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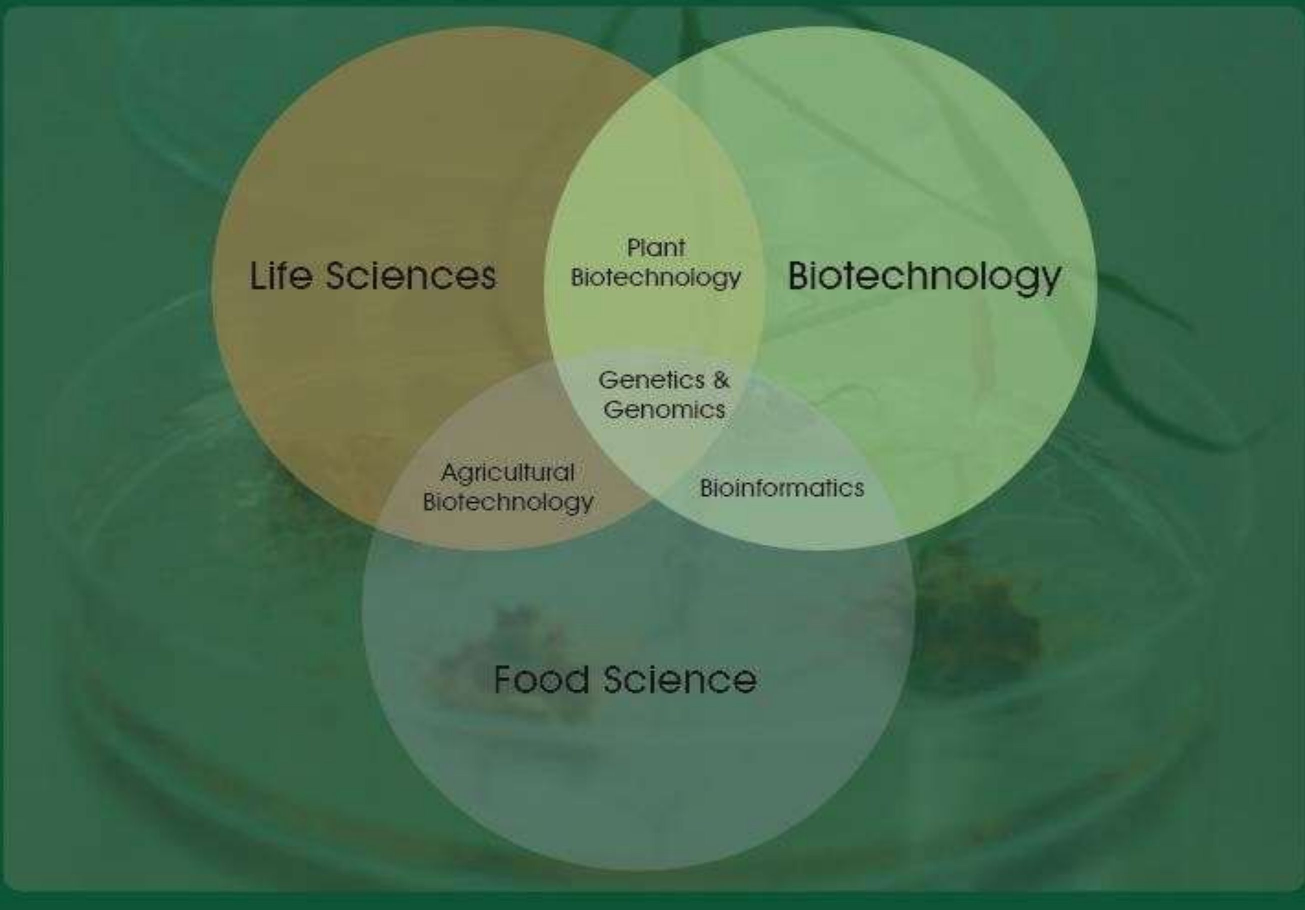

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Yildiz Technical University, Faculty of Chemical and Metallurgical Engineering.
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From The Editor;

Dear Readers and Authors,

As “International Journal of Life Sciences and Biotechnology”, we are pleased and honored to present the 22th issue of the journal. "International Journal of Life Sciences and Biotechnology" is an international double peer-reviewed open access academic journal published on the basis of research- development and code of practice.

The aims of this journal are to contribute in theoretical and practical applications in relevant researchers of Life Sciences, Biology, Biotechnology, Bioengineering, Agricultural Sciences, Food Biotechnology and Genetics institutions and organizations in Turkey, and to publish solution based papers depending on the principle of impartiality and scientific ethics principles, focusing on innovative and added value work, discussing the current and future.

With these thoughts, We are especially thankful to academicians honoring with the articles, valuable scientists involved in editorial boards and reviewers for their contributions to the evaluation processes with through their opinions/ideas/contributions/criticisms in the second issue of 2025 "International Journal of Life Sciences and Biotechnology". Hope to see you in the next issue…

15. 08. 2025
Editor in Chief
Prof. Dr. Ali Aslan

Editörden;

Değerli okurlar ve yazarlar,

“International Journal of Life Sciences and Biotechnology” olarak dergimizin yirminci ikinci sayısını yayın hayatına sunmaktan mutluluk ve onur duyuyoruz. “İnternational Journal of Life Sciences and Biotechnology” dergisi araştırma- geliştirme ve uygulama ilkeleri baz alınarak yayınlanan uluslararası hakemli açık erişimli akademik bir elektronik dergidir.

“İnternational Journal of Life Sciences and Biotechnology” dergisi Yaşam Bilimleri, Biyoloji, Biyoteknoloji, Biyomühendislik, Ziraat Bilimleri, Gıda Biyoteknolojisi ve Genetik alanlarındaki ilgili araştırmacılara, kurum ve kuruluşlara teorik ve pratik uygulamalarda katkı sağlamayı, tarafsızlık ve bilim etiği ilkelerine bağlı kalarak çözüm temelli, yenilikçi ve katma değeri olan çalışmalara odaklanan, günceli ve geleceği tartışan çalışmaların yayınlanmasını hedeflemektedir.

Bu düşüncelerle 2025 yılı ikinci sayısını yayınladığımız “Internatıonal Journal of Life Sciences and Biotechnology” dergisini, makaleleri ile onurlandıran akademisyenlere, Fikir / Görüş / Öneri / Katkı ve Eleştirileri ile değerlendirme süreçlerine katkılarından dolayı hakem ve yayın kurullarında yer alan kıymetli bilim insanlarına yürekten teşekkür ediyoruz. Bir sonraki sayıda görüşmek ümidiyle…

15.08. 2025
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Araştırma Makale/ Research article

Extraction and Comparison of Limonene Contents of Wastes in *Citrus* Species for Reuse

Tuğba Pelin Toker[1], Mustafa Hamza Mawlood Al Bayati[1,2], Mariem Bouali[1], Ümit Babacan[1], Mehmet Fatih Cengiz[1*]

ABSTRACT
Identification and extraction of limonene in fruits of the genus *Citrus* L. which has several medicinal, cosmetic, and industrial properties, is crucial for reusing fruit wastes after they have been processed into fruit juice. The aims of the current study are therefore (i) to compare limonene contents in different fractions such as peels, pulps and juices of citrus species including orange (*C. cinensis* L.), lemon [*C. limon* (L.) Osbeck], grapefruit [*C. paradisi* (L.) Macfad.] and tangerine (*C. tangerina* Tanaka)), (ii) to extract limonene from the most abundant fraction of selected citrus species and (iii) to optimize the extraction parameters of supercritical fluid carbon dioxide (SFC) extraction system. The limonene contents were determined using high performance liquid chromatography (HPLC) and extraction conditions of SFC were optimized by Response Surface Methodology (RSM). The highest limonene content was determined in the peel fraction of the lemon. The limonene was found to be <LOQ in all tested fruit juices. In the subsequent step, limonene was extracted from lemon peels using the SFC extraction system, and the optimal parameters for the system were determined to be 52°C and 147 Mpa. In conclusion, peels and pulp fractions of citrus fruits can be potential limonene extract due to the highest content of limonene after being processed into fruit juice. The results of the current study elucidated that by extracting limonene from citrus peel and pulp waste, not only environmental pollution was prevented, but also the reuse of the waste was transformed into a sustainable production model.

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Introduction

The genus *Citrus* L., which belongs to the subfamily *Aurantioideae* and the family *Rutaceae*, consists of important domesticated species such as orange (*Citrus cinensis* L.), lemon [*C. limon* (L.) Osbeck], grapefruit [*C. paradisi* (L.) Macfad.] and tangerine (*C. tangerina* Tanaka). As one of the fruits with the highest consumption rates worldwide, citrus fruits are popular for both their healthy components and health supplements[1]. According to the Food and Agriculture Organization (FAO) statistical database in 2022, the most produced citrus fruit was orange with 76.4 million tons followed by tangerine or mandarin, or clementine with a total 44.1 million tons, and lemon or lime with 21.5 million tons, while the least produced citrus was grapefruit or pomelo with 9.7 million tons in the world [2]. Despite the strong demand for citrus fruit, there is still a significant proportion has been wasted, for instance, almost 50% of lemons are utilized to make fruit juice and marmalade, and a considerable proportion of lemons, between 50% and 60% of the processed fruit, is wasted [3]. Global annual production of 25 million tons is estimated just for orange peel wastes, it is therefore omitted a significant peel waste and a chance to recover them [4]. In terms of worldwide production, citrus waste equals to around 110–120 million tons per year. The focus must be centered on valuable components made of organic acids derived from citrus wastes and biodegradable polymers in the food processing, pharmaceutical, and chemical industries [5]. Commercially damaged fruit and peeled or pressed wastes for fruit juice factories are the three primary forms of citrus waste. Since these materials are waste, they cannot be linked to the food chain. For every 1000 tons of fruit processed, commercial citrus juicing produces 450–550 tons of waste solids including peel and rag [6]. Citrus waste has a low pH (3–4), a high water content (80–90%), and a high organic matter level (95% of total solids). According to European regulations, these traits

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make citrus waste inappropriate for landfill disposal (Council Directive 2008/98/EC of 10th November 2008 on waste) [7]. On the other hand, these waste products have biological active contents that are essential for industrial properties, such as phenolics, flavonoids, and limonene. Citrus peels, which includes approximately 4% (w/w) of limonene, are appropriate product for the recovery of valuable molecules [8].

Limonene ($C_{10}H_{16}$) is a cyclic monoterpene [9] that a citrus-like odor and a colorless liquid at room temperature. In addition, it can be soluble in water 7.57 mg/L at 25°C [10]. This compound can be used as a scent in soaps and fragrances as well as a sweetener in some foods. Limonene is also extensively used in the food industry to flavor foods such fruit juices, sweets, chewing gum, soft drinks, and ice creams, such as lemon aroma in chemical industry [11]. The antibacterial activity of limonene against three bacteria and three yeasts, constituting the spoilage microflora of fruit juices, was observed [12]. Furthermore, limonene has antifungal properties were discovered as well [13]. Thus, limonene is considered as a safe food preservative. Moreover, in agricultural area, limonene is a safe and natural pesticide by directly or in combination with other compounds [14]. The anticancer effects of limonene have a positive impact on some animal cells. Regardless of the precise mechanism of limonene is unknown, it may be a promising new inquiry for breast cancer [15]. Hence the mentioned reasons, limonene is commonly preferred ingredient in numerous industrial applications.

