1}) of egg yolk of free range geese during production season (Mean±SEM)

Months	Week	Lutein	Zeaxanthin	Cis-lutein	Canthaxanthin	Apoester	Beta-carotene	Total carotene
February	3	5.66±0.64	5.07±0.58	1.91±0.21	0.64±0.0	0.50±0.05^{a}	0.58±0.06	14.39±1.64
	4	6.51±0.67	5.86±0.60	2.27±0.23	0.74±0.07	0.60±0.06	0.69±0.07	16.71±1.71
Mean		6.07±0.46^{C}	5.45±0.42 ^{B}	2.09±0.16^{C}	0.69±0.05^{C}	0.55±0.04^{B}	0.64±0.05^{C}	15.49±1.44^{B}
March	1	4.90±0.73^{b}	4.39±0.65^{b}	1.65±0.24^{c}	0.55±0.08^{b}	0.44±0.06^{b}	0.51±0.07^{b}	12.46±1.86^{b}
	2	6.19±0.76^{b}	5.55±0.68^{b}	2.09±0.26^{c}	0.70±0.08^{b}	0.55±0.06^{b}	0.64±0.08^{b}	15.74±1.95^{b}
	3	5.75±0.72^{b}	5.15±0.64^{b}	1.94±0.24^{b}	0.65±0.08^{b}	0.51±0.06^{b}	0.59±0.07^{b}	14.62±1.83^{b}
	4	13.84±1.08^{a}	12.40±0.96^{a}	4.68±0.36^{b}	1.57±0.12^{a}	1.24±0.09^{a}	1.44±0.11^{a}	35.17±2.75^{a}
Mean		7.67±0.70^{C}	6.88±0.63^{B}	2.59±0.24^{C}	0.87±0.08^{C}	0.69±0.06^{B}	0.80±0.07^{C}	19.50±1.79^{B}
April	1	12.33±0.96^{b}	13.17±1.02^{b}	4.97±0.38^{b}	1.67±0.13^{b}	1.32±0.10^{b}	1.53±0.11	35.00±2.72
	2	14.91±1.94^{ba}	15.54±1.98^{b}	8.48±0.72^{a}	2.09±0.27^{ba}	1.59±0.15^{ba}	1.94±0.54	41.76±3.44
	3	13.74±1.34^{b}	15.09±1.15^{b}	5.74±0.40^{b}	1.87±0.17^{b}	1.48±0.13^{b}	1.70±0.16	39.63±3.34
	4	18.57±1.49^{a}	19.84±1.59^{a}	7.48±0.60^{a}	2.51±0.20^{a}	1.98±0.15^{a}	2.30±0.18	52.71±4.24
Mean		14.89±0.79^{A}	15.93±0.81^{A}	6.47±0.33^{B}	2.04±0.11^{A}	1.60±0.08^{A}	1.87±0.15^{A}	42.39±1.99^{A}
May	1	14.63±1.03	19.34±1.36	11.68±0.82^{a}	2.00±0.14	1.82±0.12	1.50±0.10^{a}	51.00±3.60
	2	11.51±0.63	15.70±1.87	9.14±0.48^{a}	1.60±0.13	1.42±0.10	1.17±0.22	40.56±2.71
	3	11.76±0.71	15.54±0.95	9.38±0.57^{a}	1.61±0.09	1.47±0.08	1.20±0.07	40.99±2.50
	4	12.70±1.69	16.80±2.24	10.14±1.35^{a}	1.74±0.23	1.58±0.21	1.30±0.17	44.28±5.91
Mean		12.68±0.57^{B}	16.88±0.84^{A}	10.09±0.46^{A}	1.75±0.08^{B}	1.58±0.07^{A}	1.30±0.08^{B}	44.30±2.03^{A}
P		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
F		31.38	55.96	111.94	47.37	56.06	27.08	54.62

^{A,B,C}Capital letter shows differences among months ($p<0.05$).
^{a,b,c}Small letter shows differences among weeks in each month ($p<0.05$).

As can be seen from Table 4, total carotene, and individual carotenes such as lutein, zeaxanthin, cis-lutein, aposter, cantaxanthin, and beta-carotene were detected in the egg yolk of the goose.

When the lutein content of eggs was examined, it was seen that although they contained a fixed amount of 5-6 µg g^{-1}lutein until the 3rd and 4th weeks of February and the 3rd week of March, it doubled in the following weeks and reached its highest level in April of 12-13 µg g^{-1}. However, after the first week of May, it was seen that it tended to decrease again. While no difference was found between February and March averages, the highest lutein value was found in April ($p<0.05$). Likewise, the level of zeaxanthin increased by months, but the highest level seems to be in May. While no difference was found between February and March averages, the highest lutein value was found in April ($p<0.05$). Likewise, the level of zeaxanthin increased by months, but the highest level seems to be in May.

The cis-lutein content of the egg yolk gradually increased and reached a significant level in the 3rd week of March and the highest level recorded in May ($p<0.05$)

When the goose egg carotene results were compared with the literature, Tela et al. (2019) examined only the β-carotene and lycopene carotene levels of eggs of different species (goose, duck, quail, farm, and backyard chicken), and it is not understood why lutein, cis-lutein, and zeaxanthin, which should be found at high levels, were not investigated. Beta-carotene level was reported as 0.19 µg g^{-1}, and lycopene level as 0.08 µg g^{-1}, and it was observed that the findings were 3.64 µg g^{-1} even lower than 0.64 µg g^{-1}data of February. Lycopene carotene was not detected in our study. Because lycopene was only studied when tomatoes or lycopene were added to their feed.

The total carotene results of egg yolk compared with results of farm and wild partridge eggs (Karadaş et al., 2017). The total carotene content of farm partridge eggs was 12.19 µg g^{-1}, and wild partridge eggs was 66.58 µg g^{-1}. The total carotene content of goose eggs was 15.49 µg g^{-1} in February, which was only 3.3 units higher than farm partridge eggs and it was compatible, however, as of the 4th week of March, the total carotene content of goose eggs increased by 2.27 times and reached 35.17 µg g^{-1}. This increase continued until the end of April, reaching 52.71 µg g^{-1}, even if it did not catch the carotene content of wild partridge eggs it is also closely approached.

Carotene content of goose eggs compared with commercial laying hen eggs. It was seen that the Karageçili and Karadaş (2016) data reported as 16.85-19.95 µg g^{-1}showed close similarity with the mean values of February, and March and the values of 15.49-19.50 µg g^{-1}, but they were not similar to the values of April, and May.

Again, Alataş and Karadaş (2019) investigated the carotene contents of different commercial poultry eggs, total carotene content of egg yolk of their study was 12.48-22.40 µg g^{-1} and the results were parallel to February, and March first three weeks, but it was seen that they were not similar to the results of other months.

3.4. Ascorbic acid and GSH content of free range goose egg yolk during production season

Although ascorbic acid (AA) is not considered essential for chickens since it is synthesized in their kidneys and liver, adding it to their diet can improve their resistance to diseases, regulate stress, and help in the body's oxidation process. This ultimately enhances the laying rate, egg hatch performance, and overall poultry productivity by increasing body weight and reducing mortality rates. However, the direct impact of ascorbic acid on internal egg quality remains uncertain (Khan et al., 2012; Hieu et. al., 2022). Additionally, some reports have confirmed that ascorbic acid can enhance the immune response and antioxidant capacity of birds, especially under stress conditions (Shewita et al., 2019; Ahmadu et al., 2016; Barrio, et al., 2020). Glutathione is one of the essential natural antioxidants that can be linked to health-promoting effects on birds, humans, and plant life (Al-Temimi, et al., 2023).

Ascorbic acid and GSH contents of goose egg yolk were given in Table 5. As seen in Table 5, there was a significant change in terms of water-soluble vitamins by month. It was observed that water-soluble vitamins were lower in February, gradually increased in March, reached peaks in April, and decreased again in the last two weeks of May ($p<0.05$).

Ascorbic acid values Tela et al. (2019) reported as 0.02 µg g^{-1} in goose egg whites, 0.20 µg g^{-1} in quail egg whites, 0.17 µg g^{-1} in duck eggs, 0.02 µg g^{-1} in village chicken egg whites, and 0.11 µg g^{-1} in commercial farm egg whites.

GSH values were between 4.29-51.83 µg g^{-1}, and Tela et al. (2019) reported that GSH value of goose egg white was 75.20 µg g^{-1} and our results seem a little bit lower than these results.

Using a high concentration of vitamin E (0, 40, 200, 2000 IU kg^{-1}), in the diet of geese breeder could not increase significantly concentration of egg yolk GSH activity (32.76, 35.83, 41.37, and 38.89 µg g^{-1}, respectively p=0.0062) by Fu et al. (2022). Even though these data are in agreement with our results (33.22-45.37 µg g^{-1}), the GSH activity of February (4.29±0.83 µg g^{-1}) is quite lower than these results.

Table 5. The GSH and AA concentration of free range geese egg whites during production season

Month	Week	GSH (µg g^{-1})	Ascorbic acid (µg g^{-1})
February	3	4.29±0.825	0.69±0.171
	4	11.13±3.354	1.13±0.214
Mean±SEM*		7.71±1.856^{C}	0.91±0.143^{C}
March	1	17.19±3.364	0.75±0.080
	2	36.28±2.881	1.23±0.152
	3	41.87±3.197	2.26±0.285
	4	37.52±3.887	3.09±0.383
Mean±SEM		33.22±2.214^{B}	1.83±0.190^{B}
April	1	37.60±4.992	2.45±0.218
	2	44.18±5.303	3.20±0.276
	3	51.83±4.926	2.94±0.303
	4	47.87±3.387	2.16±0.191
Mean±SEM		45.37±2.414^{A}	2.69±0.137^{A}
May	1	41.55±1.652	1.98±0.149
	2	44.41±2.592	1.77±0.193
	3	39.65±3.114	2.15±0.265
	4	29.39±2.406	1.97±0.119
Mean±SEM		38.75±1.504^{B}	1.97±0.094^{B}
P		0.93	0.18
F		0.01	5.83

^{A,B,C}Capital letter shows differences among months ($p<0.05$).

Conclusion

At the end of this study;

1. Egg RYCF values of free-range geese got darker, especially in April and May, were positively affected by pasture, and reached a maximum value of 10.77.
2. In February, when the L* value of the Minolta results of free-range goose eggs was affected by the pasture, the highest a* (redness) and b* (yellowness) values decreased inversely as the values increased, so a* and b* values were the highest in the spring months when the green parts of forage were highest and it had been improved in proportion to the pigment level of the egg yolk.
3. While the vitamin A (retinol) content of the egg decreased according to the months, the Vitamin E content increased over time and showed a significant improvement in April and May compared to February and March.
4. Egg yolk carotene content increased significantly from February to May during production season. It was reported that individual carotenes such as lutein, zexanthin, cis-lutein, apoester, cantaxanthin, and beta-carotene were detected in free-range goose eggs, and all individual carotenes were at the highest level in April and May.
5. It was determined that the amount of GSH and AA in free-range goose egg whites was lower in February and was higher significantly in May and April ($p<0.05$).

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Research Article

The Investigation of Effect of Bacteria in Biological Control of Red Spider Mite (*Tetranychus* spp.) and Plant Yield Parameter in Cotton (*Gossypium hirsutum* L.)

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Abstract: The purpose of this study was to assess the usability of two bacterial strains, namely *Bacillus subtilis* PA1 and *Paenibacillus azotofixans* PA2, for the biological control of red spider species, and their effects on plant quality and yield in cotton under field conditions. The experiments were conducted at three different locations with multiple replications. As a control, a commercial preparation containing Lambda-Cyhalothrin as the active ingredient was used. The obtained results from the study revealed that the application of the bioagent formulation led to a significant decrease in the density of *Tetranychus* spp. at different biological stages, ranging from 59.22% to 61.07%, when compared to the control group. Additionally, several important plant growth parameters showed remarkable improvements. The number of fruit branches increased by 130.20%, plant crown diameter by 88.16%, plant height by 40.15%, the number of flowers by 21.25%, the number of wood branches by 18.13%, the average number of cocoons by 126.53%, and cocoon weights by 54.65% significantly across all three trial parcels. The successful implementation of the bacterial application for pest control had a positive impact on cotton yield. Bulk cotton yield increased by 80.03%, and fiber yield increased by 82.17%. Consequently, the bacterial formulation containing these two bacteria demonstrated its potential as a biopesticide in cotton cultivation, effectively controlling pests while also playing a crucial role in enhancing productivity. Overall, the study suggests that using the bioagent formulation consisting of Bacillus subtilis PA1 and *Paenibacillus azotofixans* PA2 could be an effective and environmentally friendly approach for pest control in cotton farming, leading to increased productivity.

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Footnote: This study was produced from the master thesis of the first author.

1. Introduction

Cotton (*Gossypium hirsutum* L.) is a very important fiber plant with a very long vegetation period of 5-6 months, adapted to the tropical and subtropical climate zone of the *Gossypium* type of the Malvaceae family of the Malvales order systematically (Anonymous, 2018). Approximately 35% of the fibers produced in the world are obtained from the cotton plant (Gündüz et al., 2020). As the world's

population continues to grow, alongside the industrialization and development of societies, the rising living standards have increased the consumption and demand for cotton fiber (Mızrak et al., 2020).

Various factors negatively affect cotton agriculture. Among these factors, yield and quality losses due to diseases, pests, and weeds have an important place. Among the pests, cotton aphids (*Aphis gossypi* Glov.), tobacco thrips (*Thrips tabaci* Lind.), red spider mites (*Tetranychus cinnabarinus* (Boisd.), and *T. urticae* Koch), and leafhoppers (*Empoasca decipiens* Paoli, *Asymmetrasca decedens* (Paoli.)) cause significant yield loss by stinging and sucking plant leaves during the basic development period of cotton plant (Güneş, 2005). Among these species, *T. urticae* is one of the pests that threaten plant production in general, the leaves that it causes damage by sucking turn yellow, the assimilation regresses because the amount of chlorophyll of the plant decreases, the leaves curl and fall, and the quality and quantity of the product taken from the damaged plants decreases (Toros, 1992) and thus it is stated that product loss is between 20-45% (Premalatha et al., 2018).

The control of red spider mites is mostly performed in the form of chemical control (acaricide/acaricide+insecticide practices), as in other agricultural pests. The value of the specific acaricide market in the world in 2013 was determined as 900 million Euros and it is noted that this value corresponds to 7% of the insecticides (excluding Fumigants) with a market value of approximately 13.3 billion Euros (Van Leuween et al., 2015). When we look at the active substance basis today, it is seen that some active substances that were put on the market in very old years are still used, as well as the latest acaricides with different effect mechanisms (spirodiclofen, spiromesifen) occupying a large place in the market share (Van Leuween et al., 2015). In Türkiye, the use of acaricide was 2 452 275 tons in 2019, constituting 4.53% of the total pesticide use of 54 098 000 tons (Anonymous, 2019). In addition to fecundational fertilization, mites can reproduce parthenogenetically, lay a large number of eggs, have a short life span, and give many offspring during the year (the development period can be as short as one week at high temperatures of about 32 °C), long term intensive use of acaricides, chemical pesticides lead to the dominance of resistant population which is not affected and will be reflected in plant yield (Fraulo and Liburd, 2007). It is stated that *T. urticae* has developed resistance to 93 different active substances and is the most resistant pest among arthropods (Anonymous, 2015). A mite population that develops resistance to an acaricide with any effect mechanism may also develop resistance to other chemicals with the same effect mechanism. Considering that the development of resistance will be delayed and the duration of use of acaricides will be prolonged if drugs with different action mechanisms are used, it is obvious that there is a need to continuously develop new drugs with action mechanisms (Dekeyser, 2004). Today, the development of new pesticides is in decline due to the high costs and the need for very long and intensive research (Sparks, 2013). In addition, the fact that the intensive use of pesticides causes negative effects on the environment and human health and the deterioration of the natural balance should not be ignored (Topakcı and Göçmen, 2008). In addition, in this respect, the biological control method, which brings plant diseases and pests under control by using microorganisms, is seen as a strong alternative to the use of synthetic chemicals (Kotan et al., 2010). Recently, important studies have been conducted on the biological control against many diseases and pests and effective results have been obtained. The fact that they have been prepared and used in many countries over time has been the best proof of how much these products contribute to biological control studies. In addition, considering the awareness of producers and consumers and the increasing trends towards organic products, it is clear that the importance of biological control in integrated control will gradually increase (Uygun et al., 2010). In agricultural production areas where chemical control is not carried out, the natural balance is preserved, although the density of diseases and pests varies from year to year. For this reason, producers should be made aware of biotechnical methods and cultural struggles to protect human and environmental health (Kaplan and Bayram, 2021). Farmers generally prefer the chemical warfare method to solve plant protection problems, and unconscious pesticide applications bring about many negativities in terms of human and environmental health (Kaplan and Saltuk, 2021).

In recent times, the use of entomopathogens in biological control against mite species in cotton fields has been considered within the framework of IPM (Integrated Pest Management) strategies. The utilization of these environmentally friendly entomopathogens, in addition to being a key component of IPM to reduce pesticide loads in the cotton ecosystem, is steadily increasing as a safer biopesticide in cotton fields.

This study, it was aimed to determine the usability of bacteria isolates which is environmentally friendly and do not threaten the health of humans and other living things that can be used as a hopeful

biological control agent against red spider mite species that cause yield losses in cotton fields both in the world and in our country. Moreover, as a result of previous studies on the yield and yield parameters of cotton plants, it is aimed to reveal how the formulation of two bacterial isolates (*Bacillus subtilis* strain PA1 and *Paenibacillus azotofixans* strain PA2) with known PGPR characteristics will have an effect on plant growth and yield in cotton under field conditions.

2. Material and Methods

2.1. Bacterial strains used in this study

In the study; *Bacillus subtilis* PA1 and *Paenibacillus azotofixans* PA2 bacterial strains isolated from the root zone of wild wheat plants, which were determined to have high potential in terms of nitrogen-fixing and phosphate dissolving properties, were used as a mixture (Table 1). This mixture has been tested in terms of its effectiveness on cotton yield in different regions of Türkiye and has been licensed as SS Super Green and offered for commercial use as a microbial product due to its high efficiency. Diagnosis of bacteria was performed using MIS (Microbial Identification System) and BIOLOG system. Bacterial cultures are preserved in the Microbial Culture Collection of Atatürk University, Faculty of Agriculture, Department of Plant Protection, Bacteriology Laboratory. The stomyl cotton variety of May Seed Company was used in the experiments.

Table 1. MIS and BIOLOG diagnostic results and some biochemical properties of bacterial strains

	Strain PA1	**Strain PA2**
MIS Identification Results	*Bacillus subtilis*	*Paenibacillus azotofixans*
MIS Similarity Indexed	0.786	0.845
BIOLOG Identification Results	*Bacillus subtilis*	*Paenibacillus azotofixans*
BIOLOG Similarity Indexed	0.56	0.87
Nitrogen Fixation	K+	+
Phosphate Solubilizing	+	+

2.2. Pathogenicity tests of bacterial strains on cotton

One-month-old cotton seedlings were used for pathogenicity tests of bacterial strains. Fresh bacterial cultures grown on Nutrient Agar (NA) for 24 hours were transferred to Nutrient Broth (NB), developed at 27 ºC in a horizontal shaker incubator rotating at 90 rpm for 48 hours, suspended in sterile distilled water (sdH_2O) and their density was arranged as 10^8 cell/ml on BIOLOG turbidimeter. The seedlings immersed in the prepared bacterial solutions were grown in pots filled with sterile soil for 1 month and then removed. Roots and ground surface parts that were thoroughly washed in tap water were examined to see if there was an infection. Infection occurrence was evaluated as + and no infection was evaluated as –.

2.3. Biological control studies

2.3.1. Establishment of field experiments

The field experiments were set up in 3 replications and each plot of 500 m^2 on 3 volunteer farmer lands selected in Şanlıurfa province, Ceylanpınar, and Harran districts. In the selected experiment areas, attention was paid to the fact that the red spider mite damage was intense in the previous year.