Extraction systems SFC appears to be a promising and different process because it operates at a low temperature, has a good mass transfer rate, and leaves no solvent residue in the finished product [16]. By employing SFC, it was possible to determine the separation of limonene from a certain plant fraction by selecting the proper temperature-pressure ratio during the extraction.

In the current study, citrus fractions compared to produce limonene, which is used in the food and agricultural industries in addition to the cosmetic business. Limonene content varies depending on environmental conditions and is affected by species of citrus and genotypes in the species. According to available literature, there is a gap on comparison of limonene content of citrus species. The current study is distinctive in that it compares the amounts of limonene found in various citrus fruits and their various components. The objectives of the current study are to (i) quantify the amount of limonene in a few *Citrus* species and (ii) identify the most precise SFC parameters.

Material and Methods

Plant materials

Citrus fruits used in the current study, orange (*Citrus cinensis* L.), lemon [*C. × limon* (L.) Osbeck], grapefruit [*C. × paradisi* (L.) Macfad.] and tangerine (*C. tangerina* Tanaka) were purchased from markets located in Antalya, Turkiye in December 2021. Collected materials were peeled and remaining parts were squeezed. In this case, three different fractions as peel, juice and pulp (remining part after squeezing) were obtained. The juice fractions were analyzed directly, whereas peel and pulp fractions were analyzed after a drying process at the conditions of 40°C for 72 hours in an incubator. The dried materials were ground by a mechanical grinder and sifted by sieves. All the materials in the current study were stored at -18°C prior to extraction to protect the loss of active ingredients.

Chemicals

Limonene analytical standards were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). All chemicals used in the current study except analytical standards were of liquid chromatographic grade. Methanol (MeOH), ethanol (EtOH), and acetonitrile were obtained from Isolab (Wertheim, Germany), while acetic acid was purchased from Merck (Darmstadt, Germany). The analytical standards at various concentrations were prepared in MeOH for HPLC analysis. The water for preparing the HPLC mobile phase was produced by an ultrapure water purification system of MP Mini pure (Ankara, Turkiye). CO_2 gas for SFC extraction was supplied by HABAŞ (Antalya, Türkiye).

Extraction of limonene by SFC extraction system

According to the HPLC analysis results, the fraction with the highest level of limonene concentration were extracted by a SFC extraction system (SuperEx F-500, Pard Engineering, Konya, Türkiye). For this aim, the fraction was prepared in a larger amount and weighed 50 grams. The material was placed to the reactor part of SFC extraction system. The system was held for 15 min in the static phase and the total extraction time was set at 75 min, under selected temperature and pressure. The experimental conditions of SFC extraction system for optimization of parameters are shown in Table 1.

Table 1. Trial design to find optimum SFC conditions

Experiments	Temperature (°C)	Pressure (bar)	Material (g)	Extract (g)
1	33	120	50.2	0.53
2	47	120	50.0	0.48
3	47	180	50.8	0.56
4	33	180	50.7	0.46
5	30	150	50.2	0.69
6	40	108	50.4	0.33
7	40	200	50.5	0.32
8	40	150	50.6	0.27
9	40	150	50.9	0.28
10	40	150	50.2	0.29
11	40	150	50.5	0.56
12	40	150	50.6	0.47

Determination of limonene concentration

Prepared fractions of citrus fruits were analyzed according to following procedures using by a HPLC method. Briefly, 0.5 g of prepared sample were transferred to a glass bottle and 10 ml of EtOH was added. The mixture was applied to ultrasonic assisted extraction system (ISOLAB, Eschau, Germany) for 10 min. The system configuration was a frequency of 23 kHz, power of 750 watts and temperature of 50°C. After the homogenization the solution was filtered via paper filter and 0.22 µM PVDF membrane filter. Clarified solution was diluted with MeOH and injected to the HPLC system (Agilent 1200 Technologies, Böblingen, Germany) with Diode Array Detector (DAD). Chromatographic separation was performed via C18 column (250 mm × 4.6 mm × 5 µm). The mobile phase composition was applied to be acetonitrile (78%) and ultra-pure water (22%). The injection volume and column temperature were 20 µL and 40°C, respectively. The total chromatographic run was 30 min with a flow rate of 2 mL/min. All peaks in the chromatogram were detected at a wavelength of 210 nm.

Statistical analyses

Response-surface method was used to optimisation of the extraction parameters. In the optimization process, the specified parameters' first and second-order effects on the concentrations of the extracted limonene and their possible interactions with each other were statistically investigated. As a result of the analysis, 3-D response surfaces graphics that show these parameters' effects were created using the models in which the process parameters were effective. Student's t-test was used to evaluate the data. The data were evaluated at a 95% confidence interval and $p<0.05$ significance level. MINITAB 18 and Microsoft Excell software were performed for descriptive images of the features.

Results

Method validation

According to the results of method validation study, the calibration curve was constructed by different concentration of limonene. It was concluded that the method was linear ($r^2=1.000$) in the specified concentration ranges (1-64 ppm). In addition, the concentrations of the analytical limonene standard at the same concentration with the LOQ level were injected six times, and the obtained peak area sizes and retention time shifts were evaluated. The results showed that the shift in the retention time of the limonene was found to be insignificant. The LOD (limit of detection) and LOQ (limit of quantification) values of the limonene were determined to be 0.07 and 0.24 ppm, respectively. In the repeatability study, the %RSD (relative standard deviation) value was determined to be 3.30%. An overview of the calibration chart is shown in Figure 1.

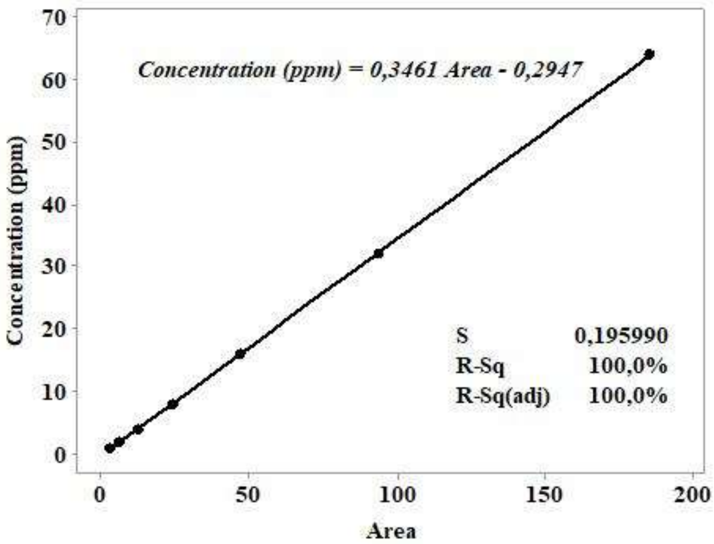

Fig 1 Calibration chart

Fractions of the citrus

The percent mass distributions of citrus fractions as juice, pulp (after squeezing) and peel were presented in Figure 2. According to the findings, the highest amount of juice fraction was determined in grapefruits (88%) where the highest portion of peel and pulp fractions in the lemon was found to be 31% and 21%, respectively.

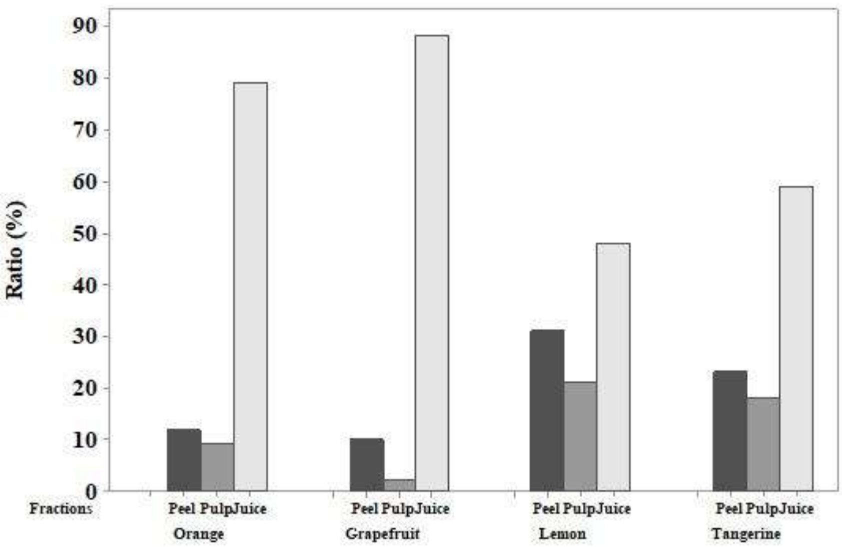

Fig 2 The percent mass distributions of citrus fractions

Obtained peel and pulp fractions of the citrus were dried prior to the extraction process and percentage reduction of weight was calculated. According to the results presented in Figure 3, the most weight reduction after drying process was found in grapefruit pulp (88%), and the least reduction was determined in tangerine peels (25%).

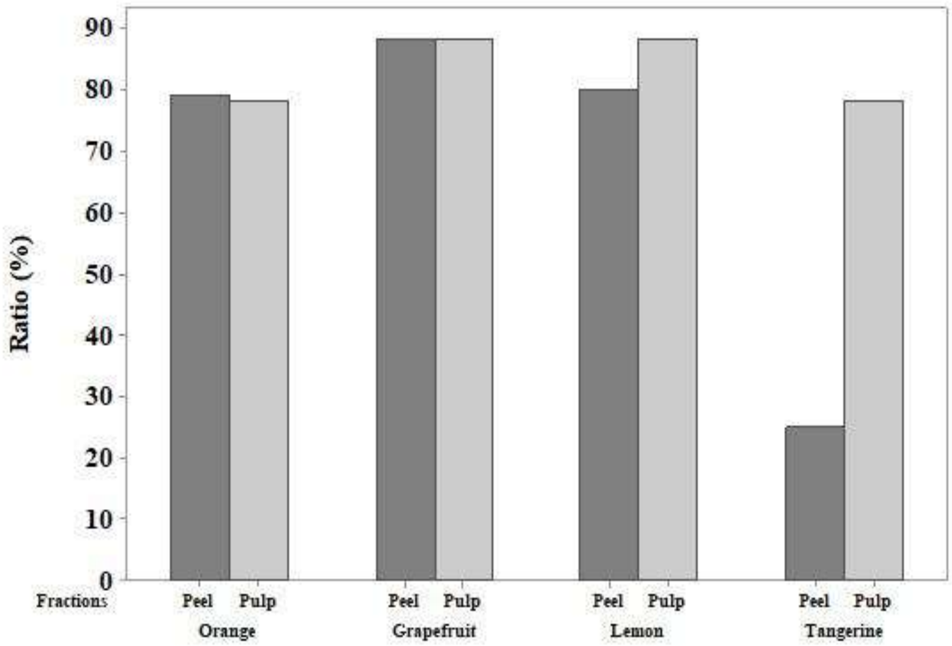

Fig 3 Weight loss of peel and pulp fractions of the citrus fruits after drying

Limonene contents of the fractions
Dried fractions of the citrus were analyzed for limonene contents by HPLC, and the results were presented in Figure 4. According to the findings the highest limonene was found in the peel fraction of lemon (58390.53 ppm), secondly in the orange peel (54768.17 ppm). Orange contains a significant amount of limonene in the pulp fraction (981.60 ppm), whereas limonene is <LOQ in fruit juices. Since fruit juices were stored at -18°C, this is thought to be due to the loss of limonene activity when citrus fruits are stored at low temperatures.