2.3.2. Preparation of the formulation of bacterial strains for field experiments

Bacterial strains (*B. subtilis* PA1 and *P. azotofixans* PA2) purely stored in long-term storage were inoculated into petris containing NA growth culture and left for incubation for 24 hours at 27°C. Colonies taken from the growing fresh cultures to the core were transferred to NB growth culture, and after they were grown in a horizontal shaker incubator for 24 hours, they were inoculated into a fluid growth culture containing NB, which was previously prepared in a fermenter and sterilized in an autoclave at 121 °C for 20 minutes. Bacteria were developed at optimum pH, oxygen, and temperature values for 24 hours and inoculated by mixing the developing cultures into the carrier fluid consisting entirely of organic substances and sterilized by steam at a ratio of 1:10. The content of this carrier

formulation consists of water, various organic substances (seaweed, whey, and herbal extracts) and various substances (Carboxymethylcellülose, Calcium carbonate, Glycerin, Magnesium sulfate) that protect and homogenize the bacterial isolate in its content. Bacteria inoculated organic fluid carrier was left for incubation at 27°C in the bioreactor. Live bacteria countings per milliliter were made and after 48 hours, when the bacterial concentration exceeded $1x10^8$ cell ml^{-1}, it was packaged under completely sterile conditions and stored in a cold room at 5°C for later use (Trinh and Lee, 2022).

2.3.3. Application of formulation of bacterial strains to cotton plant in the field

The seeds coded by soaking in the formulation prepared with bacterial strains for 24 hours, were sown in the field on 27, 28, and, 29 April 2015, respectively, at Şanlıurfa, Ceylanpınar, and Harran locations. On the 30th, 45th, and 60th days after the sowing date, bacteria were sprayed on the soil surface and the ground surface part of the plant for a total of 3 times and reapplied. Applications were made in the late afternoon hours when the weather was cool, with 250 cc of bacterial suspension in 100 liters of water, with a top-back pulverizator so that the leaves were fully covered. A commercial drug containing the active ingredient of Lambda-Cyhalothrin was used as a control.

2.3.4. Counting of red spider mite eggs, nymphs, and adults

In the evaluations made 50 days after cotton sowing, 20 leaves attached to the main stem were taken from each plot, one leaf from the upper and middle parts of the 10 plants selected randomly in the diagonal direction, and one leaf from the middle and lower parts, and the leaves were brought to the laboratory. Here, live eggs, nymphs, and adults were dropped on the vaseline glass surface with the help of a brushing device and counted under a stereo microscope. The average of the values obtained as a result of the counting was given as the average of that location, and the bacterial application was also calculated as the percent effectiveness by comparing it with the control.

2.3.5. Evaluation of plant growth and yield parameters

The efficacy of bacterial application in terms of some plant growth parameters 60 days after cotton sowing was evaluated, in 20 randomly selected plants from each parcel; average plant height (cm) was measured, wood branch (piece $plant^{-1}$), fruit branch (piece $plant^{-1}$), cocoon (piece $plant^{-1}$) and flower (piece $plant^{-1}$) countings were made. Again, in 3 plants selected randomly from each parcel and deracinated; parameters such as average number of lateral roots (number $plant^{-1}$), plant crown diameter (cm), main root length (cm), cocoon diameter (mm), and root collar diameter (mm) were also evaluated. In the evaluations in terms of yield and yield parameters made 3 days before September 24, 2015, the harvest date, the average opened and unopened cocoons were counted in 20 randomly selected plants from each parcel (pieces $plant^{-1}$), the average cocoon weight (gr) was calculated, and the cotton un-seed yield in 60 plants (gr) was taken, then the number of plants per decare and cotton un-seed yield per decare (kg $decare^{-1}$), ginning yield (%) and cotton gin yield (kg $decare^{-1}$) were determined.

2.4. Analysis of the results

All data were analyzed using the SPSS statistical package software program. The differences between the averages were determined according to the Duncan Multiple Comparison Test (Julie, 2007).

3. Results and Discussion

As a result of the pathogenicity test performed to determine whether bacterial strains are pathogenic in the cotton plant, it was determined that both strains were not pathogenic.

50 days after sowing; the results obtained in the evaluations made to determine the effectiveness of bacteria applications on red spider mite eggs, nymphs, and adults in the cotton plant are given in Table 2.

According to the counts made on the 50th day of the experiment, it was observed that bioagent bacteria applications caused significant decreases in egg, nymph, and adult numbers of red spider mites in all locations compared to control applications, and these decreases were statistically significant. In control applications, the highest number of eggs were counted in Şanlıurfa (20.25%), followed by Harran (20.05%) and Ceylanpınar (17.7%) locations. In the plots where bacteria were applied, the number of eggs decreased in Harran (8.65%), Ceylanpınar (7.95%), and Şanlıurfa (6.95%) locations,

respectively, unlike the control. In control applications, the most nymphs were detected in Harran (20.30%) compared to the nymph average, followed by Şanlıurfa (17.30%), and Ceylanpınar (15.45%) locations, respectively. In the plots where bacteria were applied, a decrease in nymph numbers was detected in Şanlıurfa (7.55%), Harran (7.25%), and Ceylanpınar (6.85%) locations. 50 days after sowing, the highest number of adults were counted in the control applications in the Center (29.05%), followed by the control applications in Ceylanpınar (26.85%) and Harran (26.80%) locations, respectively. In the plots where bacteria were applied, the number of adults decreased in Ceylanpınar (11.09%), Şanlıurfa (10.55%), and Harran locations (9.75%), respectively. On the same counting date, nymphs and adults were seen the most in Harran control application with 47.05%, Şanlıurfa with 45.35%, and Ceylanpınar control applications with 41.30% followed this location. In bacterial applications, nymph and adult countings were made at Ceylanpınar, Şanlıurfa, and Harran locations at 18.75%, 18.10%, and 17.00%, respectively (Table 2).

Table 2. Efficacy of bioagent formulation tested against Tetranychus cinnabarinus and *Tetranychus urticae* in cotton field experiments

Applications-Locations	**Egg*** **(number leaf^{-1})**	**Nymph (number leaf^{-1})**	**Adult (number leaf^{-1})**	**Nymph + Adult (number leaf^{-1})**
Control-Şanlıurfa	20.25 a	17.30 b	29.05 a	45.35 a
Control-Ceylanpınar	20.05 a	15.45 b	26.85 a	41.30 b
Control-Harran	17.7 a	8.65 a	26.80 a	47.05 a
Bioagent formulation-Şanlıurfa	6.95 b	7.55 c	10.55 b	18.10 c
Bioagent formulation-Ceylanpınar	7.95 b	6.85 c	11.9 b	18.75 c
Bioagent formulation-Harran	8.65 b	7.25 c	9.75 b	17.00 c
LSD	**33.55**	**30.57**	**19.34**	**15.82**
CV	**2.86**	**2.39**	**1.15**	**5.09**
Average of bioagent formulation	7.85 A	7.21 A	10.73 A	17.95 A
Average of control	19.33 B	17.68 B	27.56 B	44.56 B
LSD	**1.66**	**1.42**	**1.36**	**1.88**
CV	**33.73**	**31.41**	**19.6**	**16.6**

*There is no statistical difference between the values expressed with a similar letter in the same column (P<0.01).

The effect of applications 60 days after sowing on some plant growth and yield parameters in cotton plants is given in Table 3.

When the plant growth and yield parameters were evaluated in the control plots and the plots where the bioagent formulation was applied in the experiments established in three different locations, an increase was observed in the plots where the bioagent formulation was applied (Table 3). According to the location averages, in the control parcels; the average plant height is 82.06 cm, the plant crown diameter is 37.02 mm, the number of wood branches is 1.82 piece plant^{-1}, the number of fruit branches is 7.68 piece plant^{-1}, number of flowers is 6.35 piece plant^{-1}, cocoon diameter is 11.10 mm, number of cocoons is 12.36 piece plant^{-1}, root collar diameter is 4.91 mm, main root length is 29.33 cm, number of lateral roots is 9.03 piece plant^{-1}, number of opened cocoons is 35.58 piece plant^{-1}, number of unopened cocoons is 33.70 pieces and average cocoon weight is 10.10 g plant^{-1}. In the parcels where bioagent formulation is applied; the average plant height is 115.01 cm, plant crown diameter is 69.66 mm, number of wood branches is 2.15 piece plant^{-1}, the number of fruit branches is 17.68 piece plant^{-1}, number of flowers is 7.70 piece plant^{-1}, cocoon diameter is 16.14 mm, number of cocoons is 28.00 piece plant^{-1}, root collar diameter is 7.99 mm, main root length is 37.33 cm, number of lateral roots is 26.70 piece plant^{-1}, the number of opened cocoons is 69.68 piece plant^{-1}, the number of unopened cocoons is 88.71 piece plant^{-1} and average cocoon weight 15.62 g plant^{-1} (Table 3). In all three experimental areas, increases were observed in terms of all parameters evaluated in bacterial applications, and these increases were found to be statistically significant compared to the control plots. In bioagent formulation applications, increase of 40.15% in plant height, 88.16% in plant crown diameter, 18.13% in the number of wood branches, 130.20% in the number of fruit branches, 21.25% in the number of flowers, 45.40% in the cocoon diameter, 126.53% in the number of cocoons, 62.72% in the diameter of the root collar,

195.68% in the number of lateral roots, 163.23% in the number of unopened cocoons, 95.84% in the average cocoon weight and 54.65% in the number of opened cocoons were observed (Table 3).

In the evaluation made at the end of the harvest; in control parcels; the average cotton un-seed yield is 57.36 g plant^{-1} and 573.66 kg decare^{-1}, the cotton gin yield is 39.50% and fiber yield is 226.20 kg decare^{-1}. In the parcels where bioagent formulation was applied; cotton un-seed yield is 103.27 g plant^{-1} and 1032.76 kg decare^{-1}, cotton gin yield is 39.83% and the fiber yield is 412.34 kg decare^{-1}. In all three experimental areas, increases were observed in terms of cotton un-seed yield and fiber yield in bioagent formulation applications, and these increases were found to be statistically significant compared to control plots. These increases in cotton plants in bioagent formulation applications; seed cotton yield was 80.03% and fiber yield was 82.17% (Table 3).

Table 3. The effect of bioagent formulation on plant growth and yield parameters in cotton field experiments

Applications-Locations	**Plant height (cm)**	**Plant crown diameter (cm)**	**The number of**		
			Wood branches	**Wood branches**	**Wood branches**
Control-Şanlıurfa	93.05±23.16b	37.07±4.86ab	1.68±0.72a	8.00±2.53b	5.05±2.76a
Control-Ceylanpınar	89.05±23.86b	41.05±7.50b	2.10±0.78ab	9.00±2.53b	6.00±2.80ab
Control-Harran	64.10±21.28a	32.95±10.47a	1.68±0.72a	6.05±2.35a	8.00±2.61c
General average (Control plots)	**82.06**	**37.02**	**1.82**	**7.68**	**6.35**
Bioagent formulation-Şanlıurfa	120.05±26.71c	75.05±11.62d	2.15±0.66ab	17.05±2.30d	8.05±3.08c
Bioagent formulation-Ceylanpınar	118.05±20.23c	69.00±11.87cd	2.31±0.79c	21.00±3.17e	7.05±2.56bc
Bioagent formulation-Harran	106.95±19.89c	64.95±11.27c	2.00±0.56ab	15.00±3.64c	8.00±2.44c
General average (Plots with bioagent formulation applied**)**	**115.01**	**69.66**	**2.15**	**17.68**	**7.70**
Average increase (%)	**40.15**	**88.16**	**18.13**	**130.20**	**21.25**
Applications-Locations	**Diameter of cotton boll (mm)**	**The number of cotton boll (per plant)**	**Root collar diameter (mm)**	**The main root length (cm)**	**The lateral root number (per plant)**
Control-Şanlıurfa	10.29±3.15a	12.10±4.27a	4.72±1.22a	30.00±4.25b	6.00±0.79a
Control-Ceylanpınar	11.99±2.76a	13.05±3.48a	5.00±1.38a	33.00±4.53b	12.05±3.89b
Control-Harran	11.04±2.91a	11.95±3.88a	5.02±1.30a	25.00±6.83a	9.05±3.54ab
General average (Control plots)	**11.10**	**12.36**	**4.91**	**29.33**	**9.03**
Bioagent formulation-Şanlıurfa	17.53±4.68b	33.00±10.51c	7.94±2.51b	38.05±8.19c	28.05±8.56c
Bioagent formulation-Ceylanpınar	15.95±4.94b	26.00±9.53b	8.05±2.46b	42.00±7.97c	27.00±7.31c
Bioagent formulation-Harran	14.95±4.65b	25.00±6.64b	8.00±2.45b	31.95±8.99b	25.05±6.59c
General average (Plots with bioagent formulation applied**)**	**16.14**	**28.00**	**7.99**	**37.33**	**26.70**
Average increase (%)	**45.40**	**126.53**	**62.72**	**27.27**	**195.68**
Applications-Locations	**The number of**		**Mean boll weight (g plant^{-1})**		
	Boll opening (per plant)	**Not boll opening (per plant)**			
Control-Şanlıurfa	29.60±6.02b	40.00±13.02b	10.91±2.30b		
Control-Ceylanpınar	60.10±16.59c	50.10±13.65c	11.07±2.27b		
Control-Harran	17.05±11.94a	11.00±5.67a	8.02±3.13a		
General average (Control plots)	**35.58**	**33.70**	**10.10**		
Bioagent formulation-Şanlıurfa	82.05±15.44d	106.05±17.24f	17.36±3.12d		
Bioagent formulation-Ceylanpınar	75.00±13.34d	93.05±18.21e	15.52±3.11c		
Bioagent formulation-Harran	52.00±13.69c	67.05±15.85d	13.98±2.38c		
General average (Plots with bioagent formulation applied**)**	**69.68**	**88.71**	**15.62**		
Average increase (%)	**95.84**	**163.23**	**54.65**		
Applications-Locations	**Seed cotton yield (kg da^{-1})**	**Ginning yield (%)**	**Lint yield (kg da^{-1})**		
Control-Şanlıurfa	610.00b	39.50	240.95		
Control-Ceylanpınar	630.50b	39.00	245.89		
Control-Harran	480.50a	40.00	192.20		

Table 3. The effect of bioagent formulation on plant growth and yield parameters in cotton field experiments (continued)

General average (Control plots)	**573.66**	**39.50**	**226.34**
Bioagent formulation-Şanlıurfa	1049.90d	40.00	422.32
Bioagent formulation-Ceylanpınar	1120.70 d	40.00	448.28
Bioagent formulation-Harran	927.70 c	39.50	366.44
General average (Plots with bioagent formulation applied)	**1032.76**	**39.83**	**412.34**
Average increase (%)	**80.03**	**0.83**	**82.17**

*The differences between the averages indicated by different letters in the same column were found to be significant at the p=0.05 level.

The appearance of some plant growth parameters of the plants that were applied bioagent bacteria formulation and control application in cotton field experiments is given in Figure 1.

Figure 1. Appearance of the plants with bioagent bacteria formulation and control application in terms of some plant growth parameters.

In recent years, there has been a notable increase in the number of studies focusing on the utilization of bacteria for biological control, not only in Türkiye but also worldwide. A new approach in biological control is to promote plant systemic resistance by using plant growth-promoting bacteria (PGPR). Especially with the use of PGPRs for biological control, the protection of plant health and an increase in production can be achieved. Promoting plant resistance to infection by pathogen or acquired systemic resistance (SAR: Systemic Aquaried Resistance), which can be defined as the reaction of the plant against the infection of the pathogen, which is not found naturally in the plant but is physiologically

acquired by the stimulus of biocontrol organisms, is the most studied subject. In particular, SAR, promoted by bacteria is the most effective control method used against plant diseases of soil, seed, or leaf origin. With the use of PGPR for biological control, an increase in production can be achieved along with the protection of plant health. The most commonly used microorganisms in this field are different species of *Bacillus, Pseudomonas, Azotobacter, Burkholderia, Mycorrhyzae, Streptomycetes*, *Enterobacter*, *Verticillium*, *Agrobacterium*, *Aspergillus* and *Trichoderma,* especially those such as *Bacillus subtilis*, *B. cereus*, *B. pumilus*, *B. megaterium* and *B. sphaericus, Pseudomanas cepacia, P. putida, P. fluorescens* and *P. polymyxa,* and they can be used effectively in disease and pest control. (Halos and Zorilla, 1979; Guo et al., 1996; Aysan et al., 1999; Altın and Bora, 2001; Aysan et al., 2003; Guo et al., 2004; Özaktan and Bora, 2006; Akgül and Mirik, 2008).

Compant et al. (2005), emphasized that the use of bacteria in sustainable agriculture has a special status for sustainable agriculture because some of these bacteria have a characteristic that promotes plant growth as well as the ability to control pests. It has been determined that *Bacillus sphaericus* is used effectively in the control of many pests, including mites (Falcon, 1985). It has also been noted that biopesticides based on metabolites of another *Bacillus* type *B. thuringiensis* (Bt) and *Streptomyces avermitilis* bacteria have been used to control *T. urticae* in recent years (Chapman and Hoy, 2009; Brown et al., 2017). Dutton et al. (2003), reported that when Dipel obtained from *B. thuringiensis* subsp. *kurstaki* (HD-1) was applied to the corn plant in spray form, there was a slight decrease in the growth rate of *T. urticae* compared to the control. The researchers concluded that this was due to the decrease in the number of eggs laid by females in plants sprayed with Dipel. In another study, Alper et al. (2013), in their study examining the effect of a spore-crystal mixture of 31 natural *B. thuringiensis* isolates on *T. urticae* nymphs, noted that some isolates may have a certain potential in the development of biopesticides that especially retard the growth of nymphs. Moreover, Neethu et al. (2016), stated that toxin-producing bacteria such as *B. thuringiensis* have been widely used as bio-acaricide in recent years.

Abou Zaid et al. (2018), tested the efficacy of *Lysinibacillus sphaericus* and *Bacillus amyloliquefaciens* bacterial isolates, which are used as bio-control agents as well as promoting plant growth, on the harmful *T. urticae* in beans, found that 3 days after the application as a spray on the leaves, there was a 37% decrease in the *T. urticae* population. Similarly, it has been documented that *Pseudomonas aeruginosa* caused a 100% mortality among adult female *T. urticae* after 72 hours of application at a concentration of 10^7 cfu mL−1 via spray method. For *Bacillus subtilis*, the application resulted in approximately 80% mortality, while the treatment involving *Lysinibacillus sphaericus* led to slightly over 95% mortality (Emam, 2021; Jakubowska et al., 2022). In another study, Zenkova et al. (2020) conducted laboratory experiments to determine the impact of two bacterial species (*Streptomyces avermitilis* and *Bacillus thuringiensis*) on *T. urticae*. They obtained maximum mortality rates of 90% to 100% for adults and 91% to 99% for nymph stages, respectively, using biopesticides based on both *S. avermitilis* and *B. thuringiensis*. The effect of *Acinetobacter* sp., *Bacillus subtilis,* and *B. qassimus* species against *T. urticae*, which causes damage to eggplant, was tried to be determined under laboratory and greenhouse conditions, and the highest mortality rate was found in *Acinetobacter* sp. (87.15% in the laboratory, 77.29% in the greenhouse) after three days of spray application and when *B. subtilis* and *B. qassimus* were used, the mortality rates seven days after the application were 72.22% and 67.11% in the laboratory, and 70.74% and 65.19% in the greenhouse, respectively (Al-Azzazy et al., 2020).

In another study, the efficacy of *Pseudomonas putida* Biotype B belonging to the Pseudomonas type, which has been used successfully in biological control, was investigated against *T. urticae*, and for this purpose, newly emerged mated females were administered *P. putida* biotype B (108-109 colonies/ml) suspensions were applied by spraying and immersion. In the bacterial spraying process, a 100% effect on the mites was determined, similarly, the minimum number of viable eggs was also determined in the spraying process, and it was noted that this process was more effective than the immersion method (Aksoy and Yılmaz, 2008).

The use of high levels of insecticides causes the pest to show resistance to these chemicals and causes problems such as phytotoxicity caused by these insecticides (Ioannidis et al., 1991; Stewart et al., 1997; Mota-Sanchez et al., 2000). For these reasons, especially in recent years, researchers have focused on the use of bacterial and fungal pathogens in the biological control of many pests, including red spider mites. In this research; a bioagent formulation mixture consisting of free nitrogen fixer and phosphate solvent bacteria *Bacillus subtilis* PA1 and *Paenibacillus azotofixans* PA2, which were isolated from the root rhizosphere of wild and cultivated plants in various previous studies, was applied

to cotton by seed coding and ground surface hitch spray method. In this way, it has been determined that the mixture can be used successfully in the control against red spider mites that damage cotton plants in field conditions. It was determined that there was an increase in the number of cocoons in the cotton plant, the number of cocoons opened, and the average cocoon weight in bioagent formulation practices. These large increases in the number of opened and unopened cocoons show that the plant is well-fed in terms of nitrogen thanks to nitrogen fixation of the bacteria. Since this good nutritional state also prolongs the vegetative development process of the plant, it has also caused a great increase in the number of unopened cocoons. In bioagent formulation applications, an 80.03% increase in cotton un-seed yield and an 82.17% increase in fiber yield in cotton plants is because the increase in cocoon number and weight is reflected in the yield, which is of great importance.

Conclusion

This study has tried to determine the usability of the bacterial formulation used as a biological control agent that does not threaten human and environmental health in the control of *T. urticae* and *T. cinnabarinus*, which cause significant damage to cotton, and it has been determined that it can be used successfully in the control. It was also determined that this formulation significantly increased yield and quality by encouraging the development of cotton. It is envisioned that the obtained bioagent formulation can be used successfully in cotton cultivation and at the same time, it will be an alternative product against chemical pesticides and fertilizers that are used excessively in cotton. It is necessary to focus on such studies and to introduce more conscious disinfection and pest management practices. Thanks to more detailed studies on this subject that will be conducted later, alternative methods to chemicals will be put into practice.

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Research Article

Yield Prediction and Recommendation of Crops in India's Northeastern Region Using Machine Learning Regression Models

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Abstract: Agriculture has a big impact on society because it is essential for a large percentage of our food. The issue of hunger is getting worse because of a growing population in many nations, resulting in food shortages or insufficiencies. To meet the world's food needs, it is ever more crucial to provide crop protection, conduct detailed land surveys, and predict crop yields. To calculate the estimated number of crops that are produced in a year, this research focuses on the use of machine learning techniques to predict crop yield and recommend crops with the highest yield and profitability in the Northeast region of India. The crop market's fluctuations in prices may be controlled with the aid of this information. To estimate agricultural crop yields, this study accurately evaluates a range of machine learning regression models, such as Linear Regression, Decision Tree, Random Forest, Gradient Boosting, XGBoost (eXtreme Gradient Boosting), and AdaBoost. With a 0.98 R^2 score for the XGBoost and 0.96 for the Random Forest, they performed better than the other models. By evaluating crop yields along with their corresponding market prices and costs, we have determined their profitability. As a result, we have provided recommendations for the top five profitable crops in India's Northeastern region.

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1. Introduction

In the agricultural industry, crop production prediction is a crucial issue. Every farmer strives to understand crop output and whether it lives up to their expectations, which involves assessing the farmer's prior experience with the crop to anticipate the yield. To manage agricultural risk, accurate crop history data is essential. In order to effectively plan and make decisions regarding resources, which are critical to agriculture's role in supplying the world's food needs, crop yield predictions must be correct. In this context, machine learning becomes a useful technique to improve agricultural yield prediction models.

Machine learning algorithms can produce precise projections of crop yields for certain regions or farms by recognizing complex patterns and relationships within this information. The results of applying machine learning algorithms to a data collection of sugarcane crop information from Karnataka, India, are demonstrated by (Renuka and Sujata, 2019). Regression approaches are a useful technique to produce a forecast for the area. The regression method may be utilized for crop forecasts

for the area with satisfactory results, it is a useful tool for yield prediction. The R^2 statistics result is considered as good for crop production prediction (Shastry et al., 2017). Using a variety of machine learning techniques, a method for predicting the classification of production that depends on macro- and micronutrients is presented (Singh et al., 2017). The algorithm's precision was improved with the use of stacking regression (Potnuru et al., 2020). After analyzing the soil dataset category is predicted (Paul et al., 2015). It is determined that the crop yield is a classification rule from the expected soil category. For predicting crop yield, naive Bayes and k-Nearest Neighbour algorithms are employed. The indicated future study involves developing effective models utilizing other classification methods like support vector machines.

To improve classification performance, diversity measurements depending on the correlation between errors are employed to compute the classifiers' correlation approach (Yiang, 2011). Using Random Forest, (Everingham et al., 2016) proposed a method for forecasting sugarcane production. The parameters used in this work include the biomass index, climate data (such as rainfall, radiation levels, and temperature), and production data from the two years prior. In a study by (Deepa et al., 2023), the idea is to provide farmers with internet access through a smartphone application that predicts yield. In the GPS, the user enters the location and soil type. Algorithms can anticipate agricultural yields for crops chosen by the user as well as select the list of crops that will provide the greatest profits. Crop productivity estimates are made using a variety of different machine learning methods. A 95% accuracy percentage was achieved by random forest model among them. Two distinct strategies were put out (Ung and Mittrapiyanuruk, 2018) for separating the information about the plot's characteristics from the category of sugarcane production at the plot level. The strategies are based on using ensemble models Gradient Boost and Random Forest. The yield prediction model's accuracy was improved by modifying the bias, weight, and optimizer in a multilayer perceptron neural network. The suggested model predicts crop yield using an ANN with a three-layer neural network (Kale and Patil, 2019). The requirements and strategies for developing a precision agricultural software model are discussed in the paper (Babu, 2013). It thoroughly examines precision farming's fundamentals.

In (Kumar et al., 2015), the elements that influence crop choices, such as rate of production, price in the market, and governmental policies, are analyzed. This study suggests a method that fixes the selection issue of crops and raises the crop's net yield rate. It proposes that a season's worth of crops be chosen while taking the weather, crop type, soil type, and water density into account. The research (Savla et al., 2015) compares the classification algorithm's yield prediction capabilities in precision agriculture. These algorithms are used to predict the production of a soybean crop using data that has been gathered over several years. In this study, various models were employed for the yield prediction techniques. Here, the Bagging technique of ensemble learning is considered the best algorithm for yield prediction. A machine learning (ML) model is designed to forecast agricultural output. The data was gathered and educated using supervised machine learning with six different regression models to estimate crop yields. Random Forest Regressor which is an ensemble model fared better than the other models, with an MAE of 468.16 and a Cross-Validation score of 0.6087 (Panigrahi, 2023). Machine learning techniques are used to generate recommendations for crops based on geological and climatic characteristics. The dataset for the five different crops, including rice, ragi, gram, potato, and onion, has been considered when designing the recommendation crop system (Garanayak et al., 2021).

We have proposed a recommendation system that will predict how much crop will be gathered from a given agricultural area and recommend the crops having the highest crop yield value and profitability. This approach makes predictions using a variety of data sources, including area, production, previous yield records, and other relevant elements. This study uses regression models to accurately predict agricultural production in the future for the Northeast region. Regression techniques such as XGBoost, Gradient Boost, Random Forest, AdaBoost, Decision Tree, and Linear Regression have been used for predicting crop yield**.**

2. Material and Methods

2.1. Framework for our proposed model

The proposed methodology for our proposed recommendation model has been illustrated below in Figure 1**.**

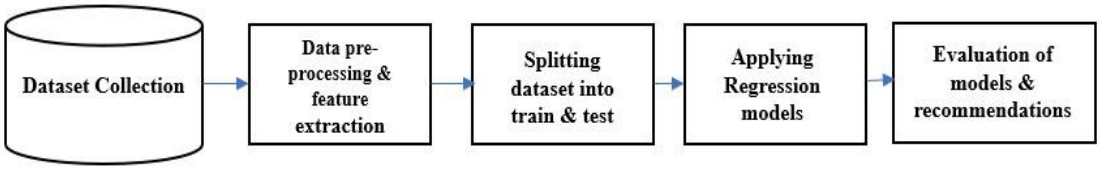

Figure 1. Framework for methodology.

2.1.1. Data collection

The dataset utilized in this research originates from (Kumar, 2018), and has been specially selected to address the unique agricultural context of the Northeastern area of India. A thorough study of crop-related trends and dynamics in this area is made possible by the dataset's wide temporal range, which extends from 1997 to 2015. This dataset is a useful tool for understanding the dynamics of the region's agriculture, improving predictions of crop yields, and making suggestions for the development of a sustainable agricultural sector. Parameters in the dataset are as follows: State Name- this field indicates the name of the Indian state from where the Northeastern region agricultural data was gathered, District Name- this field indicates the district level data of each state, Crop Year- this field indicates the year in which the data was recorded, Season- this field indicates the season for understanding the pattern of crops in different seasons, Crop- this field indicates the crop name cultivated in the respective years, Area- The area parameter indicates the amount of land (in hectares (ha)) that is being used to grow a specific crop in a given year and district. This metric is important for yield calculations as it helps in understanding the scope of agricultural activity, and Production- this metric is used to describe the overall agricultural output (measured in metric tons (MT)) for the selected crop, district, and year. It is an essential variable for crop yield analysis because it is directly related to the agricultural productivity of the area.

2.1.2. Data pre-processing

In this stage, several measures were undertaken to ensure that the data was correctly prepared for analysis. This included dealing with data ranges, missing numbers, and identifying important features. The model was implemented using high-level programming language Python which is known for its readability and simplicity. We have used the specific version 3.8.5 of Python 3.x series which includes various useful libraries such as Scikit-Learn, NumPy, and Pandas. The tool that we have used to run the Python program is Jupyter Notebook. It is an interactive open-source web tool that enables us to create and share documents with real-time code, equations, visuals, and text. Jupyter Notebook is a flexible tool that integrates interactive scripting, documentation, and visualization into a single environment. It is frequently used by data scientists, researchers, educators, and experts in domains where data analysis and interactive computing are crucial for tasks including data exploration, machine learning, scientific research, and collaborative work. The Pandas library's isnull() method was used to address missing values in the dataset. This approach identified any null values that were present in the dataset. Once identified, the same library's fillna() function was used to replace the missing data. The fillna() function was used to replace missing values in columns containing numerical characteristics with the mean values of the associated columns.

2.1.3. Feature Selection using correlation matrix

The correlation matrix employing Pearson correlation is used here for feature selection. A statistical tool called Pearson correlation is used to express the linear relationship between two continuous variables. A correlation matrix (Figure 2) was created to examine the relationships between the parameters in the provided dataset. The correlation matrix demonstrates favorable relationships between yield and production as well as production and area. These findings suggest that greater agricultural areas and higher levels of output are often associated with higher yields. Understanding these interactions can help influence decision-making processes in agricultural practices linked to optimizing yield, production techniques, and land utilization. Therefore, the crop yield which we have

calculated using the area and production that were provided in the dataset is considered an important parameter for our prediction analysis. After the process of feature selection, with a division ratio of 75% or 25%, the loaded data is divided into two sets of training and testing.

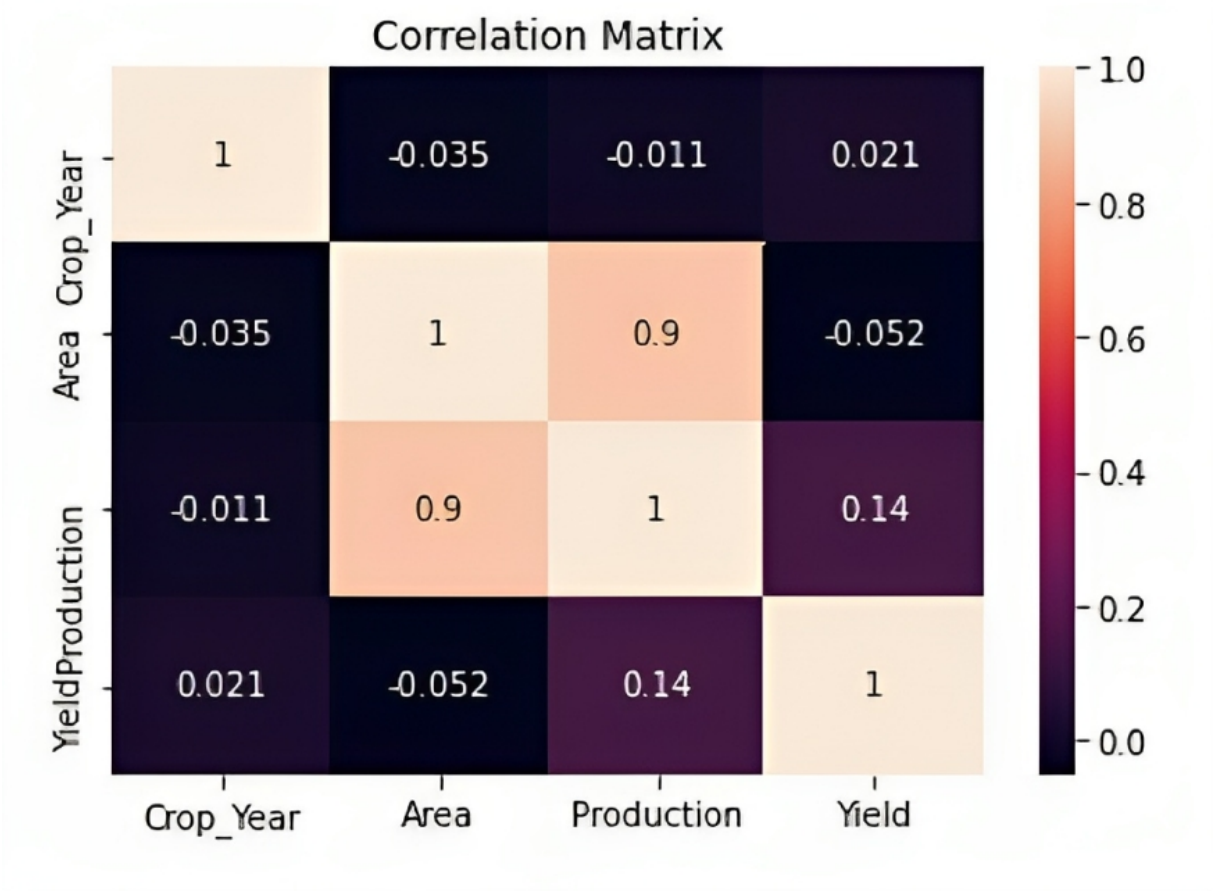

Figure 2. Correlation matrix.

2.1.4. Machine learning models used

For the purpose of predicting crop yield, we have used regression models and ensemble models. A regression model is used to examine the relationship between a target or dependent variable and one or more independent variables or characteristics. A regression model's main objective is to understand and predict the values of the dependent variable based on the values of the independent variables. And, a machine learning technique known as an ensemble model combines the predictions of various base models (commonly referred to as "weak learners") to produce a more reliable and precise prediction. The concept underlying ensemble learning is that by combining the forecasts of various models, the resulting ensemble model can frequently outperform any single base model. The regression models- Decision Tree, Linear Regression, and ensemble models- Random Forest, GradientBoost, XGBoost (eXtreme Gradient Boosting), and AdaBoost are used in this research. Brief descriptions of all the models used in our proposed approach are given below:

Linear Regression: Linear regression is used to find the relation between a dependent variable and one or more independent variables.

Random forest regressor: It is an ensemble model with a collection of decision trees. Each tree is built using a randomly selected subset of the data and features.

Gradient Boost: An ensemble model, Gradient Boosting incrementally assembles a group of ineffective prediction models, typically decision trees, to produce an effective predictive model. It operates by fitting new models iteratively to the residual errors of the previous models while attempting to reduce the mistakes caused by the prior models.

XGBoost: Extreme gradient boosting (XGBoost) Regressor is an advanced version of gradient boosting that builds extremely precise regression models. It includes various improvements to enhance efficiency and handle complex datasets.

Decision Tree: It is a popular supervised machine-learning technique used which can be used in regression and classification problems. Each internal node represents a feature, each branch represents a decision or rule based on that attribute, and results are represented by the leaf nodes.

AdaBoost: An ensemble model Adaptive Boosting or AdaBoost operates by initially assigning equal weights to each training instance's weights and then using the data to train a weak model. The weighted errors of the preceding models are the focus of the next models.

2.1.5. Evaluation of model

Several evaluation metrics, such as R^2 Score, Root Mean Squared Error (RMSE), cross-validation (CV), and Mean Absolute Error (MAE), are used to evaluate the model's performance.

Mean Absolute Error: It is calculated by adding up the absolute differences between each observation's actual and estimated values and dividing by the total number of observations.

$$\textbf{Formula for MAE,} \quad \frac{1}{n}\sum_{i=1}^{n}|y_i - y_i'| \tag{1}$$

Here (1),
n is the number of observations
y_i represents the observation's actual values
y_i' represents the estimated or predicted values

RMSE: It calculates the average squared difference between the estimated and actual values. It gives a measurement of the overall prediction error of an estimator.

$$\textbf{Formula for RMSE,} \quad \sqrt{\sum_{i=1}^{n}\frac{(y_i'-y_i)^2}{n}} \tag{2}$$

Here (2),
n is the number of observations
y_i represents the observation's actual values
y_i' represents the estimated or predicted values

R^2 Score: The coefficient of determination, also referred to as R^2 score, is a statistical metric used to evaluate the quality of fit of a regression model.

$$\textbf{Formula for } R^2\textbf{,} \quad 1-\frac{SSR}{SST} \tag{3}$$

Here (3),
SSR represents sum squared regression which is the square of the residuals.
SST is the total sum of squares which is the sum of the data's distance from the mean squared.

Cross_validation score: Here, the available dataset is split up into subsets or folds and the score is determined by averaging the results from each iteration.

3. Results and Discussion

We have proposed a recommendation model that can predict agricultural yields for several crops in the Northeast region of India and recommend crops having the highest crop yields and profitability. The data was utilized to train six different regression models, including Linear Regression, Decision Tree Regression, GradientBoost Regression, Random Forest Regression, AdaBoost Regression, and XGBoost Regression, in order to provide accurate predictions of crop yields. R^2 Score, Root Mean Squared Error (RMSE), cross-validation (CV), and Mean Absolute Error (MAE) for the models are presented in Table 1.

Table 1. Accuracy of the regression models in terms of MAE, RMSE, R^2 Score, and CV Score

Models	**RMSE**	**MAE**	**R^2 Score**	**CV Score**
Random Forest	2475	607	0.96	0.73
AdaBoost	5422	2421	0.85	0.53
Decision Tree	3128	683	0.95	0.66
XGBoost	2090	652	0.97	0.75
GradientBoost	3426	1018	0.94	0.72
Linear Regression	5039	2380	0.87	0.65

Figure 3 shows the MAE and RMSE for trained models. The RMSE value, shown in blue on the graph, is lowest for XGBoost Regression, followed by Random Forest and Decision Tree. The orange graph displays the MAE value for Random Forest Regression, which is the lowest, followed by XGBoost Regression.

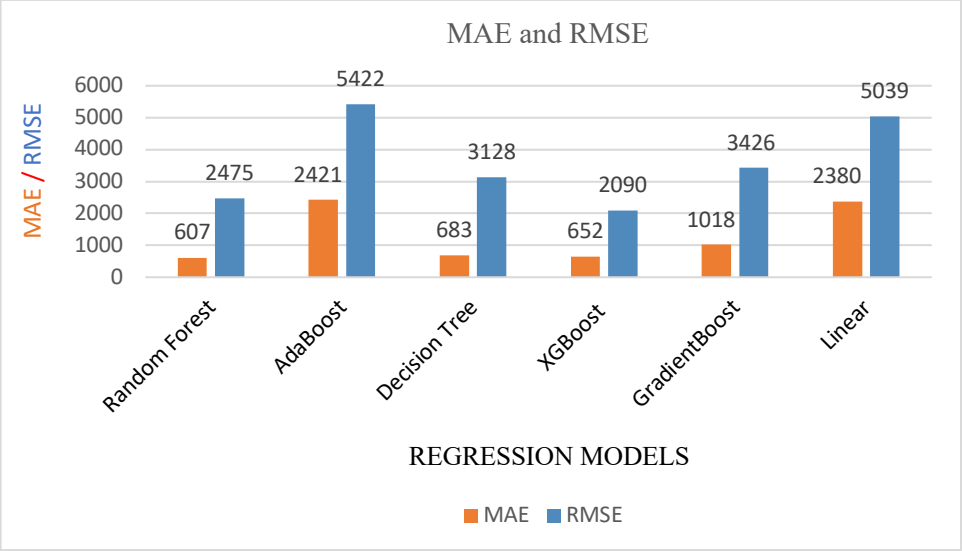

Figure 3. Graphical representation of MAE and RMSE.