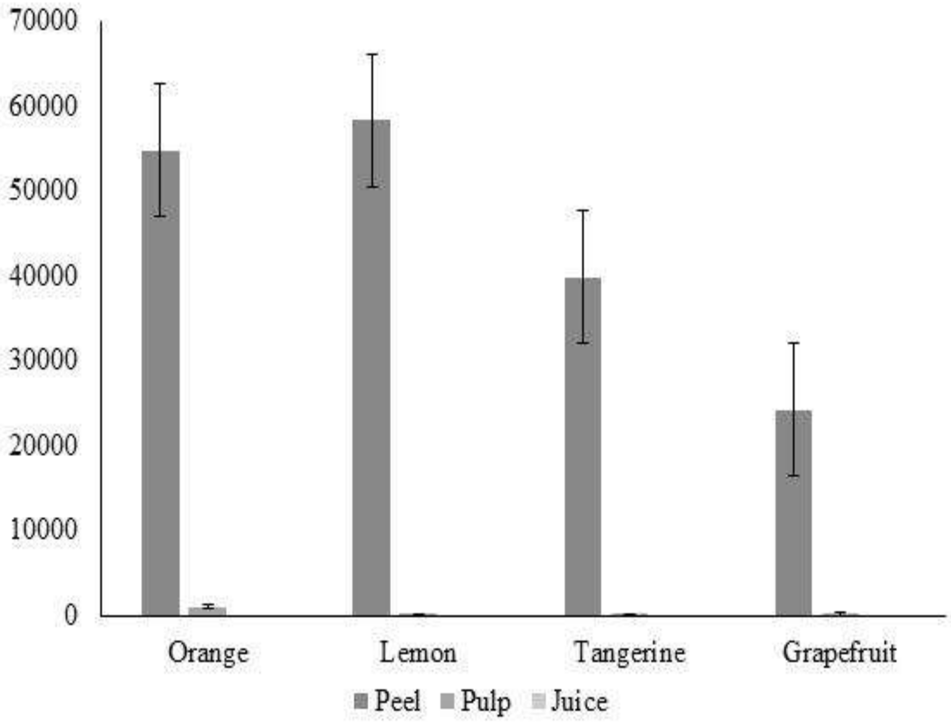

Fig 4 The fraction of limonene content of different dried citrus fruits

The study investigated the effect of a single drying method and focusing exclusively on limonene as the oil component. An overview of Limonene Content in *Citrus* spp. is illustrated in Figure 4.

Extraction of limonene from lemon peel by SFC

As shown in Figure 4. limonene was found in lemon peels at the highest level; thus, extraction was carried out with lemon peels. Lemon peels were treated like the beginning of the current study, such as dried in an oven at 40°C for 72 hours and sieved. After the experiment parameters were set up, the optimal limonene yield under different temperature and pressure conditions SFC extraction system was estimated by RSM. Obtained graph was presented in Figure 5. Optimum temperature and pressure values SFC extraction for the extraction of limonene was demonstrated in Table 2.

Table 2. Optimum conditions of SFC extraction

Limonene (ppm)	Temperature (°C)	Pressure (MPa)
450000	57	147

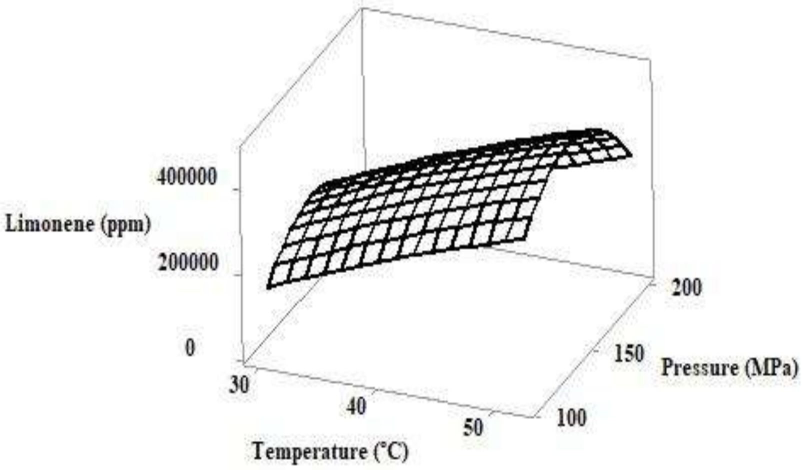

Fig 5 Optimal limonene yield in SFC

Discussion

According to a previously presented study, that worked on low density-dispersive liquid–liquid microextraction (LD-DLLME) of limonene the method demonstrated optimum value linearity with r ≥ 0.9950 with low LOD also LOQ levels between 0.00081 and 0.00269 ppm, respectively [17]. A study revealed that limonene is the most abundant compound in essential oils extracted from lemon peels, accounting for 44.74% of the oil [18]. Also, earlier studies identified limonene as one of the major components in citrus peels [19,20]. The results showed that D-limonene was the primary component in all samples analyzed. The oven-dried peel powder samples at 40°C produced a large proportion of the primary components of limonene [21]. Earlier research showed that the chemical makeup of volatile oils in Chinese citrus peels was highly influenced by drying conditions, leading to significant variability in their composition. These studies also highlighted that the primary component in orange peel oil was limonene [22,23]. According to a study, D-limonene was detected in the MeOH extract obtained from orange peels at a concentration of 4.642 mg/mL, and the yield of D-limonene was 0.4% after the extraction process. The quality of the extract and yield can be affected by various factors, including the type of solvent, production process, and equipment utilized [24]. A research study extracted limonene from orange peel waste using various solvents including cyclopentyl methyl ether, methyl-tetrahydrofuran, and hexane. The study found that the extraction yields of limonene from orange peel waste were 0.81%, 0.64%, and 0.53%, respectively [25].

In a previous study reveals that the highest amounts of limonene were measured as 1027 ppm in fresh lemonade in lemons and 402 ppm in limeade in limes [26]. Our results are not in agreement with the findings of this mentioned study, since lemon juices was not including limonene according to our results [27] revealed that the highest amount of limonene was detected in fresh lemon peels with 65000 ppm approximately. Dried and grinded lemon peels have the second highest limonene amount, while no grinded lemon peels have the least limonene 25000 ppm. It is suggested from mentioned study that grinding is essential for extraction of limonene. Another study indicates that limonene contents in oils of grapefruit, orange, mandarin and limonene were 96.2%, 94.9%, 74.7% and 69.9%, respectively, also in each of the four citrus oils analyzed, limonene was the most abundant constituent [28]. The chemical composition of four citrus oils, namely mandarin (*C. reticulata* L.), lemon (*C. limon* L.), orange (*C. sinensis* L.), and grapefruit (*C. paradisi* L.), was studied, the percentage of limonene in these essential oils was 74.7, 69.9, 94.9, and 96.2%, respectively [28]. However, the difference in the reported value could be due to several factors such as the chemical and biotype of the plant, climatic conditions, and the extraction process [29]. Additionally, the chemical composition of the starting material may vary depending on various factors such as the plant's health, growth stage, habitat including climatic factors, morphological factors, and harvest time [30].

Limonene extraction by using high pressure-temperature extraction (HPTE), an earlier study revealed that the least yield was found in 105 minutes at 100 ºC using an HPTE approach, whereas the best limonene conditions were attained in 30 minutes at 0.6 Mpa pressure and 150 ºC [27]. Telling the difference, the optimal limonene extraction demands higher pressure and lower temperature in the SFC system, as demonstrated by our studies. The research was centered on utilizing dehydrated and ground waste lemon peels to extract lemon essential oil through SFE. Various experiments were conducted at different temperatures and pressures ranging from 35-50°C and 12.5-20 MPa to determine the impact of operating conditions on total extract and D-limonene yield. The ideal conditions for extracting limonene were found to be 15 MPa and 40°C [31]. Hydro-distillation gave a higher yield in D-limonene from fresh peels than SFE from dehydrated peels, but this difference is not only due to the extraction method. Both drying and grinding pre-treatments of matrix to be extracted can significantly influence the yield on D-limonene [31], as also Asekun et al. (2007) suggested.

According to a study, limonene (61.8 %) was discovered to be the primary component of lemon peel essential oils [32]. Similarly, it was determined that the main component of the chemical composition of the essential oils of *C. limon* peel was limonene (61.68%.) [33].

So far, [34] dealt with the limonene extraction from SFC method and reported that 15 MPa is the optimum pressure, also, by the pressure increases, yield decreases. According to our current study, low pressure levels were not chosen for the experiment. At a pressure of 10 MPa, 30 °C offered the highest extract recovery (95% w/w), while the highest DL concentrations could be achieved at 60 °C (99% w/w). This was attributed to increased density at higher pressures or lower temperatures [35].

In a study, SFC extraction at 60 °C at two different pressures (30 and 20 MPa) and with two different proportions (10% and 20%) of ethanol used as the extraction solvent was used to identify the compounds in lemons and their limonene. The study showed that the use of 20 MPa and 20% ethanol gives the highest percentage of limonene, which was 30.70%. In the same study, liquid CO_2 extraction was used on limonene and showed that the use of 20% ethanol with liquid CO_2 extraction increased the percentage of limonene up to 43.84% [36]. From the current study, we conclude that the use of some solvents leads to an increase in the limonene ratio, and that the high pressure may lead to a decrease in the limonene ratio extracted from lemon peels. Raising the temperature and pressure to attain CO_2's supercritical state may cause a deterioration in the quality of limonene [19].

Conclusion

The first step in the current study was carried out with HPLC system to analyze the most abundant limonene in the fractions of some citrus fruits like orange (*Citrus cinensis* L.), lemon [*C. limon* (L.) Osbeck], grapefruit [*C. paradisi* (L.) Macfad.] and tangerine (*C. tangerina* Tanaka). The HPLC results revealed that, with a concentration of 58390.53 ppm, the lemon peel had the highest level of limonene. The following batch of orange peels contained 54768.16 ppm limonene. Fruit juices, however, did not contain any limonene that could be found; in another terms, <LOQ in fruit juices. Hence lemon peels have the highest concentration of limonene, it was extracted from lemon peels using the SFC method in the following step with an optimization chart was set up, and the most appropriate parameter was found in the SFC technique at a temperature of 52 °C and a pressure of 147 Mpa.

Limonene is a by-product of the fruit juice industry that is useful for commercial use. Moreover, citrus waste can be recovered for limonene compound. If it is handled properly, it can be utilized in numerous applications. Since our resources in the world is limited, it is predicted that the findings of the current study can be used for recovering of in citrus wastes.

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Data Availability Statement

The author confirms that the data supporting this study are cited in the article.

Compliance with ethical standards

Conflict of interest

The author declare no conflict of interest.

Ethical standards

The study is proper with ethical standards.

Authors' contributions

Tuğba Pelin TOKER: Formal analysis, Investigation, Writing – original draft, Mustafa Hamza Mawlood Al Bayati: Formal analysis, Methodology, Writing – original draft, Mariem Bouali: Formal analysis, Validation, Ümit Babacan: Formal analysis, Validation, Mehmet Fatih Cengiz: Writing – review & editing, Supervision, Investigation.

References

1. García-Salas, P., et al., Influence of technological processes on phenolic compounds, organic acids, furanic derivatives, and antioxidant activity of whole-lemon powder. Food Chemistry, 2013. *141*(2): p. 869–878. https://doi.org/10.1016/j.foodchem.2013.02.124 .
2. FAOSTAT, FAOSTAT Database. *Food and Agriculture Organization of the United Nations*, 2024. Available at: https://www.fao.org/faostat/en/#data/QCL. (Accessed: 15 Nov. 2024).
3. Wilkins, M. R., et al., Ethanol production by Saccharomyces cerevisiae and Kluyveromyces marxianus in the presence of orange-peel oil. World Journal of Microbiology and Biotechnology, 2007b. *23*(8): p. 1161–1168. https://doi.org/10.1007/s11274-007-9346-2
4. Siddiqui, S. A., et al., Extraction and purification of d-limonene from orange peel wastes: Recent advances, Industrial Crops and Products, 2022. 177, 114484. https://doi.org/10.1016/j.indcrop.2021.114484
5. Zhang, E., et al., Bio-inspired design of hierarchical PDMS microstructures with tunable adhesive superhydrophobicity, Nanoscale, 2015. *7*(14): p. 6151–6158. https://doi.org/10.1039/c5nr00356c.
6. Lane, A. G, Removal of peel oil from citrus peel press liquors before anaerobic digestion. Environmental Technology Letters, 1983. 4(2): p. 65–72. https://doi.org/10.1080/09593338309384174.
7. Ruiz, B., and X. Flotats, Citrus essential oils and their influence on the anaerobic digestion process: An overview, Waste Management, 2014. 34(11): p. 2063–2079. https://doi.org/10.1016/j.wasman.2014.06.026.
8. Pourbafrani, M., et al., Production of biofuels, limonene and pectin from citrus wastes, Bioresource Technology, 2010. 101(11): p. 4246–4250. https://doi.org/10.1016/j.biortech.2010.01.077
9. Morehouse, B. R., et al., Functional and Structural Characterization of a (+)-Limonene Synthase from Citrus sinensis. Biochemistry, 2017. 56(12): p. 1706–1715. https://doi.org/10.1021/acs.biochem.7b00143.
10. PubChem, PubChem Compound Summary for CID 22311, Limonene. National Library of Medicine (US), National Center for Biotechnology Information, 2024. (Accessed: 13 Nov. 2024).
11. Kim, Y.W., et al., Safety evaluation and risk assessment of d-Limonene, Journal of Toxicology and Environmental Health, Part B: Critical Reviews, 2013. 16(1): p. 17–38. https://doi.org/10.1080/10937404.2013.769418.
12. Bevilacqua, A., Corbo, M.R., and M. Sinigaglia, *In vitro* evaluation of the antimicrobial activity of eugenol, limonene, and citrus extract against bacteria and yeasts, representative of the spoiling microflora of fruit juices, *Journal of Food Protection,* 2010. 73(5): p. 888–894. https://doi.org/10.4315/0362-028x-73.5.888.
13. Cai, R., et al., Antifungal activity and mechanism of citral, limonene, and eugenol against *Zygosaccharomyces rouxii*, *LWT - Food Science and Technology,* 2019. 106: p. 50–56. https://doi.org/10.1016/j.lwt.2019.02.059.
14. Hollingsworth, R.G., and J.W. Armstrong, Potential of temperature-controlled atmospheres, and ozone fumigation to control thrips and mealybugs on ornamental plants for export, *Journal of Economic Entomology,* 2005. 98(2): p. 289–298. https://doi.org/10.1603/0022-0493-98.2.289.
15. Miller, J., et al., D-Limonene: A bioactive food component from citrus and evidence for a potential role in breast cancer prevention and treatment, *Oncology Reviews,* 2011. 5: p. 31–42. https://doi.org/10.1007/s12156-010-0066-8.
16. Kamyab Moghadas, B., et al., Experimental study of Dorema aucheri extraction with supercritical carbon dioxide. *Asian Journal of Chemistry*, 2012. 24: p. 3691–3694. DOI: Not available.
17. El-Deen, A.K., and Shimizu, K., Application of D-Limonene as a bio-based solvent in low-density dispersive liquid–liquid microextraction of acidic drugs from aqueous samples. *Analytical Sciences,* 2019. 35(12): p. 1385–1391. https://doi.org/10.2116/analsci.19P360.

18. Auta, M., et al., Optimization of citrus peel D-limonene extraction using solvent-free microwave green technology. *Chemical Engineering Communications,* 2018. 205(6): p. 789–796. https://doi.org/10.1080/00986445.2017.1419206.
19. Lopresto, C.G., et al., A non-conventional method to extract D-limonene from waste lemon peels and comparison with traditional Soxhlet extraction. *Separation and Purification Technology,* 2014. 137: p. 13–20. https://doi.org/10.1016/j.seppur.2014.09.015.
20. Bourgou, S., et al., Changes of peel essential oil composition of four Tunisian citrus during fruit maturation. *The Scientific World Journal,* 2012. Article ID 528593. https://doi.org/10.1100/2012/528593.
21. Ibrahim, M., and S. El-Sawi, Quality and quantity of volatile oil resulting from the recycling of different forms of orange peel using drying methods. *Journal of Materials and Environmental Science,* 2019. 10(7): p. 598–603. DOI: Not available.
22. Feger, W., Brandauer, H., and H. Ziegler, Analytical investigation of Murcott (honey) tangerine peel oil. *Journal of Essential Oil Research,* 2003. 15(3): p. 143–147. https://doi.org/10.1080/10412905.2003.9712097.
23. Kamal, G., et al., Yield and chemical composition of Citrus essential oils as affected by drying pretreatment of peels. *International Food Research Journal,* 2011. 18(4): p. 1275–1282. DOI: Not available.
24. Jha, P., et al., Valorisation of orange peel: supplement in fermentation media for ethanol production and source of limonene. *Environmental Sustainability,* 2019. 2: p. 33–41. https://doi.org/10.1007/s42398-019-00048-2.
25. Owolabi, M.S., et al., Chemical composition of Citrus limon (L.) Osbeck growing in Southwestern Nigeria: Essential oil chemotypes of both peel and leaf of lemon. *American Journal of Essential Oils and Natural Products, 2018*. 6: p. 36–40. DOI: Not available.
26. Hakim, I.A., McClure, T., and D. Liebler, Assessing dietary D-Limonene intake for epidemiological studies. *Journal of Food Composition and Analysis,* 2000. 13(4): p. 329–336. https://doi.org/10.1006/jfca.1999.0862.
27. Lopresto, C., et al., non-conventional method to extract D-limonene from waste lemon peels and comparison with traditional Soxhlet extraction. *Separation and Purification Technology,* 2014. 137: p. 13–20. https://doi.org/10.1016/j.seppur.2014.09.015.
28. Viuda-Martos, M., et al., Chemical composition of mandarin (C. reticulata L.), grapefruit (C. paradisi L.), lemon (C. limon L.), and orange (C. sinensis L.) essential oils. *Journal of Essential Oil Bearing Plants,* 2009. 12(2): p. 236–243. https://doi.org/10.1080/0972060X.2009.10643716.
29. Costa, S.S., et al., Microwave extraction of mint essential oil – Temperature calibration for the oven. *Journal of Food Engineering,* 2014. 126: p. 1–6. https://doi.org/10.1016/j.jfoodeng.2013.10.002.
30. Azmir, J., et al., Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering,* 2013. 117(4): p. 426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014.
31. Lopresto, C.G., et al., Process-intensified waste valorization and environmentally friendly d-limonene extraction. *Euro-Mediterranean Journal for Environmental Integration,* 2019. 4: p. 31. https://doi.org/10.1007/s41207-019-0115-1.
32. Kirbaşlar, Ş.I., Boz, I., and F.G. Kirbaşlar, Composition of Turkish lemon and grapefruit peel oils. *Journal of Essential Oil Research,* 2006. 18(5): p. 525–543. https://doi.org/10.1080/10412905.2006.9699161.
33. Ammad, F., et al., The potency of lemon (Citrus limon L.) essential oil to control some fungal diseases of grapevine wood. *Comptes Rendus Biologies,* 2018. 341(2): p. 97–101. https://doi.org/10.1016/j.crvi.2018.01.002.
34. Mikšovsky, P., and E.N. Horn, Continuous formation of limonene carbonates in supercritical carbon dioxide. *Organic Process Research & Development,* 2022. 26(10): p. 2799–2810. https://doi.org/10.1021/acs.oprd.2c00143.
35. Yasumoto, S., et al., Supercritical CO_2-mediated countercurrent separation of essential oil and seed oil. *The Journal of Supercritical Fluids,* 2015. 104: p. 104–111. https://doi.org/10.1016/j.supflu.2015.06.003.
36. Romano, R., et al., Pizzolongo, F., and P. Masi, Bioactive compounds extracted by liquid and supercritical carbon dioxide from citrus peels. *International Journal of Food Science & Technology, 2022*. 57(6): p. 3826–3837. https://doi.org/10.1111/ijfs.15591.

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Planting time and spacing effects on marigold (*Tagetes erecta* L.) NK1 orange: a comprehensive study in Nangarhar, Afghanistan

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ABSTRACT
Marigolds are widely valued for their ornamental beauty and versatility in landscaping, yet optimal horticultural practices for their cultivation under specific local agro-climatic conditions still need to be determined. This study aimed to elucidate the effects of cultivation time and spacing on growth, flower yield, and flowering duration of Marigolds. A field experiment was conducted on the marigold NK1 orange variety based on a factorial randomized block design. The planting times were February 4th (P1), February 21st (P2), and March 8th (P3), while the spacings were 30 cm × 30 cm (S1), 30 cm × 45 cm (S2), 45 cm × 45 cm (S3), and 45 cm × 60 cm (S4). The P2 treatment significantly ($p<0.05$) increased plant height, with no differences among the spacing treatments. The number of primary branches per plant and stem diameter varied significantly with planting time and spacing. Stem diameter positively correlated ($r = 0.41$) with plant spread under different timings. The P2-S2 treatment influenced the most significant flower size, and the maximum length of the ray floret was also observed in the P2 treatment. Flower size had a positive correlation ($r = 0.48$) with the length of the ray floret. Additionally, the highest significant ($p<0.05$) flower fresh weight and flower yield per plant were observed in the P2 treatment. Furthermore, the P2 and P3 treatments had a longer flowering duration than the P1 treatment, with the highest duration of 76 days observed in the P2 treatment. The planting time influences the vegetative growth and flower attributes which is the best time to recommend cultivation for marigold producers in the context of Afghanistan.