The R^2 and Cross-Validation scores for the trained Regression models are graphically depicted in Figure 4. The optimum R^2 score is 1. The orange graph displays the R^2 score, and the blue graph displays the cross-validation score. XGBoost has the highest cross-validation score, followed by Random Forest, Gradient Boost, and Decision Tree. The XGBoost has the highest R^2 Score followed by Random Forest and Decision Tree.

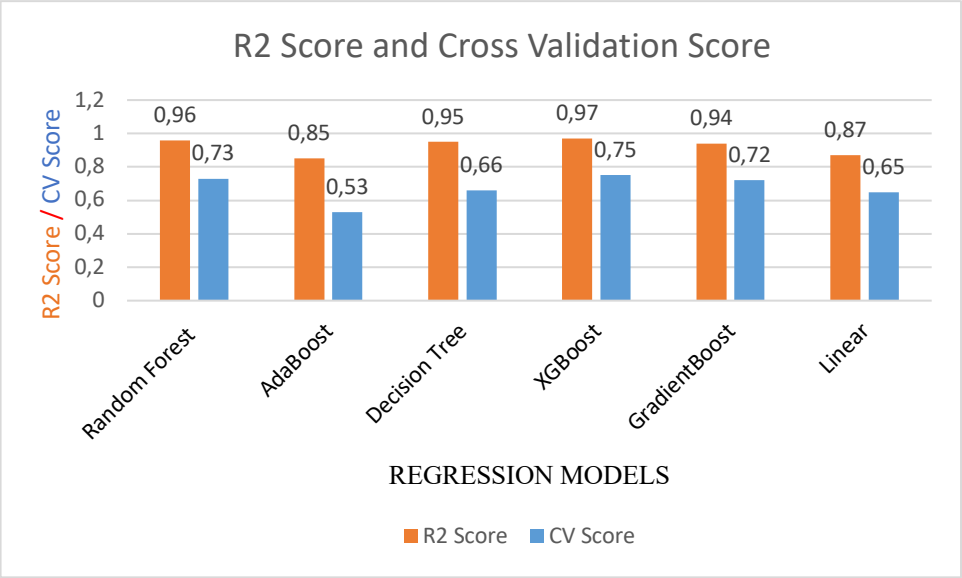

Figure 4. Graphical representation of R2 Score and CV Score.

Table 2. Accuracy of the XGBoost before and after parameter optimization

Models	**Before parameter optimization**	**After parameter optimization**
RMSE	2090	1972
MAE	652	548
R2 Score	0.97	0.98
CV Score	0.75	0.77

We further analyze and apply hyperparameter optimization to the XGBoost model as its accuracy was higher than any other model's. The accuracy was greatly enhanced once the model's parameters were optimized as shown in Table 2. In Table 2. We have represented the evaluation result for XGBoost before and after applying hyperparameter tuning for parameter optimization.

The model is further used to determine the crop having a high yield, the top five crops having high yield are presented in Table 3.

Table 3. The top 5 crops have high yields in terms of metric tons per hectare

Crop_Name	**Highest Average Yield (MT/ha)**
Sugarcane	24.374
Cabbage	11.833
Banana	10.626
Tapioca	10.472
Ginger	9.0000

Then we tried to find out the most profitable crop by considering the cost and market price of the crops having higher yield value (Table 3). The data for the market price of crops are collected from the website commodityonline.com and average cost price of the crops Ginger, Tapioca, Cabbage, Banana, and sugarcane are respectively collected from the following sources (Asia Farming, 2023), (Times of India, 2019), (Agri farming, 2023), (Patowary et al., 2022) and (Government of India, 2023). Also, a complete link of all the sources is given in the reference section. Rank wise recommendation of crops based on their profitability is shown in Figure 5.

The findings (Table 2) show that while sugarcane has the highest yield, it also has the lowest profitability (Figure 5). This finding draws attention to an important aspect of agricultural decision-making. It might not be the best course of action to focus solely on yield data when choosing crops. Instead, a thorough analysis that considers both yield and profitability becomes necessary. Farmers and

decision-makers can choose which crops to grow more intelligently by taking into consideration both criteria, ensuring not just good yields but also long-term financial gains.

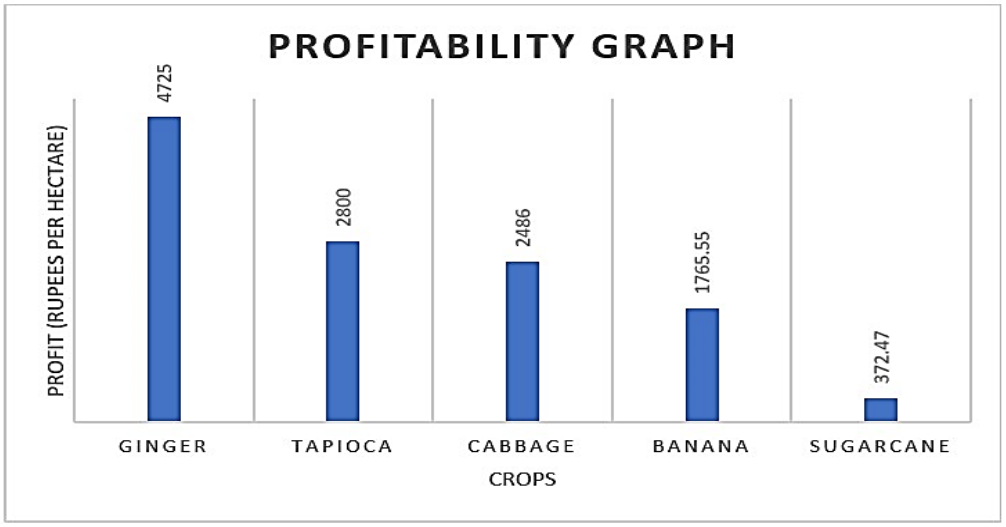

Figure 5. Crops recommendations rank wise.

4. Conclusion

The purpose of this research study is to create a recommendation model using machine learning techniques that can predict crop yield and recommend crops with the highest average yield and profitability. The dataset pertaining to the different crops grown in the Northeast region is considered by the recommendation system. The dataset for these crops is first preprocessed, and multiple regression models such as Decision Tree Regression, Linear Regression, and ensemble models, including XGBoost, Gradient Boost, Random Forest, and AdaBoost, are then employed to predict the yield and its accuracy. XGBoost outperforming the other models is then further enhanced through hyperparameter optimization to enhance the accuracy of our model. Ginger, Tapiaco, cabbage, banana, and sugarcane are the crops recommended rank wise for their maximum crop output and profitability. The highest R^2 Score that could be obtained using the models provided above is 98%. An advanced ensemble technique can be investigated in the future to increase the accuracy of yield prediction and crop recommendation.

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Research Article

Geographical Spatial Distribution and Population Density of Wheat Stem Sawfly, *Cephus pygmeus*, in Wheat Fields in Southeastern, Eastern, and Northeastern Regions of Türkiye

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Keywords

Cephus pygmeus,
Eastern Türkiye,
Population density,
Spatial distribution,
Wheat stem sawfly

Abstract: The wheat stem sawfly (*Cephus pygmeus*) is a major pest of wheat crops in many regions of the world, including Eastern Türkiye. This study aimed to investigate the spatial distribution and population density of wheat stem sawfly in Eastern Türkiye and to evaluate the damage caused by this pest on wheat yield and quality. A total of 120 wheat fields were surveyed in six provinces of Eastern Türkiye during the 2018 growing season. The results showed that the population density of wheat stem sawflies varied significantly among the surveyed fields, with an average infestation rate of 37.4%. The highest infestation rate was recorded in the province of Kilis (62.5%) and Diyarbakır (61.9%), while the lowest was in the province of Ağrı (8.3%). The study also identified some factors that affect the population density of wheat stem sawflies, including host plants (mainly wheat, barley, and rye), altitude, temperature, and geographical structure such as mountains and forests. These findings provide valuable information for developing effective management strategies to control wheat stem sawfly populations and minimize the damage caused by this pest in Eastern Türkiye, especially Southeastern Anatolia Region.

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Footnote: This study was produced from the doctoral thesis of the first author.

1. Introduction

The wheat stem sawfly [*Cephus pygmeus* L. (1767) (Hymenoptera: Cephidae)], which is a common and important pest of wheat, barley, and rye in Europe, North America, northern Asia, Middle Eastern countries, and the Mediterranean region (Morrill et al., 1992; Chen et al., 2004; Shanower and Hoelmer, 2004; Özberk et al., 2005; Kılıç et al., 2017; Mutlu, 2019; İnce et al, 2022).

The larvae of the sawfly feed on the stems of wheat plants, causing damage that can lead to yield losses and reduced grain quality. They have a biting-chewing mouth structure that allows them to rasp away at the plant tissue. This damage disrupts the vein integrity and impedes nutrient flow to the grains, resulting in delayed grain development, reduced grain weight, and increased susceptibility of weak stems to lodging (Troccoli et al., 2000; Knodel et al., 2010). It also causes losses in the protein content of the plant (Wallace and McNeal, 1966). In Türkiye, the damage caused by this pest has been estimated to be 288 kg/ha in durum wheat and 297 kg ha^{-1} in bread wheat (Özberk et al., 2005). This

translates to a financial loss of $68.8 ha^{-1} and $68.6 ha^{-1}, respectively (Özberk et al., 2005). The damage caused by the sawfly can vary depending on the variety of wheat, the time of infestation, and the weather conditions. In some cases, the damage can be as high as 23.77% (Özberk et al., 2005). *Cephus cinctus*, a straw bee species common in the Americas, is a serious pest that can cause significant crop losses. In a study by Beres et al. (2011), it was reported that this pest can cause crop losses of more than 30% and economic damage of $350 million annually.

Cephus pygmeus has one generation in a year and initiates its reproductive activities when the average air temperature reaches 18 °C and the relative humidity reaches 76%. Following a period of winter diapause, the males are the first to emerge from this dormant state within 2-4 days (Mutlu, 2019). These adult insects satisfy their dietary needs by consuming nectar from weed flowers found at the periphery of the wheat fields, while their preferred mating locations are yellow flowers. Once the temperature reaches at least 20 °C approximately 26-27 days after reaching adulthood, the females begin laying their initial eggs inside wheat stems (Fulbright et al., 2017; Mutlu, 2019). Usually, one egg is deposited per talk, and these eggs hatch within a span of 8-12 days. The emergence of the larvae coincides with the milk production period of the wheat plant. Upon reaching maturity, the larvae leave the culm and enter diapause within stubble or other plant debris, where they form a transparent cocoon within two weeks (Gol'berg, 1986; Mutlu, 2019).

In this study, it was aimed to investigate the spatial distribution of *Cephus pygmeus*, a damaging pest in wheat fields, specifically the eastern regions (including Southeastern Anatolia, Eastern Anatolia, and Eastern Black Sea) of Türkiye. The objectives include assessing population densities in areas where the pest is spreading and enhancing the efficacy of integrated pest management programs (IPM) developed against this pest. By understanding the distribution patterns and population dynamics of *Cephus pygmeus*, valuable insights can be gained to support targeted pest control strategies and mitigate the impact of this destructive insect on wheat crops.

2. Material and Methods

The study encompassed 30 provinces and a total of 167 districts within the Southeastern Anatolia, Eastern Anatolia, and Eastern Black Sea regions in eastern Türkiye. The provinces included in the study were Adıyaman, Ağrı, Ardahan, Artvin, Batman, Bayburt, Bingöl, Bitlis, Diyarbakır, Elâzığ, Erzincan, Erzurum, Gaziantep, Giresun, Gümüşhane, Hakkâri, Iğdır, Kars, Kilis, Malatya, Mardin, Muş, Ordu, Rize, Siirt, Şanlıurfa, Şırnak, Trabzon, Tunceli, Van.

Samplings occurred from March to August 2019 and were conducted at two different times for each region, based on the tillering-stemming and spike-up periods of the plant, according to the Zadoks growth scale (Zadoks et al., 1974). In order to determine the distribution and density of *Cephus pygmeus* in wheat fields, a sweepnet with a diameter of 35 cm was used in each field. The sweeping process was repeated 200 times in each field. The sampling locations were accurately marked using a GPS device (Garmin BaseCamp) (Figure 1).

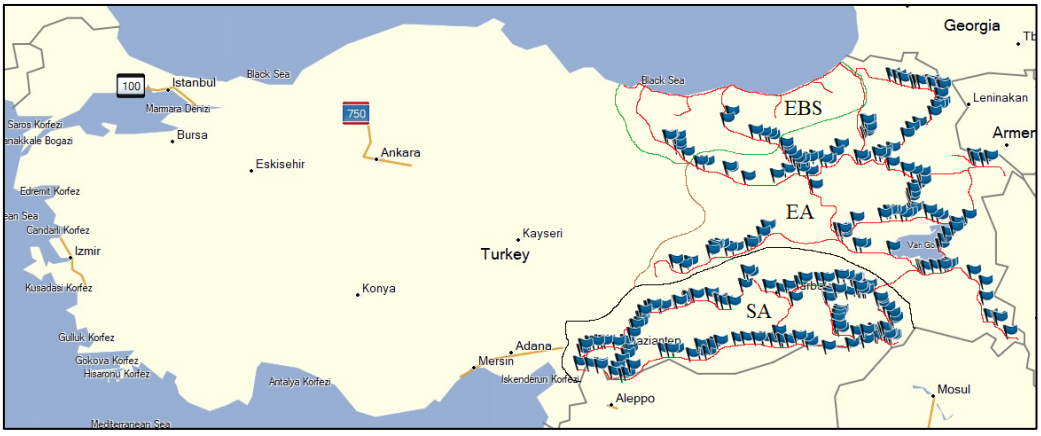

Figure 1. Sampling locations in Southeastern Anatolia (SA), Eastern Anatolia (EA), and Eastern Black Sea (EBS) regions of Türkiye (Garmin BaseCamp v4.6.2) (The red lines seen on the map represent the routes taken during the survey).

3. Results and Discussion

During the study, 330 wheat fields were surveyed and specimens of *Cephus pygmeus* were collected in 125 fields, 992 females and 571 males were collected within these areas. Distribution and densities of the *C. pygmeus* in the surveyed areas were represented in Figure 2 and Figure 3. It has been determined that the pest was common in all of the Southeastern Anatolian region and in other provinces of the Eastern Anatolian region except Tunceli and Hakkâri provinces. Sampling could not be conducted in Artvin, Rize, and Trabzon provinces located in Eastern Black Sea region since wheat-producing areas were not encountered enough in these areas. Wheat stem sawfly was not encountered in the sampling in Bayburt, Giresun, and Gümüşhane provinces. Figure 3 shows that the pest is most intense in the Southeastern Anatolia Region, with a small presence in the Northern parts of the Eastern Anatolia Region. There has not been a comprehensive study on the distribution and population density of the pest in these study areas, apart from a few studies that showed that it spread in some provinces in these regions and caused various damages (Özberk et al., 2005; Budak, 2012; Karaca et al., 2012; Durel, 2016; Mutlu, 2019; Mutlu et al., 2019; Özgökçe et al., 2022).

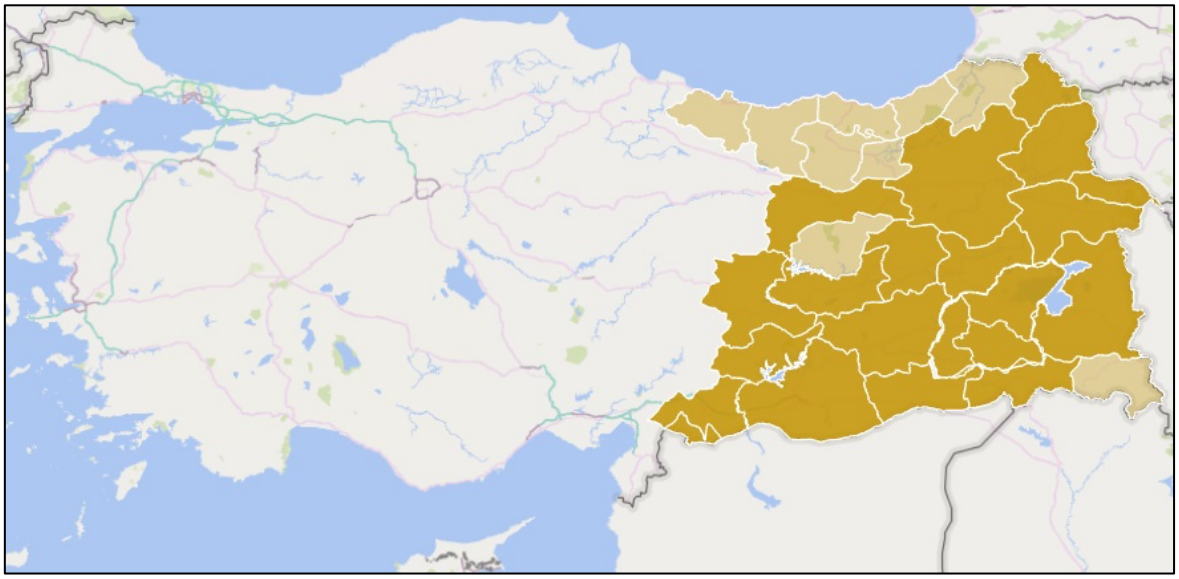

Figure 2. Observed (dark) and not observed (light) places of *Cephus pygmeus* in Southeastern Anatolia, Eastern Anatolia, and Eastern Black Sea regions of Türkiye.

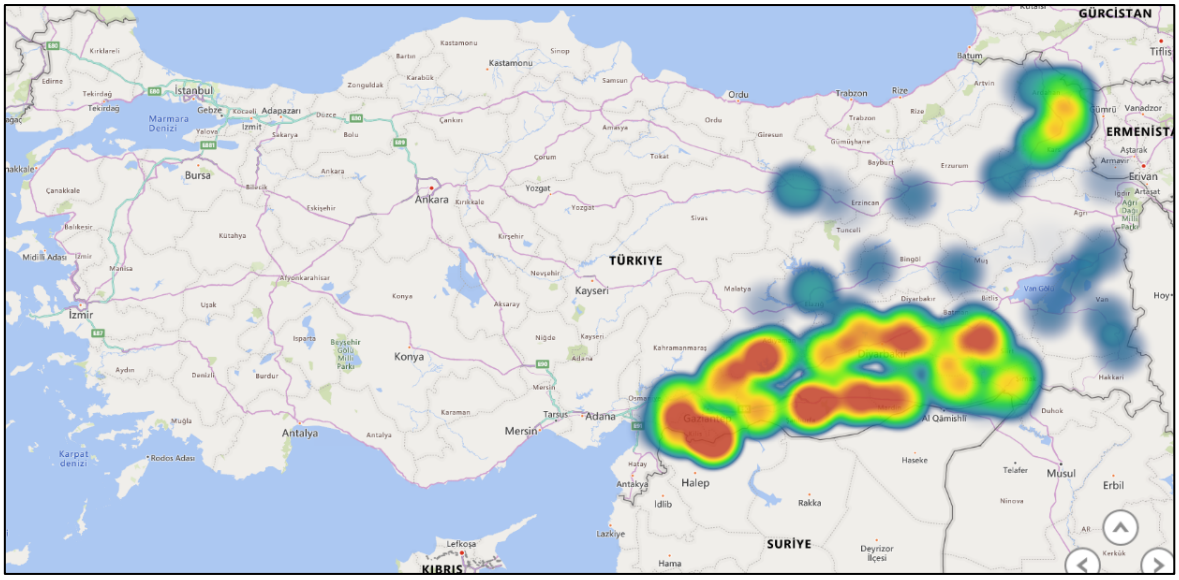

Figure 3. Heatmap of the density and distribution of *Cephus pygmeus*.