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Introduction

Ornamental plants are cultivated for their decorative purposes in gardens and landscape designs, as houseplants, cut flowers, and specimen displays. With the recent focus on agricultural diversification, floriculture has been considered an enterprise that can give farmers higher income per unit area [1]. Among the many ornamental plants grown worldwide, marigold (*Tagetes* spp L.) stands out as a significant commercial flower belonging to the Compositae/Asteraceae family [2]. Native to South and Central America, especially Mexico [3]. It is highly popular in tropical and subtropical regions as a garden plant, potted plant, and for creating herbaceous borders to enhance the beauty of gardens and landscapes [4]. The plants of *Tagetes erecta* are tall, but their height varies depending on the species. The plant canopy is mostly erect and features numerous branches, large flowers, and a variety of colors [5], [6]. The most common colors of marigolds are yellow and orange, with multiple shades such as bright yellow, light yellow, golden yellow, bright orange, and dark orange [3]. Moreover, the Tagetes varieties are adaptable to diverse environmental conditions and can be grown in any soil with good drainage. Growers prefer marigolds due to their accessible cultural practices, short juvenility, profuse flowering, considerable blooming period, attractive color, shape, size, and good quality [7]. Marigold is also a potential source of pharmaceutical components for natural color preparation, oil extraction, etc. [8].

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In marigold plants, the planting times and plant spacing are considered as important factors that affect plant growth, yield, flowering duration, and oil production [9]. The cultivation of marigold in May caused a substantial increment of plant height and plant spread [10]. According to [11], planting time, spacing, and pinching at 60 DAT showed significant effect on the number of days to first flowering and the number of flowers per plant.

The successful commercial production of marigolds depends upon many factors, such as variety, planting time, fertilizer, spacing, irrigation, etc. Planting time and spacing are important factors that affect yield and flower-related parameters. In the context of Afghanistan, no information is available on the time of planting, i.e., seed sowing/transplanting of seedlings and spacing for transplanting to determine optimum plant density in marigolds to raise a better crop. Also, the agroclimatic conditions are favorable, offering significant potential for the commercial cultivation of marigolds for flowers, landscaping, and other ornamental uses. However, horticultural practices under local conditions have yet to be standardized to ensure high-quality flower production. Therefore, this study was conducted in 2021 at the Experimental Farm of the Faculty of Agriculture, Nangarhar University, Nangarhar, Afghanistan, to determine this region's optimal cultivation time and spacing.

Material and Methods

Cultivation condition

This experiment was conducted in 2021 at the Experimental Farm of Agriculture Faculty, Nangarhar University (Nangarhar, Afghanistan). Geographically, Nangarhar province is located in the eastern region of Afghanistan. Mainly, Nangarhar University is located at latitude 34° 28' 20" N and longitude 70° 22' 9" E, with a 599 m elevation above sea level. Its climate is semi-arid with hot summers, and the hottest months are June, July, and August, as shown in Fig 1. This experiment was laid out in a factorial randomized block design (FRBD) with three replications. Seeds of marigold NK1 Orange variety were sown in the nursery at three planting times and subsequently cultivated in a four-planting space. The planting times were February 4th (P1), February 21st (P2), and March 8th (P3), while the spacings were 30 cm × 30 cm (S1), 30 cm × 45 cm (S2), 45 cm × 45 cm (S3), and 45 cm × 60 cm (S4). One seedling per hill was transplanted in all plots. The field was thoroughly ploughed and properly leveled, and all grasses, stubbles, and crop residues were removed. During bed preparation, chemical fertilizers were applied at 50 kg per hectare of Urea (containing 46% N) and 100 kg per hectare of DAP (Diammonium phosphate). Additionally, 150 kg per hectare of Urea was applied one week before the flowering stage. The first irrigation was provided immediately after transplanting, followed by regular watering when the soil became sufficiently dry.

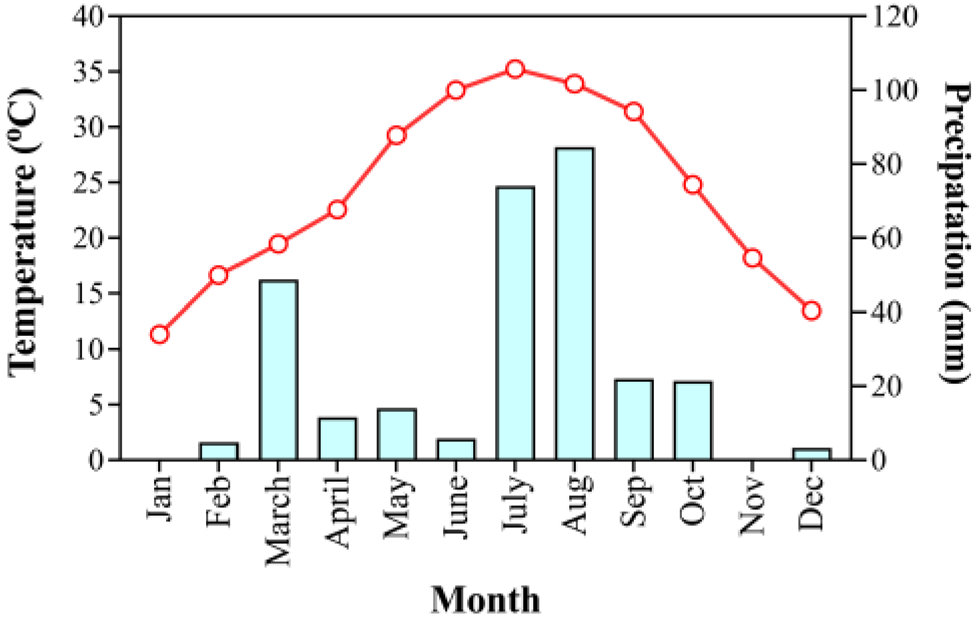

Fig 1. The experimental location's temperature and precipitation (mm) during the marigold growing season (Meteoblue, 2023).

Pinching and weeding

Pinching was performed one month after transplanting in the P1 and P2 treatments, while it was done 40 days after transplanting in the P3 treatment. Weeding was carried out as needed across all treatments.

Growth and flowering parameters

Growth parameters; plant height (cm), plant spread (cm), number of primary branches per plant, stem diameter (mm), and length and width of leaf (cm). Parameters related to the flowers included flower size (cm) (measured using Digital Vernier Calliper), length and width of the ray floret (cm), number of flowers per plant, fresh weight of the single flower (g), flower yield per plant (g), flower yield per plot (kg), dry weight of the single flower (g), and days to bud initiation and first flowering. The flower yield per plot was measured using the following formula.

Flower yield per plot = flower yield per plant × total number of plants per plot

Statistical analysis

The data were analyzed using the two-way analysis of variance (ANOVA) and Pearson's correlation analysis with language R 3.6.2 statistical software. The means were considered using Tukey's test at the 0.05 level.

Results

Growth attributes

Planting time significantly ($p<0.01$) affected plant height, whereas no significant differences were observed among the spacings. The P2 treatment resulted in the tallest plants compared to the other treatments. In the P2 treatment, the highest plant height was 48.72 cm in S1, followed by 47.13 cm in S2 and 46.52 cm in S4 (Fig 2). No significant differences ($p>0.05$) in plant height were found among the planting spacings in the P1 and P3 treatments. The planting time significantly affected the plant spread ($p<0.01$). P2 had a higher plant spread than P1 and P3 treatments, as shown in Table 1. In P2 treatment, S4 had a higher plant spread (42.94 cm) compared to other treatments, followed by S1, S2, and S3, sequentially. The highest value of plant spread was 42.38 cm in S2 of P3 treatment. Furthermore, the results presented in Table 1. revealed that spacing had no significant influence ($p>0.05$) on plant spread. However, the plant spread was comparatively affected by higher spacing.

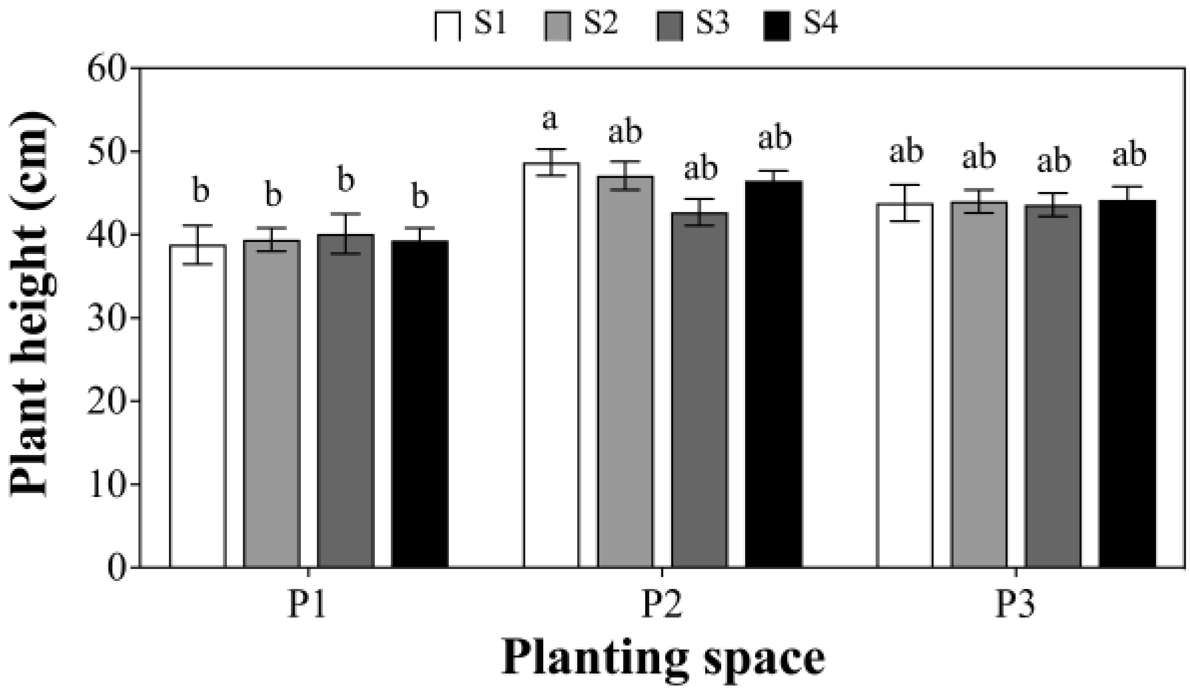

Fig 2. Effect of planting time and spacing on plant height of marigold (n = 10). According to Tukey's test, different letters are shown on bars at 0.05 level.