When examining the sampling sites where *Cephus pygmeus* individuals were collected, it was determined that the lowest elevation recorded was 371 m (Şırnak-Silopi), while the highest elevation reached 2222 m (Van-Başkale). Notably, the wheat stem sawfly individuals were predominantly found in the field margins of wheat fields. Adult individuals of the wheat stem sawfly, known for their slow flight, displayed a distinct pattern when disturbed during sampling, making them easily recognizable. Furthermore, it was observed that the adults of the wheat stem sawfly could be captured using a sweepnet at any time of the day, as they tended to rest on the stalks.

In terms of activity, the male individuals appeared to be more active than the females. Morphological examinations revealed that female individuals were larger and had a darker coloration compared to their male counterparts (Figure 4a, 4b). Furthermore, the male individuals predominantly exhibited a yellow coloration, particularly in the ventral part of the thorax (Figure 4c, 4d).

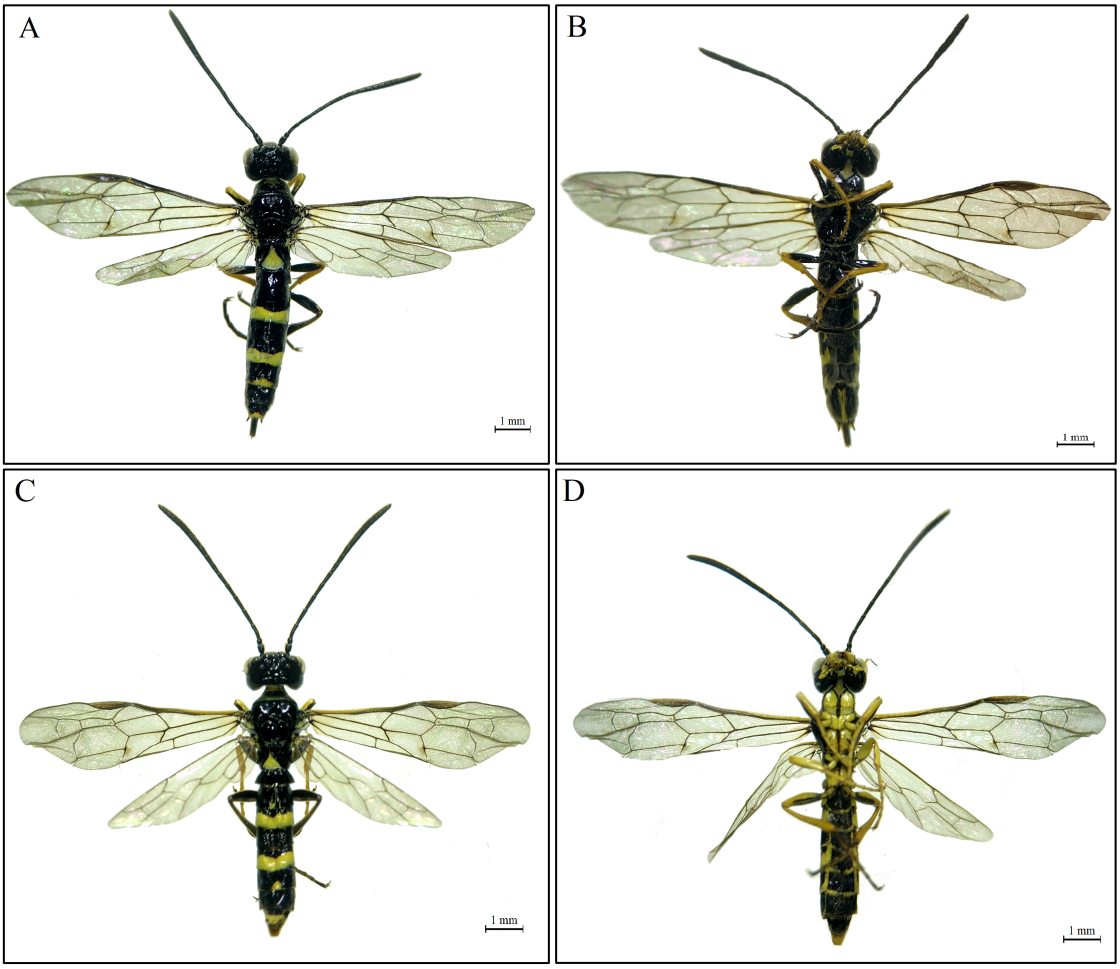

Figure 4. Morphological views of adult individuals; dorsal (A) and ventral (B) views of females, and dorsal (C) and ventral (D) views of males.

The population densities of the pests according to provinces and the pest infestation rates of the fields in each province are represented in Figure 5 and Figure 6. In the Southeastern Anatolia Region, the average number of adult individuals collected varied across the provinces. The average numbers of the individuals ranged from 3.70 (Batman) to 17.63 (Kilis) per 200 sweeping, indicating variations in the population densities of adult *C. pygmeus* individuals according to provinces. Within this region, the infestation rates of the wheat stem sawfly in the wheat fields exhibited a similar pattern to the pest densities across the provinces (Figure 5). The lowest recorded infestation rate was observed in Batman at 30%, while the highest adult infestation rate was found in Kilis and Diyarbakır, reaching 62.5% and 61.9%, respectively (Figure 5).

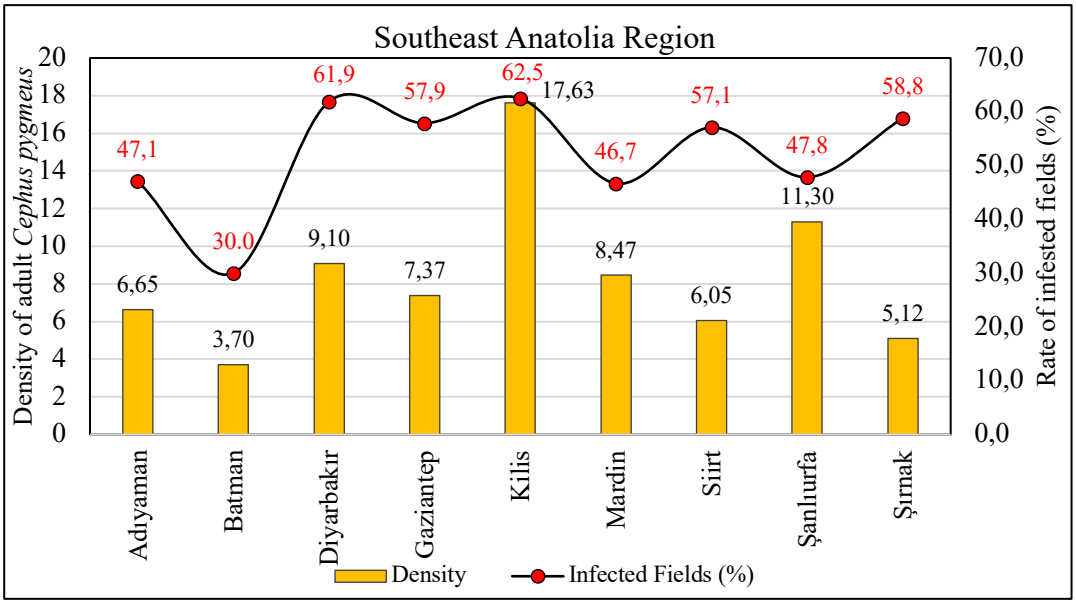

Figure 5. Density of adult *C. pygmeus* (adults/200 sweepnet) (primary axis) and rate of infested fields (%) (secondary axis) in the Southeast Anatolia region.

The infestation rate of *C. pygmeus* adults in wheat fields in the provinces of the Eastern Anatolia Region were found between 8.3% (Ağrı) and 50% (Kars). However, the population density of this pest in these provinces was significantly lower compared to the Southeastern Anatolia Region. The densities of the pests were determined from 0.14 to 7.21 individuals per 200 sweeps in Bingöl and Kars, respectively.

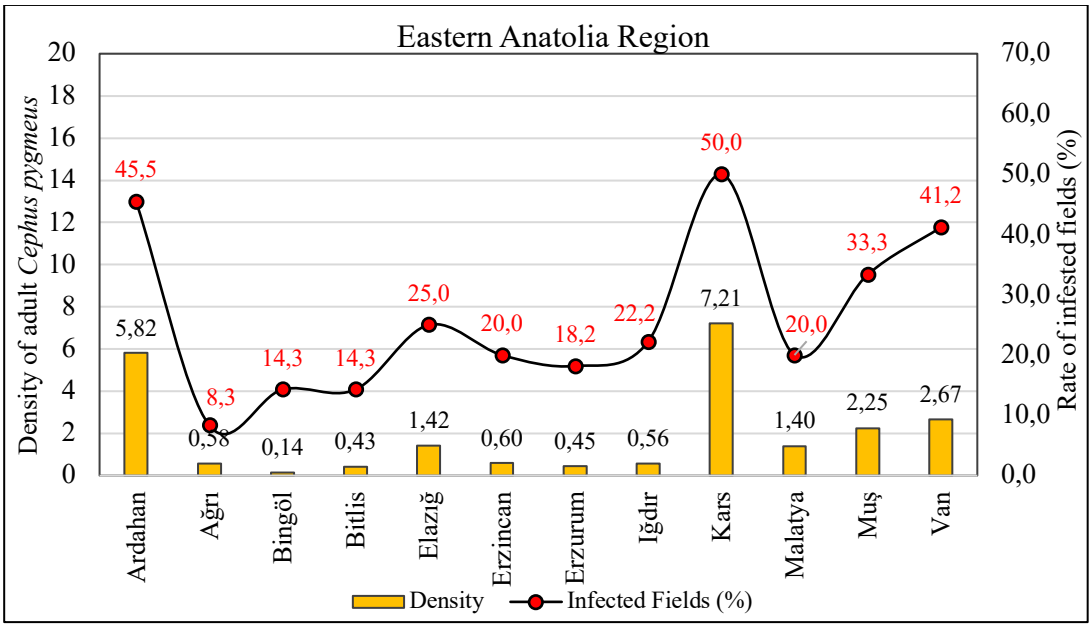

Figure 6. Density of adult *C. pygmeus* (adults/200 sweepnet) (primary axis) and rate of infested fields (%) (secondary axis) in the Eastern Anatolia region.

This study presents the first large-scale, comprehensive survey of the pest's distribution and density in the study areas, providing valuable insights into its distribution, population dynamics, and potential impact. According to the results obtained, it has been determined that the pest is more common and intense in the Southeastern Anatolia Region compared to other regions. Considering the geographical structures and climatic characteristics of the regions, it is seen that the spread and density of the pest increases from mountainous and forested areas to plains, from cold regions to warmer regions.

This situation also creates differences in wheat production areas and amounts between regions. According to the last 5 years data of TUIK, the Southeastern Anatolia Region has an area of 9.3% and a production amount of 11% in terms of wheat production, while the Eastern Anatolia Region is 5.5% and 4%, and the Black Sea Region has an area of 0.3% and 0.2% wheat, respectively (TUIK, 2023).

In this study, the adult infestation rates and densities of the pests in the wheat fields were compared according to the regions. In the Southeastern Anatolia Region, the adult infestation rate was 52.2%, with an average of 8.4 individuals/sweeping. On the other hand, the adult infestation rate in the Eastern Anatolia Region was determined as 26.0% and an average of 1.96 individuals/sweeping.

In a study conducted by Mutlu et al. (2019) in some provinces of the Southeastern Anatolia Region, it was determined that pests' infestations at a rate of 5.73%, 54.66, 15.27 and 27.05 in Adıyaman, Diyarbakır, Mardin and Şanlıurfa, respectively. In this study, the adult infestation rates in the provinces in question were found to be 47.1%, 61.9, 46.7 and 47.8%, respectively. No other research on the infestation rate of the pest in our study area was found. However, Korkmaz et al. (2010) determined the infection rate of *Cepheus* species (mostly *C. pygmeus*) in wheat fields as approximately 60% in their survey study covering all provinces of Central Anatolia Region. In addition, İnce et al.(2022) determined the infection rate of *C. pygmeus* as 77.4% in their study conducted in wheat fields in Yozgat province. According to these results, there is a similarity between the pest infestation rates in the Central Anatolia and Southeastern regions of Türkiye, while a relatively lower level of infestation was found in the Eastern Anatolia Region. This difference may be due to the parallelism between wheat production areas and amounts and pest infestation rates across the regions.

4. Conclusion

At the end of the study, it was found that the wheat stem sawfly, *Cephus pygmeus*, was distributed in the vast majority of wheat fields in the Southeastern Anatolia and Eastern Anatolia regions of Türkiye.

In the density of pest populations in the areas where the wheat stem sawfly and *Cephus pygmeus* are distributed, the Southeastern Anatolia region is at the lead. The pest was found to be spreading in all provinces of this region, with Kilis being the province with the highest pest density among these provinces. According to these provinces, it was found that wheat production is quite high and the provinces with large cultivated areas are Şanlıurfa, Diyarbakır, Mardin, and Gaziantep (including 2018-2022) (TUIK, 2023). *Cephus pygmeus* was found to be widespread in almost all provinces of the Eastern Anatolia region, which accounts for 10% of Türkiye's wheat production. The provinces with the highest density of *Cephus pygmeus* populations in this region were identified as Kars and Ardahan. *Cephus pygmeus* was not encountered during surveys in the eastern Black Sea region, which does not have a soil structure suitable for wheat cultivation.

According to the results of this study, it was determined that the pest significantly increased its population density and distribution in the Southeastern Anatolia Region compared to previous years. It is reported that the current global climate change increases the density and harmfulness of wheat stem sawflies in the West Siberian pest focus within Altai Territory (Kaplin and Lysikov, 2022). The increase of the pests in the Southeastern Anatolia Region from the population density and distribution areas determined in 2014-2015 (Mutlu et al., 2019) to the increase rate determined in the current study may be related to the same reasons. Although it is less common and dense in the Eastern Anatolia region, it is difficult to have an idea about the development of the distribution and density of the pest, since no research has been done in the region before. Although there is no direct control in the Southeastern Anatolia Region or other regions against the pest in the current situation, it is extremely important to carefully monitor the pest population and economic damage in the coming years.

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Research Article

Turkish Consumers' Purchase Motivation towards Erzurum Stuffed-Kadayif with Protected Geographical Indication (PGI) at the Dessert Retailers

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Etnocentrism,
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Purchase motivation

Abstract: It was planned to determine the main factors affecting Erzurum Stuffed-kadayif purchase motivation of Turkish consumers in the study. The main material of the research was obtained from 385 households residing in Erzurum, Türkiye. Exploratory Factor Analysis and Two-step Cluster Analysis were used to explore Turkish consumers' Erzurum Stuffed-kadayif purchase motivation at the dessert retailers. The results of the research highlighted that consumers consuming this product at the local restaurants were satisfied highly with the food images under cultural integration. On the other hand, those consuming this dessert at the local patisseries also attituded a big importance to the entrocentrism approach based on cultural integration. Similarly, consumers purchasing Erzurum Stuffed-kadayif as a ready-made local dessert from local manufacturer vendors tried to contribute considerably to sustainable food supply and consumption with an entrocentrism approach under cultural integration. It should be improved appropriate positioning and segmentation strategies according to the purchase motivation of each consumer segment, and then they should be implemented by policy makers.

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1. Introduction

In recent years, there has considerably maintained a change in consumers' food consumption preferences and purchase motivations under the negative effects of global climate change due to lower yield and quality attributes suffered in plant and livestock products, biodiversity losses, possible risk factors on food safety and security at food life cycle from the farms to the retailer shelves, negative consumer perceptions about emotional food quality attributes, as well as negative impacts on human health and the environment (Bernabeu et al., 2023; Bouranta et al., 2023; Mesias et al., 2023).

Under the impact of the Covid-19 pandemic and the Ukraine and Russia war along with the negative effects of climate change, the production of wheat being the main raw material of stuffed-kadayif has considerably decreased for the last few years in the world and Türkiye. As considered global wheat supply and demand trends, while global wheat production and stocks decreased from 764 and 284 million tons in 2019 to 769 and 271 million tons in 2022, wheat consumption increased from 741 million tons to 782 million tons (TEPGE, 2022). In response to the decreases in both global wheat production

and current stocks, a significant increase in global wheat consumption was also observed in view of the trend figures. Consequently, this situation has indicated the existence of a serious problem in meeting consumer demands of wheat supply worldwide and a large supply gap in the future if the necessary preventive and adaptation studies are not carried out to an adequate extent.

Wheat production in Türkiye was 19.00, 17.65, and 19.80 million tons in 2019, 2021, and 2022 respectively, whereas domestic wheat consumption was given as 20.00, 19.01, and 19.00 million tons (TEPGE, 2022). In particular, it abnormally caused product prices to increase with the effects of panic buying by narrowing the supplies of wheat and bakery products under the negative impacts of ongoing climate change and the Covid-19 pandemic hitting 2019 (Arafat et al., 2021). Indeed, while the average annual wheat price was ₺1.5 kg^{-1} in 2018, it increased to about ₺5.5 kg^{-1} in 2022 (PTB, 2022). The dramatic increases in wheat prices at commodity markets caused wheat flour prices to trade from ₺1.76 kg^{-1} in 2018 to increase by ₺7.7 kg^{-1} in 2022 (PTB, 2022a). Manufacturing cost increase resulting from excessive rises in the prices of Stuffed-kadayif ingredients such as sugar, walnuts, pistachios, and hazelnuts, along with the price of the flour being the main input of Erzurum Stuffed-kadayif, therefore, caused the price per kg to rise from ₺15 ($2) in 2019 to ₺140 ($7) in 2023.

On the other hand, besides the natural risk factors having a negative impact on agriculture and the agricultural food industry, when the macroeconomic data are taken into consideration for 2022-2023 years in Türkiye, the consumer price index (CPI) and food price increases (food inflation) were annually realized as 50.51 and 67.89% (TUIK, 2023). The annual increases in the producer price index (PPI) and food input prices were calculated as 62.45% and 88.38%, respectively (TUIK, 2023a). The pressures of these inflationary and natural risk factors have todays caused the food prices to increase dramatically with the contraction in the economy by increasing the production costs, and then the formation of social welfare losses created by the contraction in demand resulting from the real decline in consumer incomes. This situation has indeed caused an excessive increase in the share of consumer incomes allocated to mandatory food needs in the expenditure budget, and thus their consumption motivations have also changed considerably depending on the marketing mix.

It was reported that consumers' psychographic factors on their food consumption motivation had a much greater impact than their socioeconomic ones such as gender, age, education, and profession on their attitudes and behaviors patterns (Graham and Ambrahamse, 2017; Harguess et al., 2020). Consumers' individual factors, therefore, (attitude and value, knowledge and skill, emotion and cognitive level, taste and flavour, demographic factors), their sociocultural attributes (culture and belief, social norm and status), and the external factors (political and economic factors related to food marketing environments) must be assessed rationally how their food purchase motivations are impacted under current conditions (Chen and Antonelli, 2020; Harguess et al., 2020). Therefore, consumers trying to meet their food needs under the effects of climate change have rationally tried to shape their food choices and purchase motivations at retail levels by taking into account not only the hedonic and sensory food attributes but also the negative progressions in the Turkish economy in the last years.

It was reported in the prior researches that it was firstly attempted to determine consumers' purchase motivations by taking into account the hedonic food attributes, a part of the marketing mix focused on consumers' visual sense (price, brand, labeling, package weight, and size, geographical indications, purchase convenience, reaching to retailers, conformity and comfort at retail stores, health claims) (Edenbrandt and Nordström, 2023; Fakhreddine and Sanchez, 2023; Petrontino et al., 2023; Yeh and Hirsch, 2023; Zanchini et al., 2023; Zeng et al., 2023), and then the sensorial food attributes based on a variation of the nutritional composition at farming and manufacturing process (taste, aroma, flavor, colour, texture, appearance, sound, content or ingredient, juiciness, sweetness) (Bejaei and Xu, 2023; Fakreddine and Sanchez, 2023; Giannoutsos et al., 2023; Kleih et al. 2023; Lavui et al., 2023) impacting on their purchase models at retail levels.

Especially, when making consumers' food purchase decisions based on their hedonic experience perceptions, it was emphasized that they make purchasing decisions to a large extent by taking into account the marketing mix such as the region of origin and prices (Topcu and Çavdar, 2022; Bernabeu et al., 2023; Chaffee and Ross, 2023), the food brands and their communication tolls (Bernabeu et al., 2023), food packaging and label knowledge (Chaffee and Ross, 2023) and the retailers and their positioning strategies (Bytyqi et al., 2023; Curutchet et al., 2023; Seo and Kim, 2023).

In these studies based on consumers' food purchasing motivations, it was pointed out that the extrinsic/hedonic food attributes were the major determinants of their purchase motivation at the food

retailers, and also provided vital information about their socioeconomic attributes. Similarly, it was also reported that there were much stronger interactions between the intrinsic/sensory food attributes and hedonic/extrinsic ones on consumers' purchase motivations.

On the other hand, differentiated product types of traditional food products registered by PGI and manufactured by traditional production models are heavily preferred by target consumer mases. Because they are not exposed to an intensive manufacturing process and the chemical pollutants creating a negative impact on human health and the environment. Similarly, it was also reported that the factors such as the high sensory quality and core benefit attributes of the food products with PGI, the use of natural inputs free of chemical additives and preservatives with the region of origin, their traceability and sustainability at manufacturing process, the ethnocentrism approach contributing to regional and rural development affected positively consumers' purchase motivation (Sanchez-Bravo et al., 2020; Devia et al., 2021; Rahman et al., 2021; Topcu and Çavdar, 2022).

Within the scope of the current research, the extrinsic and intrinsic food motives impacting consumers' consumption preferences and purchase decisions towards Erzurum Stuffed-kadayif could considerably shape their purchase patterns. In this context, the aim of the study is to determine consumers' purchase motivations based on the intrinsic and extrinsic food motives for Erzurum Stuffed-kadayif with protected geographical indication bought from the food retailers in Erzurum and then to create customer-oriented marketing strategies for each consumer segment.

2. Material and Methods

2.1. Material

The main material of the study consisted of primary data obtained from face-to-face questionnaires conducted with the households in Erzurum; Yakutiye, Aziziye, and Palandöken Central Districts, consuming Erzurum Stuffed-kadayif with PGI in 2019 by taking into consideration the questionnaire form approved by Ataturk University Ethics Committee with 2021/14 number. In addition to primary data, secondary data were obtained from the data of various statistical institutions and organizations (TUIK, FAO, Erzurum Chamber of Commerce, Commodity Exchanges), as well as domestic and foreign scientific research project reports and article findings and results.

2.2. Methods

2.2.1. Method used to determine the sample size

In order to ensure the homogenous participation of the households consuming Erzurum Stuffed-kadayif in Erzurum, the city was divided into three central districts; Yakutiye, Aziziye, and Palandöken (44.325, 14.818, and 38.674 households), respectively and then the sample size in Equation 1 was calculated with the Simple Random Sampling Method (Malhotra, 1993).

$$n = \frac{Z^2 \cdot p \cdot (1-p)}{c^2} = \frac{1.96^2 \cdot 0.05 \cdot (0.05)}{0.05^2} = 385 \quad (1)$$

In Equation 1,
n: Sample size
Z: Standardized Z value (at 95% confidence interval, 1.96)
p: Erzurum Stuffed-kadayif consumption probability (0.50)
c: Error term (0.05 = ±5)

The survey numbers under the proportional techniques were calculated as 175 in Yakutiye, 58 in Aziziye, and 152 in Palandöken, and a total of 385 in Erzurum by taking into account the sample size and the number of households in each district.

2.2.2. Method used for preparation of questionnaire forms

In order to design the attitude scale related to the intrinsic and extrinsic food attributes that determine consumers' purchase motivation consuming Erzurum Stuffed-kadayif in Erzurum were utilized from the domestic and foreign studies related to the research scope and context. The scale was

firstly designed with 43 marketing mix attributes (product mix: 25 items, price mix: 6 items, communication mix: 3 items, distribution mix: 9 items) impacting on their Erzurum Stuffed-kadayif purchase decisions, it was asked consumers participated in the survey to mark each statement on the attitude scales with 5-point Likert Scale (1: no important, 3: neutral/undecided, 5: very important) allowing consumers' attitudes to be perceived more accurately at the scale dimension (Kotler and Amstrong, 2018).

2.2.3. Methods used in statistics analyses

In the first step, Explanatory Factor Analysis (EFA) due to having not applied any approved research scale was used to determine the main factors impacting their Erzurum Stuffed-kadayif purchase motivation (Hair et al., 2013). The EFA is a multivariate statistical dimension reduction technique trying to create a small number of unrelated, but conceptually meaningful new factors (Bursal, 2019; Civelek, 2020). Hierarchical steps for the EFA were followed to test the suitability of the data, determine the main factor number, perform the rotation (transformation) techniques, identify main factors, and calculate the explained and cumulative variances for each factor dimension, respectively.

In order to investigate the data suitability of the sample mass according to the main population for the EFA, Kaiser-Meyer-Olkin (KMO) and Bartlett's test of Sphericity were used in the research. KMO, the adequacy criterion of the sample size should be in an acceptable confidence interval (between 0.50 and 1.00). On the other hand, the correlation matrix should be different from the unit matrix in Bartlett's test of Sphericity explaining the relationship among the variables depending on the correlation matrix calculated between each pair of variables.

Whereas determining the main factor number with the EFA used Maximum Likelihood (ML) extraction method in the study, the factors with Eigenvalues greater than 1 or equal to 1 were statistically taken into consideration. The rotation technique was also used to be able to give the factor names and eliminate the variable overlaps in factor matrices. In the rotation process, the factors in the axes are rotated so that reducing the variable loads to optimal levels. Rotation could be applied in two groups as vertical (orthogonal) and oblique rotation. While it could be minimized the relationships among the factor dimensions at vertical rotation, it could be accepted the relative relations among them at oblique rotation. It is often used the varimax, quartimax, and equamax methods for vertical rotation techniques, however, it is generally used direct oblimin and promax methods for oblique rotation ones. In this study, therefore, it was applied the vertical rotation technique and its varimax method to minimize the relationships among the factors.

On the other hand, to retain and select the items under each factor dimension on a rotated component matrix in the EFA, the factor loads with a range of 0.30 and 0.50 scores are generally accepted for the cut-off threshold of the items depending on number of the items on scaling instrument and sample size reflecting main population (Quy and Ha, 2018; Bursal, 2019; Civelek, 2020). These authors suggested that, thus, if the sample size was more than 300 cases, the cut-off threshold of factor load was accepted as 0.30, also if the sample size was between 300 and 200 cases and between 200 and 150 cases, the cut-off thresholds of factor loads would be considered as 0.40 and 0.50, respectively.

In the second step, it was used the cluster analysis, a two-step cluster analysis, dividing a heterogenic target mass into two or more homogeneous segments by taking into account their attributes such as socioeconomic, psychological, and individual characteristics (Topcu and Baran, 2017; Karagöz, 2019). Two-step cluster analysis considering the ideal numbers of clusters and yielding the relationships between the main factors obtained and the consumption groups desired to be created is one of the most effective clustering techniques. In the present study, the main factors impacting the Turkish consumers' Erzurum Stuffed-kadayif purchase motivation were used in a two-step clustering analysis (CA) taking into consideration their retail selling points. It was thus segmented target consumers into three groups consuming at the restaurant (29.1% of overall consumers) and the patisserie (30.4% of those) and buying from the manufacturer stores (40.5% of those).

3. Results

3.1. Consumers' demographic and socioeconomic profiles

Participants' gender, age, life cycle, education and occupation status, monthly income, and expenditure groups at each cluster were presented in Table 1. The results of the study indicated that 59%

of the target consumer mass consisted of men, and the consumers with college graduates and white collars concentrated generally at each consumption segment of Erzurum Stuffed-kadayif.

Table 1. Consumers' various demographic and socioeconomic attributes

Consumers' attributes		Consumption segments of Erzurum Stuffed-kadayif						Overall consumers	
		Patisserie		Manufacturer		Restaurant			
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Gender	Male	77	66	85	55	65	58	227	59
	Female	40	44	71	45	47	42	158	41
	$(Pearson\ Chi-kare) = \chi^2_{(2603;2)} = 24{,}746$ *p=0.000*								
Education	Literate	4	4	9	6	3	3	16	4
	First school	20	17	39	25	31	28	90	23
	High school	45	39	39	25	35	31	119	30
	College	48	41	69	44	43	38	160	42
	$(Pearson\ Chi-kare) = \chi^2_{(2606;4)} = 77.378$ *p=0.000*								
Occupation	Businessman	11	9	27	17	13	12	51	13
	White-collar	50	43	49	31	42	38	141	37
	Blue-collar	18	15	12	8	17	15	47	12
	Retailers	27	23	40	36	26	24	93	24
	Pensioners	9	8	15	10	12	11	36	9
	Farmers	1	1	7	5	1	1	9	2
	Housewife	1	1	6	4	1	1	8	2
	Total	117	100	156	100	112	100	385	100
		$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$	*n*
Age group	+ < 30 years (young)	30.76	37	30.43	30	30.56	16	30.60	83
	30-50 years (mature)	42.23	69	43.33	84	42.59	64	42.76	217
	+ > 50 years (more mat.)	56.64	11	58.05	42	56.94	32	57.45	85
	Group means	39.96	117	44.81	156	44.97	112	43.38	385
	$F_{(382,2)} = 10.559$ *p=0.000*								
*Income**	+ < $400 (low-income)	321.43	14	360.00	24	365.00	12	350.40	50
	$400-1000 (middle-income)	416.04	91	751.56	109	750.91	88	740.14	288
	+ > $1000 (high-income)	1398.33	12	1421.74	23	1384.67	12	1406.30	47
	Group means	738.80	117	790.13	156	777.46	112	770.85	385
	$F_{(382,2)} = 0{,}903$ *p=0,406*								
*Expenditure**	+<$400 (low-expenditure)	320.00	21	318.60	43	310.00	19	316.99	83
	$400-700 (middle-expend)	591.94	62	582.90	69	596.56	61	590.16	192
	+>$700 (high-expenditure)	848.82	34	885.36	44	890.94	32	875.69	110
	Group means	617.78	117	595.36	156	632.05	112	612.85	385
	$F_{(382,2)} = 0.903$ *p=0.404*								
Family size	+< 4 person (core family)	2.55	56	2.63	40	2.68	25	2.60	121
	4-6 person (small family)	4.18	57	4.64	108	4.67	75	4.54	240
	+> 6 person (large family)	11.00	4	8.50	8	9.17	12	9.25	24
	Group means	3.63	117	4.32	156	4.71	112	4.22	385
	$F_{(382,2)} = 10.084$ *p=0.000*								

$\bar{x}$: arithmetic means, *n*: sample size, *%*: relative rate, *exchange rate is ₺ $^{-1}$ 5.75 on September 15, 2019.

On the other hand, the results also highlighted that the average age of overall consumers was 43.38 years, the family size consisted of 4.22 individuals, and the middle age group and large families showed intensity in each consumption segment. Similarly, the average income and expenditure levels for all consumption groups were $1406.30 and $875.69, and these economic indicators also were of the highest shares in each consumer segment.

3.2. Results of the EFA

The goodness fit statistics results and five factor dimensions that consider 31 items impacting the consumers' Erzurum Stuffed-kadayif purchase decisions in the EFA by being eliminated their load overlap and meaningless loads were given in Table 2. KMO that compares the observation and partial correlation coefficients in the EFA was calculated as a value of 0.911 ($p < 0.001$). The test score was acceptable at an excellent level due to much closer to the 0.99 threshold value, thus, providing the confirmation of sampling adequacy for the EFA. Bartlett's test of Sphericity statistics for the main factors related to consumers' purchase decisions, then, was calculated as $\chi^2_{(190;0.05)} = 6650.10$ ($p = 0.000$), and the unit matrix hypothesis was rejected ($p < 0.001$). Two statistics evaluating the data indicated that the data was at an excellent level for the EFA.

Table 2. The results of the EFA related to consumers' Erzurum Stuffed-kadayif purchase motives and their item loads

The items and factors interpretations	The factors and items loads*			
	F_1	**F_2**	**F_3**	**F_4**
Food image and value				
Price and quality relation	0.928			
Product quality	0.903			
Product brand and package label	0.860			
Product price	0.802			
Packaging appeal	0.750			
Package weight and size	0.690			
Advertisement impact	0.624			
Ready-made local dessert				
Serving at social meetings		0.835		
Preparing in a pratical way		0.765		
Being a light dessert		0.761		
Longer storage possibilities		0.746		
Offering along with tea		0.689		
Reference group impact		0.682		
Entrocentrism approach				
Contribution to the regional food retailers			0.897	
Contribution to the region economy			0.804	
Contribution to regional development			0.774	
Contribution to mitigate migration			0.634	
Cultural integration				
Being a part of the regional diet culture				0.862
Being a food with the region of origin				0.841
Being a part of cultural integration				0.640
Eigenvalues	*5.153*	*3.806*	*3.017*	*2.217*
Explained share of variance (%)	*25.764*	*19.028*	*15.085*	*11.085*
Cumulative share of variance (%)	*25.764*	*44.792*	*59.876*	*70.961*
KMO (Kaiser-Meyer-Olkin) statistic				*0.911*
Bartlett's test of sphericity	$[Chi - square\ (\chi^2_{190;\,0.05} = 6650.10\ (p = 0.000)]$			
Maximum Likelihood (goodness-of-fit test)	$[Chi - square\ (\chi^2_{116;\,0.05} = 494.860\ (p = 0.000)]$			

*It was suppressed the smaller *coefficients than 0.350.*

The results of the EFA indicated that the four-factors solution with Eigenvalue scores greater than 1.0 were derived from 20 items impacting the consumers' Erzurum Stuffed-kadayif purchase motivation in Table 2. The four factors were logically identified as the food image and value, ready-made local dessert, entrocentrism approach, and cultural integration, and their explained total variance was found as 70.96% (Quy and Ha, 2018). The first factor referring to the food image and value explained 25.76% of the total variance. It was thus assessed that the food image and value consisted of the loaded items measuring a wide range of real food images and value based on the relationships among

the product mix, price mix, and communication mix, which strengthened consumers' Erzurum Stuffed-kadayif purchase motivation.

Similarly, the second factor explained by 19.03% total variance identified as a ready-made local dessert that is often serviced practically along with meals or tea at social meetings under the effects reference groups. At the social meetings organized by consumers with Erzurum-originated, Erzurum Stuffed-kadayif is one of the most preferred desserts, and it has been also consumed by overall consumers with great satisfaction in diets. The third factor supported the first and second factors was named as entrocentrism approach (15.09% explain rate), that is, Erzurum-originated consumers consuming Erzurum Stuffed-kardayif have also tried to obstacle regional migration by orientating to the local food retailers to trigger regional economic development. On the other hand, the last factor was determined as the cultural enragration explaining 11.09% of the total variance. Erzurum Stuffed-kadayif, indeed, a crucial dessert of traditional culinary culture in Erzurum, created a cultural integration by being a part of the diets with protected geographical indication (PGI) under the region of origin.

3.3. Results of the CA

The main factors derived from the EFA, and shaping the purchase motivations of Turkish consumers who bought Erzurum Stuffed-kadayif from the local food manufacturer stores, patisseries, and restaurants were given in Table 3. The results of the CA indicated that consumers consuming Erzurum Stuffed-kadayif at the local restaurants focused on the food image and value representing a part of the regional cultural integration that triggered their purchase motivation. Especially, in order to maintain the regional culinary culture for Erzurum Stuffed-kadayif, consumers oriented consciously to these dessert images with the cultural consumption motives together with daily meals at the local restaurants.

On the other hand, it was analyzed that consumers buying Erzurum Stuffed-kadayif from the local food manufacturer stores also contributed meaningfully to the entrocentrism approach by serving the ready-made Erzurum Stuffed-kadayif at the social-cultural meeting strengthening the cultural integration on their purchase motivations. Similarly, it was assessed that consumers buying from or consuming Erzurum Stuffed-kadayif at the local patisseries also attributed a greater priority to entrocentrism approach triggering their purchase motivation through interactive cultural integration.

Table 3. The cluster center values related to the consumers' Erzurum Stuffed-kadayif purchase motives and the sample sizes in each cluster

The main factors	**Consumer segments***					
	Restaurant		**Manufacturer**		**Patisserie**	
	$\bar{x}$	**p**	**$\bar{x}$**	**p**	**$\bar{x}$**	**p**
Food image and value	0.24	*0.002*	-0.05	*0.002*	-0.10	*0.002*
Ready-made local dessert	-0.17	*0.001*	0.18	*0.001*	-0.07	*0.001*
Entrocentrism approach	-0.30	*0.002*	0.14	*0.002*	0.18	*0.002*
Cultural integration	0.17	*0.001*	0.12	*0.001*	0.23	*0.001*
Number of total cases at each cluster (n)	*112*		*156*		*117*	
Population ratio for each cluster (%)	*29.1*		*40.5*		*30.4*	

*Bold values indicate the highest final cluster center scores in each segment.
**Total sample size (n): 385 households.

4. Discussion

The most effective factors on consumers' food purchase motivation during their purchase period are accepted as the drivers of the marketing mixes covering the product, price, communication, and buying convenience mixes (Kotler and Amstrong, 2018). Especially, the local food image and cultural values impacting the consumers' purchase decisions are shaped by appealing motives of the product, price, and communication mixes under cultural integration. Previous researches also informed that the food image and value under culinary culture linked firstly with the various combinations of the major extrinsic product motivation drivers on consumers' local food purchase decisions (Chong et al., 2022;

Khan and Pandey, 2022; Liu et al., 2022; Shi et al., 2022; Topcu, 2022; Giannoutsos et al., 2023; Kaçmaz et al., 2023; Kumar et al., 2023).

Of these studies related to the food image and value on consumers' local food purchase motivation, Kushwah et al. (2019), Akay (2021), Kadirhanoğulları et al. (2021), Shi et al. (2022), Khan and Pandey (2022), Apak and Gürbüz (2023), Huddleston et al. (2023), Kumar et al. (2023) and Perumal et al. (2023) highlighted that the cultural attitude, the region of origin, food brand, advertising, food packaging and labelling, optimum pricing, social media platform, consumption ethically and culinary culture, social responsibility consciousness affected positively consumer perceived local food image and cultural appreciation (subjective value) linking with the cultural integration on their local food buying intentions at the food stores or online platforms, and thus there was a strong correlation among local food image and cultural appreciation under the cultural integration, and it could be also provided a major contribution to sustainable food consumption with the local or the region of origin in the context of cultural integration.

Similarly, Fakreddine and Sanckez (2023), Magalhaes et al. (2023), Mesias et al. (2023), and Siddiqui et al. (2023) also emphasized that the brands, labels, and the region of origin information presented on food packages were generally considered as the important determinants on consumers' purchase decision and motivations, and thus local food image and cultural value judgments for consumers impacted directly on their repurchase decisions. In the current study, indeed, the Erzurum Stuffed-kadayif image and cultural value appreciation under the cultural integration were found to be the most impact stimuli on purchase motivation of consumers preferring the local diners.

On the other hand, Erzurum Stuffed-kadayif promoting to maintain cultural integration among Erzurum-origined consumers purchasing from the manufacturer vendors has still functioned as a ready-made local dessert and has also provided a fairly significant contribution to the entrocentrism approach at the research region. The results of the study, indeed, highlighted that Erzurum Stuffed-kadayif as a part of Erzurum culinary culture buying from the manufacturer stores was serviced more practically along with the daily meals or tea presentations as a ready-made local dessert at social meetings organized by Erzurum-origined consumers residing in all the provinces of Türkiye, and thus not only was it sufficient to ensure a stronger cultural integration among the younger generations, but the entrocentrism approach was also activated. Consequently, it was pointed out that there was a strong correlation among three factors impacting on buying motivation of the consumers purchasing the local dessert from the local manufacturer vendors.

Focused on the entrocentrism approach driving consumers' buying motivation and decision towards the local foods, Chen and Antonelli (2020), Migliore et al. (2021), Miguel et al. (2022), Maro et al. (2023), Siddiqui et al. (2023), Skalkos and Kalyva (2023) and Sundqvist (2023) pointed out that consumer entrocentrism was of a strong and positive relationship with trust in the local and organic foods, to their vendors, cultural integration and convenience food, and thus it was also found to be a vital motivator of their willingness to buy and consume the local foods due to instilling a pride sense that leads to an overestimation of local food appreciation belonging to their culinary culture or ethnicity and satisfiying through consumers' discriminatory sensitive buying behaviour towards the local food with the region of origin.