The number of primary branches per plant was significantly influenced ($p<0.01$) by planting time and planting space. Among the different planting times, the highest value of the number of primary branches per plant (7.66) was observed in the P1-S2 treatment. Also, the production of primary branches was suppressed in P3 compared to P1 and P2. After S2 (7.66) and S1 (6.88) in the P1 treatment, S2 (6.77) and S1 (6.66) had a higher value in the P2 treatment (Table 1). The results revealed that stem diameter was significantly different ($p<0.01$) in planting time and planting space ($p<0.05$) treatments. The data for stem diameter is in accordance with plant spread between planting time treatments and had a positive correlation ($r = 0.41$) between plant spread and stem diameter. The highest values of stem diameter were observed in P2 as compared to P1 and P3 treatments. In the P2 treatment, stem diameter's highest value (18.17 mm) was in S4

and followed by S3 (16.88 mm), S1 (15.23 mm), and S2 (14.12 mm), Sequentially (Table 1). The effect of planting time revealed that during plants' growth in P2 treatment, due to appropriate climatic conditions, the growth of plant spread and stem diameter was enhanced, as shown in Table 1.
Marigold's transplanting at the third week of P2 treatment is the proper time for transplanting for the better physical appearance of the plant's canopy. Statistically, there was no significant difference ($p>0.05$) in the leaf length under different planting times and spacing. At the same time, there was a difference among planting treatments, as shown in Table 2. The results revealed that the width of leaves showed a significant difference ($p<0.01$) between planting times, while there was no significant difference between spacing. The highest values were observed sequentially in the P1 treatment, followed by P3 and P2. In the P2 treatment, planting space S3 (6.77 cm) showed the highest value, followed by S4 (6.50 cm), S1 (6.44 cm), and S4 (6.44 cm), as shown in Table 2.

Flower characteristics

The results of this experiment revealed that different planting times had a significant ($p<0.05$) effect on the flower size of the marigold plant (Fig 3A). Comparing the mean values between treatments, P2-S2 (9.01 cm) had the highest flower size values, while the flower size was lower (6.21 cm) in the P3-S3 treatment. The higher plant spread and stem girth observed in the P2 treatment resulted in larger flower size, as shown in Fig 3A.
The length of florets showed a significant difference ($p<0.05$) between planting times, while there was no significant difference ($p>0.05$) between spacings and their interaction with planting time. The maximum length of the ray floret (4.8 cm) was found in the P2 treatment, and the minimum (3.73 cm) was recorded in the P3 treatment. As shown in Fig 3B, the longest floret length was observed in the P2 treatment at the S2 spacing, followed by the S3 and S1 spacings. The flower size was directly influenced by the length of the floret, with a positive correlation ($r = 0.48$) between the length of the ray floret and flower size. Similarly, there was a significant difference ($p<0.05$) in the width of the ray floret among planting times but no significant difference between spacings. However, spacing had a comparatively minor effect on the ray floret width in marigold plants. There was a positive correlation between the width of the ray floret and flower size ($r = 0.62$) and between the width and length of the ray floret ($r = 0.65$). The highest width of the ray floret was observed in the P2-S2 treatment (1.70 cm), followed by S3 (1.61 cm) and S1 (1.52 cm), as shown in Fig 3C.
The statistical analysis revealed that different planting times and spacings had a significant ($p<0.05$) effect on the number of flowers per marigold plant. The highest number of flowers per plant was observed in P3, followed by P2 and P1, respectively (Fig 3D). In the P3 treatment, S2 spacing resulted in the highest number of flowers (53.00) compared to other treatments. In contrast, the second-highest number of flowers per plant was recorded in S4 spacing (49.82) of the P2 treatment (Fig 3D). This study also demonstrated a positive correlation between plant spread and the number of flowers (Fig 4). According to Fig 4D, the highest plant spread was observed in the S2 and S4 spacings of the P3 and P2 treatments. Therefore, in marigold plants, a wider spread leads to more flowers per plant. Compared to planting time, the influence of planting time on flower production was greater. Additionally, the wider plant spread observed in the P2 treatment contributed to a higher number of flowers per plant, aligning with the primary objective of the experiment.
The highest fresh weight of a single flower was recorded in the P2 treatment compared to other treatments. Within the P2 treatment, the highest fresh weight of a single flower was observed in S2 (13.41 g), while the lowest was in S4 (12.82 g). Additionally, even the lowest fresh weight of a single flower in the P2 treatment's subgroup (planting space) was higher compared to the other spacings in the P1 and P3 treatments. Sowing and transplanting times directly affect plant growth and flower parameters in marigold plants. The results of this experiment indicate that early (P1) and late (P3) planting of marigold plants can decrease the fresh weight of flowers (Table 3).
Flower yield per plant increased significantly ($p<0.01$) with the P2 treatment. The flower yield per plant showed a positive relationship with flower size ($r = 0.62$), length of the ray floret ($r = 0.44$), width of the ray floret ($r = 0.47$), number of flowers per plant ($r = 0.35$), and fresh weight of a single flower ($r = 0.35$). The highest flower yield per plant was observed in the P2 treatment (639.91 g), while the lowest values were recorded in the P3 treatment (328.21 g). Within the P2 treatment, the maximum flower yield per plant was observed in S4 (639.91 g), followed by S2 (598.31 g), S3 (566.83 g), and S1 (566.61 g), respectively (Table 3). Planting time and planting space had a significant effect on flower yield per plot ($p < 0.01$). The number of plants per plot is the most important factor for total flower yield in a specific area.

Table 1. Effect of planting of planting time and spacing on plant spread, number of primary branches, and stem diameter.

Treatments	Plant spread (cm)					Number of primary branches per plant					Stem diameter (mm)				
	S1	S2	S3	S4	Spacing Mean	S1	S2	S3	S4	Spacing Mean	S1	S2	S3	S4	Spacing Mean
P1	36.88	38.72	38.44	35.44	37.37	6.88	7.66	6.44	6.44	6.86	12.28	13.10	13.92	13.52	13.20
P2	41.50	41.33	40.55	42.94	41.58	6.66	6.77	6.22	6.00	6.41	15.23	14.12	16.88	18.17	16.10
P3	37.50	42.38	36.72	39.83	39.11	5.11	5.44	4.55	4.88	5.00	12.66	16.14	14.57	15.43	14.70
Planting time mean	38.62	40.81	38.57	39.40		6.22	6.62	5.74	5.77		13.39	14.45	15.12	15.71	
SD	2.50	1.88	1.91	3.76		0.96	1.11	1.03	0.80		1.60	1.54	1.55	2.34	
P	**					***					***				
S	ns					***					*				
P × S	ns					ns					ns				

The data is represented as mean (n = 9). SD means standard deviation. Significant differences at the 0.05% level are shown above in the table. *** $p<0.001$, ** $p<0.01$, *$p<0.05$, and ns: not significant. The P and S stand for planting time and spacing, respectively.

Table 2. Effect of planting time and spacing on the length and width of the leaf.

Treatments	Length of leaf (cm)					Width of leaf (cm)				
	S1	S2	S3	S4	Spacing Mean	S1	S2	S3	S4	Spacing Mean
P1	11.00	12.27	12.32	10.94	11.63	7.22	8.22	7.55	7.05	7.51
P2	11.22	11.88	11.00	12.11	11.55	6.44	6.44	6.77	6.50	6.54
P3	11.61	12.44	11.57	12.38	12.00	6.72	7.11	6.50	7.44	6.94
Planting time mean	11.27	12.20	11.63	11.81		6.79	7.25	6.94	7.00	
SD	0.30	0.28	0.66	0.76		0.39	0.89	0.54	0.47	
P	ns					**				
S	ns					ns				
P × S	ns					ns				

The data is represented as mean (n = 9). SD means standard deviation. Significant differences at the 0.05% level are shown above in the table. ** $p<0.01$, and ns: not significant. The P and S stand for planting time and spacing, respectively.

Among the planting time treatments, the highest yield was observed in P2, followed by P1 and P3. The number of plants per plot is determined by spacing, with S1 (36 plants per plot) having the highest value compared to S2 (24 plants per plot), S3 (16 plants per plot), and S4 (12 plants per plot) (Table 3). Flower yield per plot significantly increased with decreasing planting space between plants. Different spacings S1 (30 cm × 30 cm), S2 (30 cm × 45 cm), S3 (45 cm × 45 cm), and S4 (45 cm × 60 cm)-resulted in decreasing flower yields per plot as spacing increased. However, decreasing plant spaces negatively affected the number of flowers per plant, flower size, length of the ray floret, and width of the ray floret (Table 3).

Table 3. Effect of planting time and spacing on fresh weight of the single flower, flower yield per plant, and flower yield per marigold plot.

Treatments	**Fresh weight of the single flower (g)**					**Flower yield per plant (g)**					**Flower yield per plot (kg)**				
	S1	**S2**	**S3**	**S4**	**Spacing Mean**	**S1**	**S2**	**S3**	**S4**	**Spacing Mean**	**S1**	**S2**	**S3**	**S4**	**Spacing Mean**
P1	11.95	12.22	12.54	12.45	12.29	433.41	496.11	459.82	484.72	468.53	15.66	11.90	7.35	5.81	10.17
P2	12.90	13.41	13.16	12.82	13.07	566.61	598.31	566.83	639.91	592.92	20.39	14.36	9.07	7.67	12.87
P3	8.55	7.60	7.811	7.78	7.93	359.01	380.62	328.21	353.10	355.21	12.92	9.13	5.25	4.23	7.88
Mean Planting	11.1	11.0	11.1	11.0		453.0	491.7	451.6	492.6		16.30	11.80	7.22	5.91	
SD	2.28	3.06	2.92	2.80		105.1	108.9	119.5	143.5		3.78	2.61	1.91	1.72	
P	***					***					***				
S	ns					ns					***				
P × S	ns					ns					ns				

The data is represented as mean (n = 9). SD means standard deviation. Significant differences at 0.05% level are shown above in the table. *** p<0.001 and ns: not significant.