Attributed similarity to the trends of consumers' willingness to buy Erzurum Stuffed-kadayif from manufacturer stores, consumers adopted only the entrocentrism approach formed by cultural integration by consuming the local dessert at the local patisseries. The identify-based motivation (IBM) model based on the food consumption studies revealed that, indeed, it was differentiated with longitudinal cultural processes and situational activation by contextual cues, each with different implications for the availability and accessibility of ethnic cultural knowledge, and thus the motivation model also associated by consumers' cultural integration with a linear and positive correlation on their food consumption motivations (Aguirre-Rodrigez et al., 2022; Shi and Jiang, 2022; Apak and Günbüz, 2023; Hedriana et al., 2023).

As a result of the cultural integration based on Erzurum culinary culture, consumer entrocentrism promoted their purchase intention and motivation towards the local cultural foods. Erzurum-origined consumers' motivation to purchase Erzurum Stuffed-kadayif was reflected in their attitude towards loving and taking pride in unique local foods and cultures as compared with the others, and thus this phenomenon affected positively their purchase motivation. Dhewi and Oktaviani (2023), Maro et al. (2023), Siddiqui et al. (2023) and Sundqvist (2023) revealed that consumer entrocentrism

was of a positive and significant impact on buying attitude and motivation, and thus consumers entrocentrism was also accepted as a market segmentation tool in most developed countries.

Conclusion

The EFA results of the study revealed that the main factors impacting the Turkish consumers' Erzurum Stuffed-kadayif purchase intension and motivation were the food image and value, a ready-made dessert, entrocentrism approach, and cultural integration. The CA results also highlighted that while middle-income consumers consuming this traditional dessert at the local restaurants were satisfied fairly higher with the local food image under cultural integration, low-income consumers consuming Erzurum Stuffed-kadayif at the local patisseries also attributed big importance to the entrocentrism approach under cultural integration. On the other hand, high-income consumers purchasing Erzurum Stuffed-kadayif a ready-made traditional dessert from local manufacturer vendors tried to contribute considerably to sustainable food supply and consumption with an entrocentrism approach under cultural integration.

Therefore, it should be implemented differentiation and positioning strategies based on the food image along with the manufacturing and processing strategies strengthing cultural integration at the local restaurants for middle-income consumers, and the intensified multy-segment marketing strategies contributing to regional development by acting entrocentrism approach through cultural integration at the local patisseries for low-income consumers, respectively. Similarly, it should be applied the manufacturing and penetrating to new markets strategies focused on Erzurum Stuffed-kadayif as a ready-made dessert reflecting Erzurum culinary culture as a crucial tool of cultural integration and entrocentrism approach for high-income consumers purchasing this local dessert from local manufacturer vendors.

Although this study was one of the first research conducted on consumers' Erzurum Stuffed-kadayif purchase motivation in the economics literature, there were also some limitations. In the study, thus, these limitations could be addressed for the next research. Firstly, the study focused on only consumers in Erzurum due to funding and time constraints. The future researches, hence, could be planned for larger sample sizes accounting for consumers residing at more important trade and consumption centers. Secondly, it was applied the EFA as the research model in the study, but it could be utilized from Confirmatory Factor Analysis (CFA) for the next research, as well.

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Review Article

Bioprocessing of Agricultural and Agro-Industrial Wastes into Value-Added Products

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Lignocellulose

Abstract: Agricultural wastes are one of the most abundant lignocellulosic wastes on Earth. Inevitably, this number will increase due to the increasing population needed to be fed. Unfortunately, this substantial amount of resource is underutilized and ends up in different routes: a) incineration b) left in the field to decay, and c) landfill. In all these possible scenarios, it is obvious that they are both non-ecofriendly or unsustainable for society and related industries. Agricultural wastes are noteworthy "inputs" for the circular economy since they possess high nutritional composition. The circular economy is defined as a system in which the "output" of an industry is reused as a "resource" for another industry. Agricultural and agro-industrial wastes can be converted into value-added products such as enzymes, biofuels, pharmaceuticals, food/feed enhancers, green chemicals, bioplastics, etc. In this way, we can eliminate the problems related to waste management and lower our environmental impact. In addition, a circular bioeconomy can lower the production cost of bioprocesses, create regional job opportunities, and support farmers. This review discusses industrially important products produced via bioprocessing agricultural feedstocks and related examples from the literature are given.

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1. Introduction

Since the first modern oil drill was opened in 1859 in Titusville, Pennsylvania, crude oil has been the feedstock for chemicals, polymers, and other industrial products (Yadav et al., 2020). Unfortunately, crude oil resources are not renewable and they will be depleted in the near future. Moreover, the utilisation of crude oil greatly damages the environment since underground carbon is being released into the atmosphere in a short time.

Lignocellulosic biomass (LCM) is the most abundant, renewable organic matter on Earth. It is composed of lignin, cellulose, and hemicellulose. Photosynthesis is the route for the formation of LCM. LCM constitutes hydrogen, oxygen carbon, and lower concentrations of nitrogen compared to former elements. Extractives, minerals, and ash are the other components found in the structure of LCM.

LCM defines agricultural, agro-industrial, and forestry residues. But in this chapter, its scope will be limited to agricultural waste. 181.5 billion metric tons of LCM are produced every year (Singh et al., 2022). Unfortunately, most of them end up decaying in the field or are burned. Bioprocessing these materials into a value-added product is crucial since it is a considerable route for lowering the cost

of bioprocesses and also for regional economical development. “Figure 1” classifies the agricultural wastes for the production of value-added products (Philippini et al., 2020).

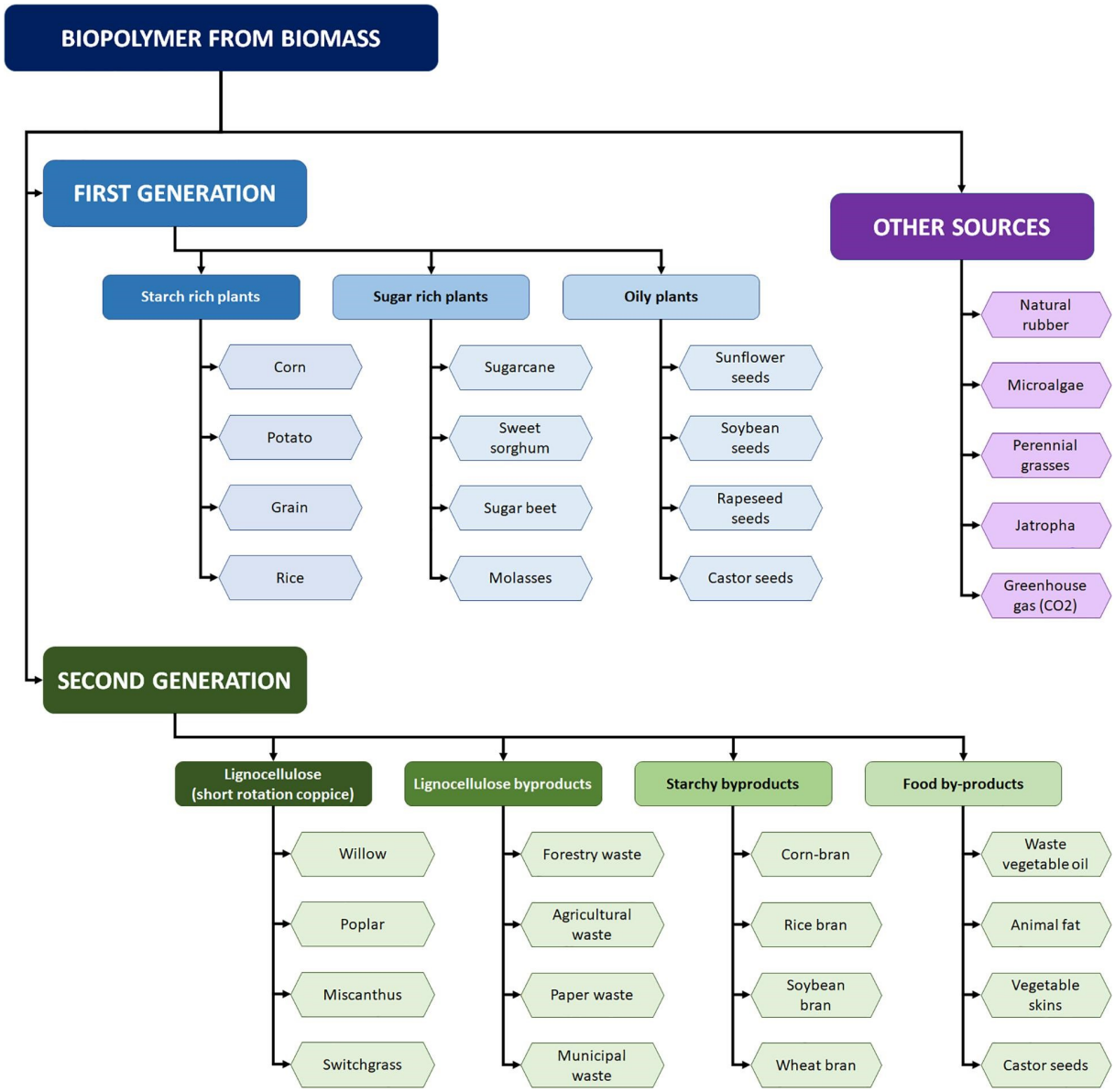

Figure 1. Classification of agricultural biomass for the production of many value-added products (Philippini et al., 2020).

As bioeconomy become prominent in recent years, researches related to the utilisation of LCM for the production of valuable industrial products accelerated and several countries have developed bioeconomy strategies to incent their industries to convert into a sustainable production (Hess et al., 2016). “Figure 2” shows the countries with bioeconomy policies.

Wastes from agriculture and the food industry using LCM are crucial feedstocks for bioeconomy. Agricultural wastes are noteworthy substrates for the production of industrial and medicinal enzymes, biodegradable polymers, and many other chemicals.

In this review, examples of enzyme, biofuel, pharmaceutical, food/feed enhancer, and green chemicals production from LCM are given.

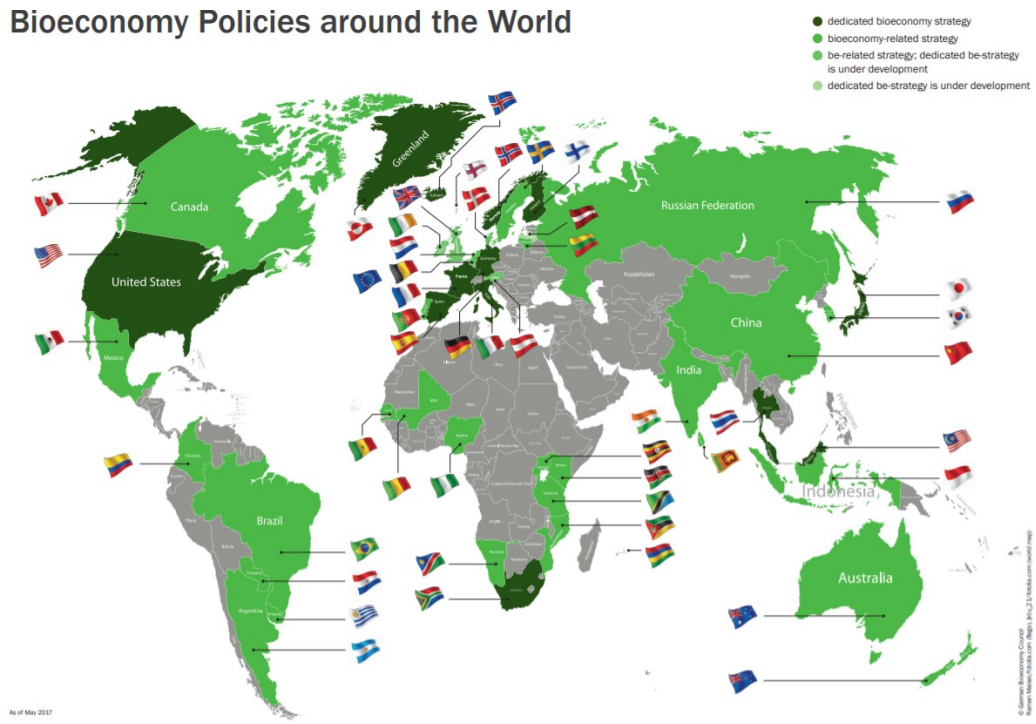

Figure 2. Countries with bioeconomy policies (Hess et al., 2016).

1.1. Enzymes

Lignocellulosic enzymes (LCE), such as cellulases, xylanases, and lignin modifying enzymes, can be produced through whole cell biocatalysis of LCM. These enzymes find application to a broad extent, such as bioremediation (Yesilada et al., 2010; Yadav and Yadav, 2015; Falade et al., 2018; Hu et al., 2018), detergent (Azmi et al., 2016), pulp and paper (Couto et al., 2006; Freitas et al., 2009), textile (Menendez et al., 2015), biosensors (Mate and Alcalde, 2017), animal feed (Shraddhaet al., 2011; Sharma and Arora, 2014; Moreno et al., 2020).

Cellulose and hemicellulose (i.e. plant carbohydrates) are exploited by microorganisms for their survival and also for the production of industrially important products, such as enzymes. This conversion is the keypoint of the carbon cycle in nature (Ruiz-Dueñas and Martínez, 2009; Jönsson and Martín, 2016).

There is a broad range of LCM spread over different kinds of geographies of Earth, and so are LCE and microorganisms growing on them (Castanera et al., 2012; Hasunuma et al., 2013).

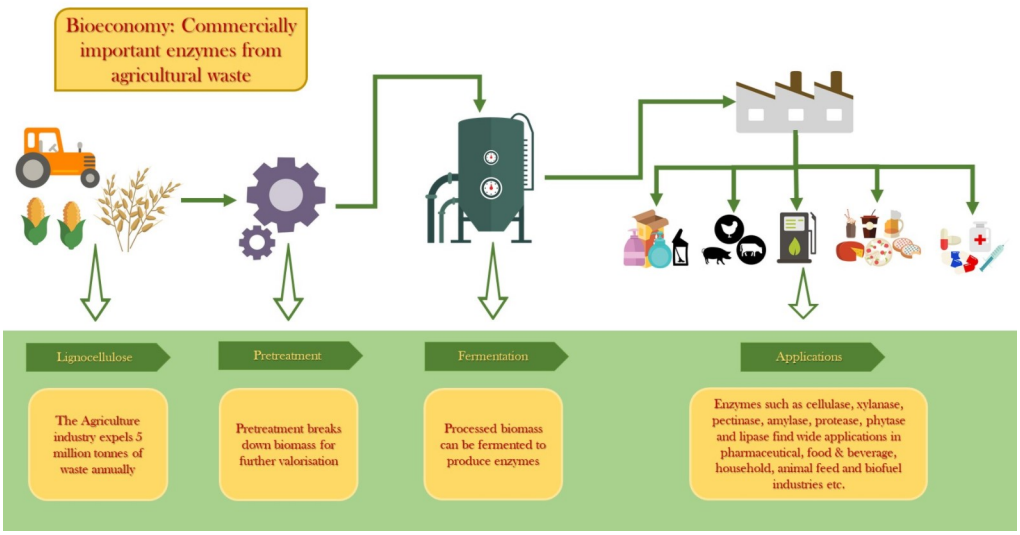

Figure 3. Production of industrial enzymes from agricultural wastes (Ravindran et al., 2018).

Cellulases (such as beta-glucosidase and endoglucanases) are used in many industries, such as animal feed (Cunha et al., 2017), food, energy (Olofsson et al., 2017), fermentation, pulp and paper, agriculture, and textile (Madhu and Chakraborty, 2017).

In a recent study, pea hulls were used to produce cellulase. Pea hulls are the main waste from the frozen food processing of pea and represent 60% of the total pea waste. *Trichoderma reesei*, which is accepted as the most efficient cellulase producer, was used as a biocatalyst for the biotransformation of pea hulls into cellulase (Sirohi et al., 2019).

Bacteria can also be used for the production of cellulase from different kinds of lignocellulosic biomass. Swathy and colleagues used waste date palm seeds for the production of high grade cellulase, where *Cellulomonas uda* NCIM 2353 was used as biocatalyst. They compared the efficiency of the produced cellulase to commercial cellulase, Cellic® CTec2 (Novozymes corporation, Denmark), and found that saccharification efficiency for acid-pretreated sugarcane bagasse was as high as 60.5%, which was equivalent to the efficiency of Cellic® CTec2. After the saccharification, resulting hydrolysate (rich in reducing sugars) was fed to *Clostridium thermocellum,* and the production of biohydrogen of maximum concentration 187.44 mmol L^{-1} was achieved at the end of 24 h of fermentation (Swathy et al., 2020).

Not only cellulase but also xylanase is another enzyme of paramount industrial importance. They are used together with cellulase in biorefineries to promote ethanol fermentation. Kaur and colleagues tried to enhance the xylanase production efficiency from *Bacillus pumilus* 3GAH via optimization of the process variables. They used Central Composite Design (CCD) to find these optimal conditions of concentration of xylose, yeast extract, peptone, and optimal concentrations were found to be 0.2%, 0.2%, and 0.2% respectively, with a fixed concentration of wheat bran (0.3%). Optimized conditions resulted in an approximately 24 fold increase in the activity of produced xylanase, compared to conditions before optimization (Kaur et al., 2016).

Carboxymethylcellulose production by *Bacillus sp. 313SI* under submerged and stationary fermentation conditions were investigated in the study of Goyal et al. Rice straw is one of the largest agricultural wastes with a capacity of 1.35 tonnes per 1 tonne of harvested rice. They investigated the effect of different concentrations of inoculum, and rice straw. Additionally, various carbon and nitrogen supplementation with a fixed concentration, temperature, and pH range were tested for their effect on carboxymethylcellulose production. Prior to fermentation, rice stalks were pretreated by two-stage pretreatment: alkali treatment (for removal of lignin) followed by acidic treatment (for removal of cellulase) (Goyal et al., 2014). The optimum conditions of these studies were tabulated below (Table 1).

Table 1. Optimum conditions for carboxymethylcellulose production in the study of Goyal et al. (2014)

	Submerged	**Stationary**
Carbon Source	Carboxymethylcellulose	Carboxymethylcellulose
Nitrogen Source	Ammonium nitrate	Ammonium sulphate
Inoculum (v/v)	0.4	1
pH	8	8
Temperature	30	35
% of rice straw	0.75	1
Maximum activity (U L^{-1})	4150	3080

Lignin moiety is responsible for the recalcitrance nature of lignocellulosic biomass. It hinders the access of microorganisms to the carbohydrate moiety (which is cellulose) of lignocellulose. Luckily some of the microorganisms, especially white rot fungi, can grow on lignocellulosic biomass due to their ability to secrete extracellular lignin modifying enzymes, such as laccase, and by the action of these enzymes, the recalcitrance of lignocellulosic biomass loosens, and microorganism can effectively use cellulose as a carbon source to survive. Laccase is one of the lignin-modifying enzymes, which is capable of oxidizing lignin. Utilization of laccase is not limited to biological pretreatment of lignocellulosic for second generation bioethanol production (Rico et al., 2014). In addition, laccase can be used in other sectors such as bioremediation (Bilal et al., 2019), biomedical technologies (Mate and Alcalde, 2017), the food industry (Osma et al., 2010), and even in cosmetics (Dana et al., 2017).

In the study of Asgher et al., *Schizophyllum commune* was used to produce laccase from rice straw. *Schizophyllum commune* is a white-rot fungus, which is capable of producing lignin-modifying

enzymes from agricultural wastes (Asgher et al., 2016). They reported that rice straw was a cheap source of producing lignin-modifying enzymes, including laccase. They tested the ability of the enzyme cocktail for reduction of lignin content of different agricultural residues such as sugarcane bagasse and banana stalk and found out that after 48 hours of treatment, the reduction was by 72.3% and 61.7%, respectively.

A new approach - two step cultivation strategy - to increase fungal laccase production was reported by Hazuchova and colleagues in 2017, where the first step is the propagation of fungi, and the second step is the production of laccase via using lignocellulosic biomass as an inducer. *Pleurotus ostreatus* was used for laccase production. They used different kinds of lignocellulosic biomass, including corn straw being one of the highly produced agricultural waste around the world. The highest laccase activity was achieved with the corn straw, which is almost two times higher than the media which are supplemented with pine straw dust and alfa alfa steam. This result was achieved with two-step cultivation, which was better than one-step cultivation by 9 to 16 fold (Hazuchová et al., 2017).

It is important to mention that enzyme cocktails produced from a specific agricultural waste will obviously be better in the degradation of that specific agricultural waste (Cunha et al., 2017).

Although fungi are thought to be a good producers of laccases, some bacteria are also good at the production of laccases. In addition, due to their thermotolerant nature and ability to retain their activities in alkaline conditions, bacterial cellulases are important in industrial applications.

Muthukumarasamy and colleagues (Muthukumarasamy et al., 2015) reported that *Bacillus subtilis* MTCC 2414 was good at the production of laccases from wheat and rice bran. They used solid-state fermentation as the production type. Rice bran (345 ± 3.14 U mL^{-1}) was found to be a better substrate than wheat bran (265 ± 4.44 U mL^{-1}) for a higher yield of enzyme production.

Beta-glucosidase is another valuable enzyme that is used in industry. It is not only the key enzyme in the degradation of lignocellulosic biomass for bioethanol production (Saritha Mohanram, 2015; Sukumaran et al., 2009), but it is also used in many other sectors, such as juice and alcohol production sectors, as clarification and taste enhancer agent (Claus et al., 2018).

Yilmaz-Sercinoglu and Sayar have reported optimal medium compositions for the production of laccase and beta-glucosidase from hazelnut husk using *Pycnoporus sanguineus* DSMZ 3024 as the producer strain. They stated that beta-glucosidase production was better in a medium supplemented with yeast extract and sodium nitrate as a nitrogen source (111.9 U L^{-1}), while the maximum activity of laccase was observed in a medium supplemented with malt extract (581.7 U L^{-1}) (Yilmaz-Sercinoglu and Sayar, 2020).

The thermophilic fungus *Myceliophthora heterothallica* was used as the biocatalyst for beta-glucosidase production. Wheat bran and sugarcane bagasse were the substrates in solid-state fermentation, while cardboard was used in submerged cultivation. The results showed that maximum beta-glucosidase production was 244 ± 48 U g^{-1} in submerged fermentation and 0.9 ± 0.3 U g^{-1} in solid-state fermentation (Teixeira Da Silva et al., 2016).

Phytase is another important enzyme that is used in the animal feed industry as an enhancer. Legumes and cereals seeds contain phosphorus as phytate. Phytate can not be easily digested by animals and for this reason, external supplementation of phosphorus to the feed is indispensable. Moreover, phytate reduces the adsorption of essential nutrients from the feed, since it binds to amino acids and ions such as Zn^{+2}, Ca^{+2}, Cu^{+2}, Fe^{+2}, and Mn^{+2} and insoluble salt forms (Coban et al., 2015). Additional phosphorus and other nutrients increase the cost of feed production, and excess phosphorus through animal feces leads to accumulation in the aquatic environment.

The addition of phytase to animal feed, therefore, is pivotal to enhance the availability of phosphorus in seeds and legumes and reducing the environmental impact of excess phosphorus.

In a study, researchers used corn cope and corn brain for phytase production via solid-state fermentation. The biocatalyst is *Penicillium purpurogenum* GE1 and was isolated from soil around bean root nodules. Corn cope was better in the production of phytase (46 ± 2.8 U g^{-1} ds (units per gram dry substrate)), compared to corn bran (41 ± 4.2 U g^{-1} ds) (Awad et al., 2014).

In a recent study, two different organisms were used for the production of phytase. Medium supplemented with corn meal favored the production of phytase when the fungus *Acremonim zeae* was used as a biocatalyst. Rice bran was the best substrate for phytase production by the yeast *Kluyveromyces marxianus* (Pires et al., 2019). They also mentioned that the effect of different substrates on production

efficiency could have resulted from the environment where microorganism was isolated. This also indicates the possibility of the production of different enzymes from a broad range of substrates.

1.2. Green chemicals and pharmaceuticals

Production of platform chemicals via biochemical processes is a great alternative since they possess a lower impact on environments and use cheaper feedstock. The utilisation of agricultural wastes and byproducts/wastes of food processing industries are valuable feedstock for the product of green chemicals and pharmaceuticals (Perlatti et al., 2014; da Silva, 2016).

Hydrolysates of agricultural waste can also be used for the production of value-added chemicals, such as lactic acid. Lactic acid is the building block of polylactic acid (PLA), which is gaining attention in the industry due to its biodegradable and hydrophobic nature. Almost 40% of the production cost of lactic acid results from the expense of the substrates and nutrients (Krull et al., 2020).

In their study, Krull and colleagues (Krull et al., 2020) tested the ability of *Lactobacillus casei* ATCC 393 for the production of lactic acid. They changed 70% of the nutrient sources with hydrolysed rapeseed meal or distillers' dried grains with solubles (DDGS) and reached the same production yields. Although there is no increase in productivity, the total cost of the lactic acid production process was reduced by almost 25%. This result is encouraging to see that agricultural wastes are a perfect substitute for expensive nutrients, which are the main obstacle to the biochemical routes for large-scale production of such chemicals.

Molasses and corn steep liquor were used to produce D (-) lactic acid via *Lactobacillus delbrueckii*. The organism was able to grow on these substrates and after 48 hours of cultivation 162 g L^{-1} D(-) lactic acid was produced (Beitel et al., 2020).

Resveratrol is an important phenolic bioactive compound produced as a secondary metabolite in plants, especially in grapes and berries (Costa et al., 2022). It is highly studied because of its pharmaceutical importance in cardiovascular and neural diseases (Gordish and Beierwaltes, 2014; Yetik-Anacak et al., 2015). It is produced via genetically engineered organisms, but microbial extraction from plants is another way to obtain resveratrol from plants.

An immobilized microbial consortium (Yeast CICC 1912, *Aspergillus oryzae* 3.951, and *Aspergillus niger* 3.3148) was used to extract resveratrol from grape seed wastes from the winemaking process. Researchers reported that microbially treated samples resulted in approximately 6 times higher yield of resveratrol (305.98 ± 0.23 μg g^{-1}) than untreated samples (Jin et al., 2021).

1.3. Biopolymers

Integration of agricultural wastes into a biorefinery is a profitable route for the production of not only enzymes and fuels but also biopolymers. Petrochemical substances are being used to produce many polymers, especially synthetic plastics. But growing concerns about plastic pollution accelerated the research for biodegradable polymers synthesized by various microorganisms using different agricultural wastes.

Pullulan is one of those biopolymers. It is an α-linked linear glucan exopolysaccharide and has unique physicochemical properties, such as non-toxicity, non-mutagenicity, tasteless, odorless, and edible nature. Films with different strengths can be produced from pullulan and these films are recalcitrance to slight thermal changes. All of these properties make it suitable candidate to be used in the food, biomedical and pharmaceutical industries. Comprehensive summary of pullulan applications was schematized by Coltelli and colleagues and it is given in "Figure 4" (Coltelli et al., 2020).

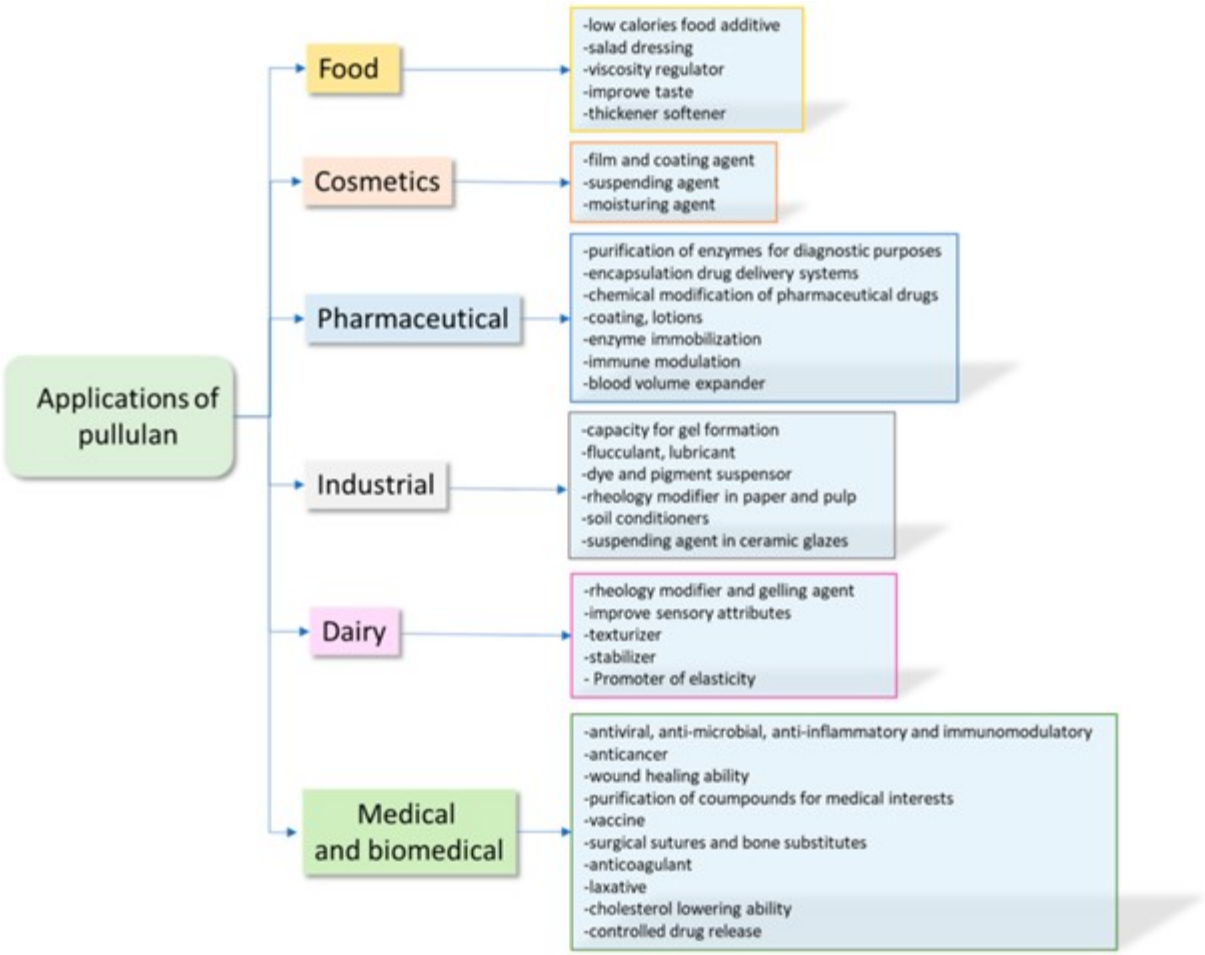

Figure 4. Comprehensive schematisation of pullulan applications (Coltelli et al., 2020).

Major obstacle in front of commercialization of pullulan production is expensive process cost (mostly due to substrate cost) and low yield.

Many studies can be found in the literature which were conducted to find the optimum media compenents for production of pullulan with high yield. In the study of Viveka and colleagues, cassava waste was investigated as substrate for pullulan production from *Aureobasidium pullulans* MTCC 1991 (Viveka et al., 2020). They achieved to produce pullulan with a yield of 6.45 g pullulan L^{-1}.

Another strain of *Aureobasidium pullulans* (MTCC 6994) was used as biocatalyst to produce pullulan in a laboratory scale stirred tank bioreactor. De-oiled rice bran was used as a carbon source. They achieved the maximum yield of pullulan as 8.32 ± 0.02%, *w/v* (Singh et al., 2020).

In a recent study, kitchen waste (KW) was used as a source of reducing sugars. An in-house produced enzyme cocktail was used to hydrolyze KW. Hydrolysate of KW was then fed to *Aureobasidium pullulans* MTCC 2013 and the production yield was achieved as 20.46 ± 2.01 g L^{-1} pullulan. Further analyses showed that produced pullulan is biodegradable and water soluble and similar to commercial pullulan (Rishi et al., 2020).

The melanin-deficient strain of *Aureobasidium pullulans* KY767024 was used to produce pullulan from sesame seed oil cake (SSOC), as a novel substrate, without the addition of other nutritional sources. 52.50 ± 0.73 g pullulan was produced per unit kilogram of sesame seed oil cake (Mirzaee et al., 2020).

Annual production of fossil-based plastics reached 300 million metric tonnes. Plastic pollution is a growing concern around the world, but attempts to switch to biodegradable plastics are slow since the production cost is a major obstacle in the mass production of biodegradable plastics. Agricultural wastes, side streams of biodiesel and bioethanol industries, and also dairy industry effluents can be used as potential carbon sources for the production of biodegradable plastics, such as polyhydroxyalkonates (PHA) (Figure 5) (Koller, 2017; Winnacker, 2019; Hon Kee et al., 2022). PHA are well-known bacterial

storage polymers and a well-studied type of PHA is polyhydroxybutyrate (PHB). They share similar properties with petrochemically derived plastics (Penkhrue et al., 2020). Due to its brittle nature with low thermal and relatively low mechanical properties, PHB is usually used in composites with other biodegradable polymers such as polylactic acid (Arrieta et al., 2017; Jamwal et al., 2023).

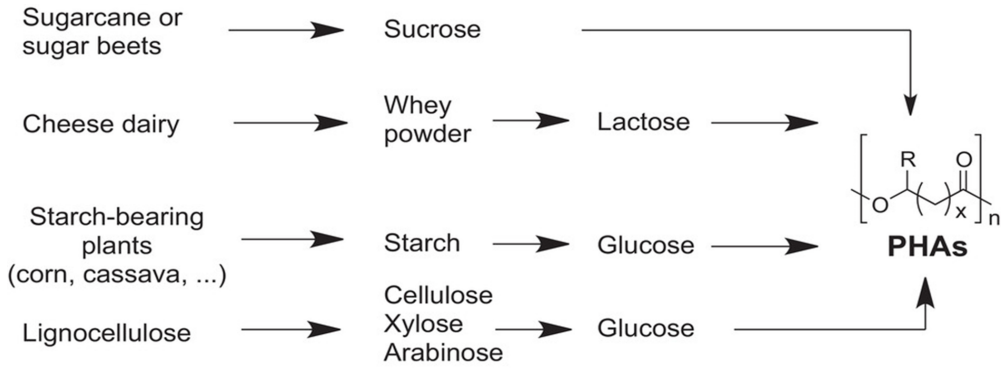

Figure 5. Potential feedstocks for PHA production (Winnacker, 2019).

Lysinibacillus sp. was used as a biocatalyst for production of PHB production from sugar cane bagasse (SCB) hydrolysates supplemented with corn steep liquor. Characterisation of produced PHB showed that it is identical to commercial PHB. They included that detoxification of SCB hydrolysate was not necessary. It is noteworthy in terms of reducing process steps. Devoid of detoxification, overall process cost will eventually reduce, together with cheap substrate (Saratale et al., 2021).

Hassan et al. used different agricultural wastes such as corn cob, wheat bran, corn bran, rice bran, and sugarcane molasses for PHB production. Rice bran outperformed the other carbon sources for PHB production. They optimised cultural parameters using Plackett–Burman and Box–Behnken designs. A six-fold increase in the PHB content was achieved when production was carried out under optimized conditions (Hassan et al., 2019).

In another study, wheat bran, rice bran, and corn cob were again used as substrates for both PHA and enzyme production (Israni and Shivakumar, 2020), in addition to other substrates, such as bajra straw and bagasse, wheat husk, wheat straw, ragi husk (RH), rice husk, rice straw, oat straw, jowar straw, mustard oil cake, sesame oil cake (SOC), groundnut oil cake and coconut oil cake. *B. megaterium* strain Ti3 (accession number HF968632) was used as a biocatalyst for PHA production.

RH and SOC were better at supporting maximum PHA production among other substrates. They also studied the effective pretreatment method of those substrates to obtain higher reducing sugar content. Although acid and alkali hydrolysis resulted in significantly higher initial reducing sugar levels for RH and SOC, the use of microorganisms' enzymes for the treatment of the substrates was offered to reduce the cost of the overall process and also for avoiding possible inhibitors released during chemical treatments of substrates (Kim et al., 2011; Mithra and Padmaja, 2016).

Bacillus drentensis (*B. drentensis*) BP17 was used for the production of PHB production from various fruit peels, including dragon fruit, apple, pineapple, mango, sugarcane, and banana. Peels were soaked in hot water to get extractions of peel and those extracts were supplied to *B. drentensis* BP17 as a cheap carbon source. 40.8 g L^{-1} reducing sugar was achieved after the hot water treatment of pineapple. Pineapple peel juice outweighed the other substrates in PHB production (5.6 g L^{-1}) in batch fermentation (Penkhrue et al., 2020).

In the latest study, researchers used finger millet straw (FMS) hydrolysates for the production of PHB. They used a combination of physicochemical and enzymatic treatment (enzymatic hydrolysis of ultrasound-aided alkaline treated FMS) to collect reducing sugars. PHB production was 4.81 g L^{-1}. Neutral pH favored the production of PHB (Silambarasan et al., 2021).

Conclusion

In this review, the importance of agricultural waste as a potential substrate for biotechnological production of industrially important chemicals is emphasized. Agricultural residues, side streams, and wastes of food processing industries are often treated as waste. On the contrary, they are valuable

feedstocks that can be used to produce value-added chemicals due to their diverse nutritional composition. When the microbial and vegetational diversity on Earth is taken into consideration, it is obvious that there are numerous possibilities to match the agricultural residues and related microorganisms for the production of value-added chemicals.

While the potential of agricultural waste as a feedstock for bioprocesses is undeniable, several challenges hinder its commercialization. Chief among these obstacles is the cost of the substrate itself, which can be prohibitive for large-scale production of value-added products. Additionally, the collection cost, encompassing activities such as harvesting, logistics, and storage, present another significant hurdle.

The collection cost of agricultural waste represents a significant aspect that warrants careful consideration in any biotechnological valorization strategy. The process of gathering these residues involves multiple stages, starting with the efficient and timely harvesting of agricultural crops. Properly coordinating harvesting activities to coincide with peak waste generation periods is essential to optimize collection efficiency.

Logistics also play a crucial role in managing the collection cost. Establishing well-designed transportation networks that connect agricultural regions to processing facilities can streamline the movement of waste materials, reducing both time and expenses. Implementing innovative solutions such as regional collection centers or mobile collection units can further enhance the efficiency of waste retrieval and minimize the burden on individual farmers.

Another factor contributing to the collection cost is the storage of agricultural waste. Since waste generation may not always align with the immediate requirements of processing facilities, proper storage facilities become necessary to maintain the quality and quantity of the collected materials. Investing in suitable storage infrastructure can prevent waste deterioration and ensure a consistent supply of feedstock throughout the production process.

Moreover, the collection cost can vary based on the type of agricultural waste being generated. Different crops or food processing by-products may have distinct characteristics that affect handling and transportation methods. Understanding these variations and tailoring collection approaches accordingly can significantly impact the overall cost-effectiveness of the biotechnological production process.

Addressing the collection cost challenge requires a collaborative effort among farmers, waste management companies, and relevant industries. By promoting knowledge-sharing and providing technical support, farmers can improve waste segregation practices and facilitate more efficient waste collection. Concurrently, partnerships with waste management companies can lead to the development of specialized collection techniques and the implementation of cost-saving measures.

Overall, optimizing the collection cost of agricultural waste is crucial for establishing sustainable biotechnological processes that unlock the immense potential of these valuable feedstocks. By focusing on innovative approaches, strategic planning, and community engagement, we can create a robust and economically viable system that transforms agricultural waste into a valuable resource for the production of essential value-added chemicals.

In conclusion, it is evident that agricultural residues must no longer be dismissed as "waste." Through strategic and collaborative efforts involving farmers, industries, governments, and the public, we can effectively transform these materials into valuable resources that drive the production of essential value-added chemicals, all while fostering sustainable practices and contributing to a greener and more prosperous future.

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