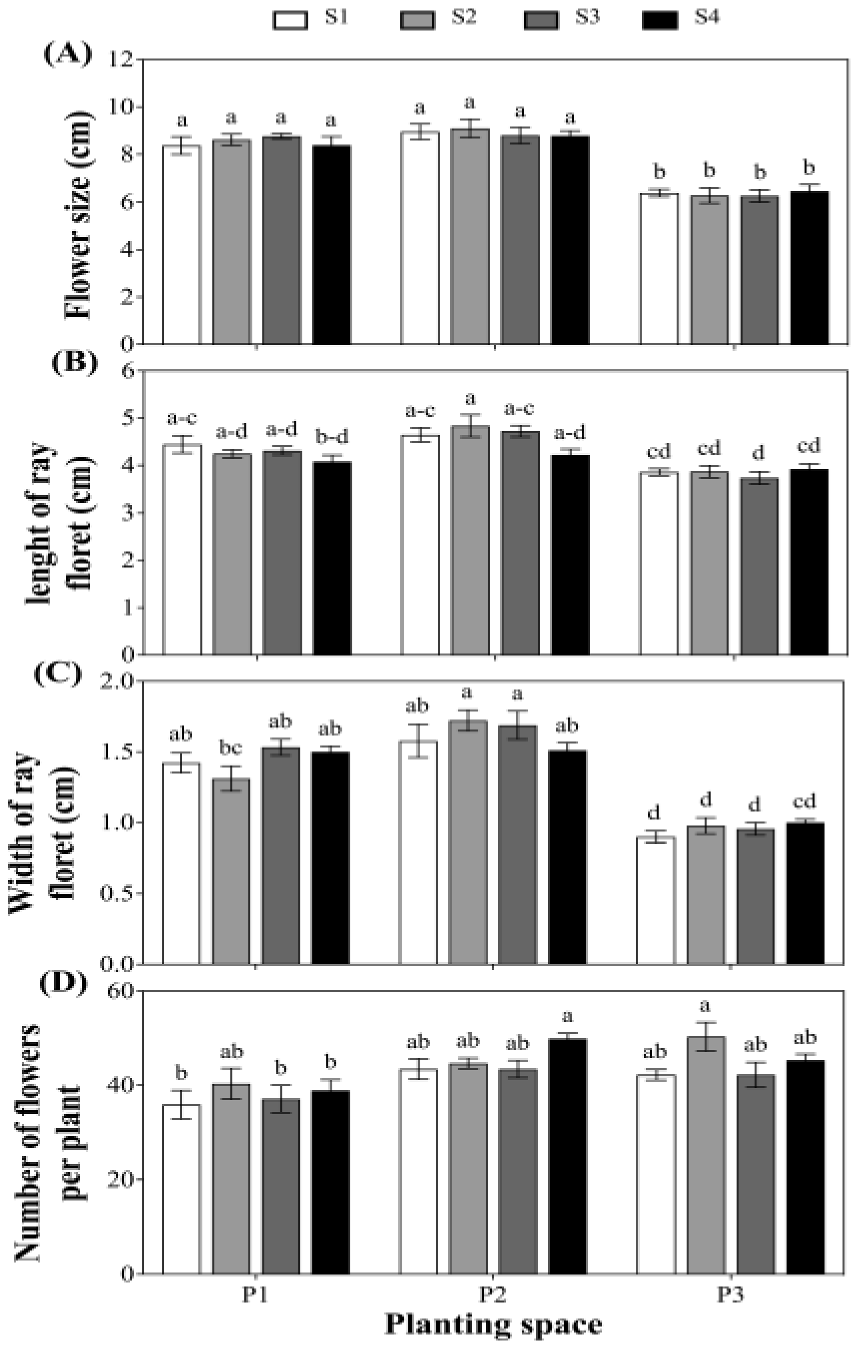

Fig 3. Effects of planting space and sowing time on flower size, length of ray floret, width of ray floret, and number of flowers per plant of marigold shown in (A), (B), (C), and (D), respectively. The letters on the bars are shown differently among treatments based on Tukey's test at 0.05% level. S indicates sowing and P planting time.

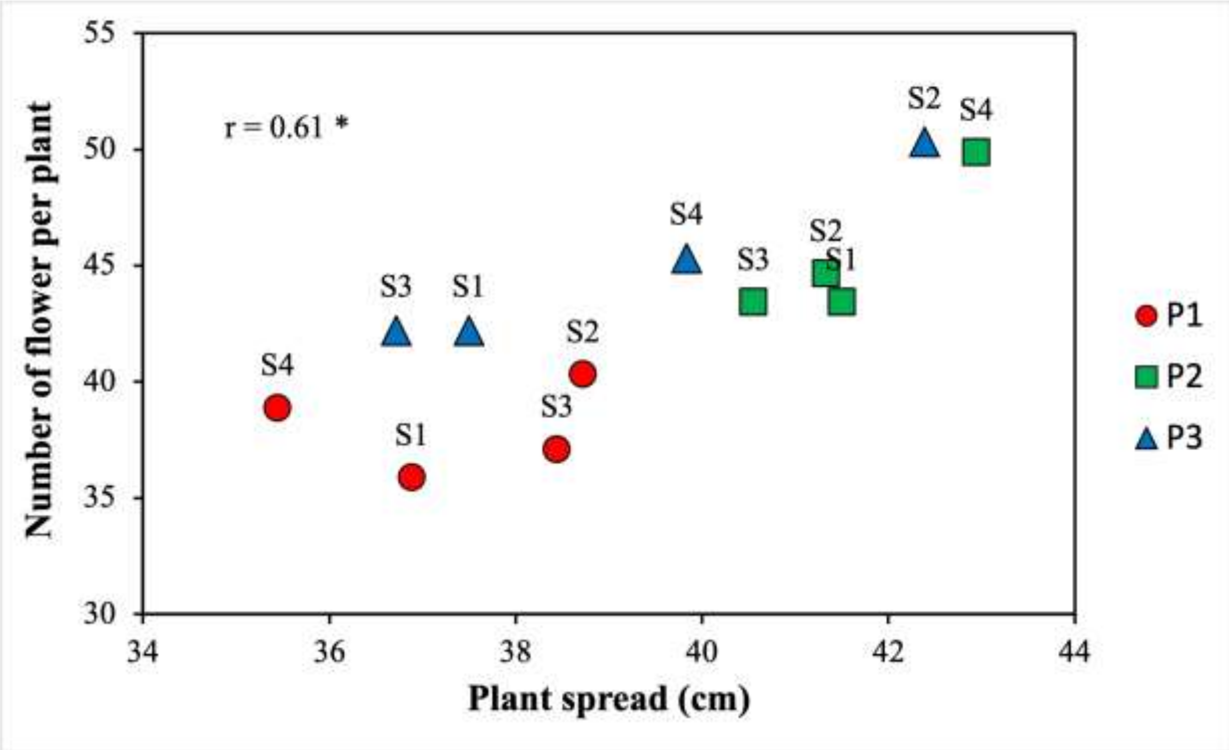

Fig 4. The relationship of plant spread with the number of flowers per plant under different planting times and spacing of marigolds.

The maximum dry weight of marigold flowers was observed in the P2 treatment compared to other treatments. Within the P2 treatment, the highest value was recorded in S2 (2.37 g), followed by S1 (2.33 g), S3 (2.16 g), and S4 (2.14 g). The increase in the dry weight of a single flower varied significantly ($p<0.001$) between planting times. However, there was no significant difference ($p>0.05$) due to planting spacing or the interaction between planting time and spacing, as shown in Table 4.

Table 4. Effect of planting time and spacing on the dry weight of marigold flowers.

Treatments	Flower dry weight (g)				
	S1	**S2**	**S3**	**S4**	**Spacing Mean**
P1	2.06	2.07	2.13	2.25	2.13
P2	2.33	2.37	2.16	2.14	2.25
P3	1.72	1.53	1.44	1.58	1.57
Planting time mean	2.03	1.99	1.91	1.99	
SD	0.30	0.42	0.40	0.35	
P	***				
S	ns				
P × S	ns				

Effect of planting time and planting on flower attributes

The effect of different planting times on the days taken to reach various phenological stages of marigold is presented in Table 5. The results revealed that marigolds differed in the days taken to bud initiation, flowering, and the duration of the flowering period. P1 cultivation had the maximum days to bud initiation (56 days), significantly higher than P2 and P3 treatments. The days taken to flowering corresponded with the days taken to bud initiation.

The most important parameter of this experiment was the duration of flowering. The results showed that the P2 and P3 treatments had longer flowering durations than the P1 treatment. Among all treatments, P2, with 76 days of flowering duration, was the most favorable for cultivating marigolds in the Nangarhar province of Afghanistan (Table 5).

Table 5. Effect of planting time on days to bud initiation, days taken to flowering, and duration of flowering.

Treatments	Days to bud initiation	Days taken to flowering	Duration of flowering days
P1	56.2	63.4	69.2
P2	48.3	54.4	76.7
P3	40.1	47.2	70.1

Discussion

This study investigates the impact of different cultivation times and plant spacing on the growth, floral yield, and phenology of marigold plants under the climatic conditions of Nangarhar province, Afghanistan. Plant height is a crucial growth component representing a crop's growth behavior and influencing the marigold plant's physical appearance. It results from genetic makeup and environmental conditions, particularly temperature and plant-to-plant competition during growth. The results indicated significant variation among treatments under different cultivation dates and plant spacing, as shown in Fig 2. Plant height was noticeably affected by plant spacing across all three cultivation dates, with the highest plant height observed in P2 treatments. In the P2 treatment, plant heights increased sequentially with spacings of 30 × 30 cm, 30 × 45 cm, and 45 × 60 cm. In contrast, P1 and P3 treatments showed no substantial difference between spacing treatments, but different spacing relatively affected plant height due to adjacent crop competition. These findings align closely with those of [13] who reported that closer spacing (30 × 30 cm) resulted in greater plant height compared to other spacing treatments (30 × 45 cm, 45 × 45 cm, and 45 × 60 cm) in marigolds. Similar results were also observed in marigold plants by [14]. Various spacing treatments showed a significant difference in plant spread, while planting dates did not significantly affect plant spread. However, there is still a substantial difference between planting space treatments. The maximum plant spread (42.9 cm) was recorded in the P2 treatment of the cultivation date and S4 (45 × 60 cm) of planting space, followed by S2 (30 × 45 cm) of P3 and P2, as shown in Table 1. The increased plant spread can be attributed to a greater number of flowers per plant and a higher number of branches per plant, which results in a wider plant canopy. These findings are supported by [9], who reported that marigold cultivation after February decreased plant spread. Conversely, wider spacing increases plant spread due to reduced competition for water, light, and available nutrients in the growing area. Similar results have been reported by [10], [15], [16], [17] in marigold plants. The results of this experiment revealed that the effect of planting date and plant spacing on the number of branches per plant of *Tagetes erecta* was significantly different. Still, the interaction of the aforementioned treatment effect was not significant. The number of branches per plant varied based on the planting dates. There were more branches in the February cultivation than in the March cultivation, but it was relatively close to the late February cultivation. The branching process of marigolds tended to be faster in P1, P2, and P3 sequentially (Table 1). Increasing spacing did not affect the number of branches per plant, as there was more planting space. The plant spread showed good results in providing a wider canopy and utilizing the nutrients to proliferate produced branches. The maximum number of branches in Tagetes erecta was produced in 30 × 45 cm spacing in all cultivation dates. These results are in accordance with the [10], [15], [18], [19], [20] in marigold plants. The stem diameter differed significantly in all cultivation dates and spacing. The stem diameter positively correlated with the plant spread in all cultivation dates. The highest value of stem diameter (18.1 mm) was in the P1 cultivation date with 45 × 60 cm, while the closer spaces (30 × 30 cm) showed less stem diameter in all three cultivation dates. The variation of stem diameter of different genotypes was also revealed by [21]. The results of this experiment for stem diameter of marigold plants under different spacing are supported by the same result of [22].

The size of flowers was significantly different between cultivation dates, while not significant in between spacing. Among cultivation dates, the P2 treatment showed a wider flower size diameter than the P1 and P3 treatments. The maximum flower size (9.0 cm) was recorded in P2 treatment, while a minimum (6.2 cm) was observed in P3. This decrease is due to late cultivation in P3, as the days from cultivation to harvest were shorter than other treatments. Moreover, in the interaction of cultivation dates and spacing, P2 showed the maximum flower size (30 × 45 cm) in the S2 planting space and followed by S3 (45 × 45 cm), S1 (30 × 30 cm), and S1 (45 × 60 cm). The diameter of flower size can always be affected by the length of the floret ray; herein, the length of the floret ray showed a positive correlation with the flower size. Similar results were reported by

[1], [11], [23] in African marigolds. The various cultivation dates and spacing significantly influenced the floral parameters, particularly the length and width of the ray floret. The length of the ray floret was in line with the flower size; this means if the size of the ray floret is affected by the cultivation date and spacing, many flowers can be affected. The maximum length of ray floret was found in P2 and followed by P1 and P3 treatments (Fig 4.4). The planting space of S2 (30 × 45 cm) showed the longest floret length compared to other spacing treatments. Reduced spacing might increase competition during vegetative growth, resulting in shorter floret length and smaller flower size. Herein, the balanced planting space and best cultivation time for the maximum length of the floret and flower size are found in the P2 cultivation date and S2 planting space. Similar results were found by [24] in marigold and [25] in gladiolus. Likewise, regarding flower size and the length of the ray floret, the cultivation date and spacing significantly affect the width of the ray floret. The maximum width of the ray florets (1.7 cm) was observed in the P2 treatment, followed by P1 and P3 sequentially. The width of the ray floret was significantly affected by the cultivation date in P3 treatment. At the same time, this is a good confirmation of the late cultivation (after February) catastrophic effect on marigold plants. Based on the spacing, this experiment revealed that the S2 treatment showed more width of the ray florets in the P2 and P3 treatments, while in the P1, S3 (45 × 45 cm) showed wider florets in the flowers. The P1 cultivation date showed a wider ray of the florets due to the long plant growth period and relatively good weather compared to the P3 treatment. These results conform with the findings which are described by [7], [14], [26], [27], [28], [29] in marigold plant.

Different cultivation dates and planting significantly affected the number of flowers per plant. More flowers per plant were sequentially observed in P3, P2, and P1. The maximum number of flowers in the S2 of P3 was recorded and followed by S4 of P2 treatment. The wider planting space of 45 × 60 cm had a higher number of flowers as compared to other treatments in P2; this seems to be mainly due to a greater number of branches per plant and also less competition among the plants. The fewer flower plants were in P1 cultivation treatment in S1 (30 × 30 cm) planting space treatment. This is proof of the effect of high competition between plants in a closely-spacing environment in marigold plants. The plants in different cultivation dates varied between all treatments, but the effect of the environmental conditions, such as temperature, was stable on all planting space treatments. There was a strong correlation ($r = 0.61^*$) between plant spread and the number of flowers per plant as shown in Fig 4. These results were similar to the findings of [3], [10], [30], [31], [32], [33], [34], [35], [36] in marigold plants. The flower yield per plant was greatly affected by flower size, length and width of the ray floret, number of flowers per plant, and fresh weight. There was a significant difference between cultivation dates, but no significant difference between spacing or the interaction of cultivation dates and spacing. The maximum flower yield per plant was recorded in P2 and the minimum in P3 treatment. In P2 treatment, the highest values of flower yield per plant (639.9 g) were observed in S4 (45 x 60 cm), followed by S2 (598.3 g), S3 (566.8 g), and S1 (566.6 g) sequentially (Table 3). The S4 planting space of the P2 treatment showed higher flower yield per plant due to the greater number of flowers per plant, while S2 (30 × 45 cm) showed higher fresh flower weight and better performance in other floral parameters.. The number of flowers per plot differed significantly between cultivation dates and spacing, but not in their interaction. The maximum value of flower fresh weight per plot (20.4 kg) was recorded in P1 with (30 × 30 cm), and the minimum (4.2 kg) was in P3 with (45 × 60 cm) (Table 3). In all P treatments, the treatment with the least spacing (30 × 30 cm) showed the highest values of fresh flowers per plot, which was due to the high number of plants per area.

Data recorded on flowering attributes (Table 5) showed that the earliest bud initiation occurred in P3 (40.1 days), P2 (48.3 days), and P1 (56.2 days) sequentially. The early cultivation revealed that the temperature was low and the days taken to initiate flowering were longer compared to other treatments. In contrast, later (after February, as in the P3 treatment), the temperature was higher, which caused early bud initiation in marigold plants. In this experiment, data presented in Table 5 revealed that the days taken to flowering were in line with the days taken to bud initiation in different cultivation times. In contrast, the duration of the flowering was varied among cultivation dates. The maximum number of days to keep flowers fresh in plants was recognized as P2 cultivation, while the P1 treatment with the earlier cultivation had shorter flowering periods than other treatments. From these results, we conclude that optimal timing for bud initiation and flowering can lead to a longer flowering duration in marigold plants under different cultivation dates. These results are similar to the results of [37], [38] in marigold plants. The planting time and spacing had a substantial impact on the vegetative growth and flowering attributes of marigolds in this study. However, further research is needed to examine additional marigold genotypes and gain a deeper understanding of this process and its sustainability.

Conclusion

In this experiment, we found that planting time and spacing have a significant impact on the growth and flowering attributes of marigold plants under the climatic conditions of Nangarhar, Afghanistan. The data from the P2 cultivation and S2 (30 × 45 cm) spacing showed notable effects on plant spread and the number of primary branches per plant. The largest flower size was observed in the P2-S2 treatment. Additionally, this treatment resulted in the highest fresh flower weight and flower yield per plot. The findings revealed that cultivation time had a strong influence on the timing of bud initiation, the number of days to flowering, and, most importantly, the duration of the flowering period. For ornamental plants, the duration of the flowering period is a priority for growers compared to other parameters. The most extended flowering duration (76.7 days) was recorded in the P2 (late February) treatment, indicating that this planting time is most favorable for extending the flowering period in marigolds under the study conditions. Thus, it is advised that marigold farming in Nangarhar, Afghanistan, implement a planting schedule of late February (P2) and a spacing of 30 × 45 cm (S2) to enhance growth, flower size, yield, and particularly the duration of flowering.

Compliance with ethical standards
Conflict of interest / Çıkar çatışması
The author declare no conflict of interest.
Ethical standards
The study is proper with ethical standards.

References

1. Nain, S., et al., Effect of pinching and spacing on growth, flowering and yield of African marigold (Tagetes erecta L.) under semi-arid conditions of Haryana. Journal of Applied and Natural Science, 2017. 9(4), p. 2073–2078. doi: 10.31018/jans.v9i4.1491.
2. Lalit, B. C., et al., Effect of pinching on yield and yield attributing characteristics of marigold (Tagetes erecta L.): A review. Tropical Agrobiodiversity, 2020. 1(2), p. 57–60. doi: 10.26480/trab.02.2.
3. Singh, R., et al., A Review on Effect of Pinching on Growth, Flowering and Flower Yield of Marigold. International Journal of Pure Applied Bioscience, 2019. 7(4), p. 493–501. doi: 10.18782/2320-7051.7760.
4. Singh, V., A. K. Singh, and A. Sisodia, Growth and Flowering of Marigold as Influenced by Pinching and Spraying of Nitrogen. International Journal of Current Microbiology and Applied Sciences, 2017. 6(7), p. 2283–2287. doi: 10.20546/ijcmas.2017.607.268.
5. Ganai, S.A., et al., Diversity of arthropod fauna associated with marigold (Tagetes erecta L.) in Jammu. Joural of Entomology and Zoology Studies, 2017. 5(5), p. 1940–1943.
6. Bosma, T. L., et al., Sowing Dates and Priming Influence African Marigold Field Emergence. Horticulture Technology, 2003. 13(3), p. 487–493. [Online]. Available: http://horttech.ashspublications.org/content/13/3/487.abstract
7. Jyothi, K., et al., Studies on the Effect of Planting Dates and Levels of Pinching on Growth, Flowering and Yield in Marigold (Tagetes erecta) cv. Arka Agni. International Journal of Current Microbiology and Applied Sciences, 2018. 7(11), p. 2705–2713. doi: 10.20546/ijcmas.2018.711.309.
8. Biswal, B., et al., Evaluation of phytoremediation capability of French marigold (Tagetes patula) and African marigold (Tagetes erecta) under heavy metals contaminated soils. International Journal of Phytoremediation, 2021. doi: 10.1080/15226514.2021.1985960.
9. Ismail, R. F., et al., Effect of planting date and plant spacing on growth, yield and essential oil of Mexican marigold. The Journal of Applied Sciences Research, 2013. 9(1), p. 330–340.
10. Mohanty, C. R., Influence of various planting distances on growth and flowering of Annual Chrysanthemum (Chrysanthemum coronarium L .). International Journal of Creative Research Thoughts, 2021. 9(2), p. 5081–5086.
11. Meena, Y., et al., Effect of planting time, spacing and pinching on growth and seed yield traits Effect of planting time, spacing and pinching on growth and seed yield traits in African marigold (Tagetes erecta) cv . Pusa Narangi Gainda. Indian Journal of Agricultural Sciences, 2015. 85, p. 797–801.
12. Weather Meta - meteoblue. Accessed: Apr. 03, 2023. [Online]. Available: https://www.meteoblue.com/en/weather/week/meta_italy_3173524
13. Pratibha, C., et al., Effect of planting dates and spacing on growth and flowering of French marigold Sel. FM 786. African Journal of Agricultural Research, 2018. 13(37), p. 1938–1941. doi: 10.5897/ajar2015.10227.
14. Raju, D. V. S., K. P. Singh, and K. Swaroop, Performance of French marigold (Tagetes patula Linn.) in different planting months under Delhi conditions. Journal of Ornamental Horticulture, 2006. 9(3), p. 215–217.
15. Chanda, S. and N. Roychoudhury, The effect of time of planting and spacing on growth, flowering and yield of African marigold (Tagetes erecta L.) cv. Siracole. Horticultural Journal, 1991. 4(2), p. 53–56.
16. Mishra, H. P., Performance of french marigold as influenced by time of planting under North Bihar situation. Indian Journal of Horticulture, 1997. 54(3), p. 265–269.
17. Chaturvedi, A. K., et al., High temperature stress during flowering and grain filling offsets beneficial impact of elevated CO_2 on assimilate partitioning and sink-strength in rice. Scientific Reports, 2017. pp. 1–13. doi: 10.1038/s41598-017-07464-6.
18. Anju P. and A. K. Pandey, Effect of plant spacing on growth and flowering in African marigold (*Tagetes erecta* L.) under Budnelkhand Region. Progressive Research, 2007. 2(2) p. 70–72,

19. Pandey, R., et al., Studies on effects of planting dates and spacing in African marigold (*Tagetes erecta* L.)," Progressive Horticulture, 2014. 46(1), p. 149–152.
20. SINGH, A. K., U. KUMAR, and A. KUMAR, Effect of planting date and spacing on performance of marigold (*Tagetes erecta* Linn) cv. PUSA NARANGI under North Bihar agro-ecological conditions," International Journal of Forestry and Crop Improvement, 2015. 6(1), p. 16–20. doi: 10.15740/has/ijfci/6.1/16-20.
21. Prashanth, M. KK, P., et al., Evaluation of different African marigold (*Tagetes species* Linn.) genotypes for vegetative, floral and yield attributes under Southern Telangana condition. International Journal of Chemical Studies, 2018. 6(5), p. 3311–3315.
22. Halagi, M.S., et al., Effect of plant spacing and pinching interval on growth, yield and flower quality of African marigold (*Tagetes erecta* L.) under semi-arid conditions, 2023. The Pharma Innovation Journal, 12(4), 2246-2254.
23. Ahirwar, M. K., K. Ahirwar, and M. Shukla, Effect of plant densıtıes, nitrogen and phosphorus levels on growth, yıeld and qualıty of afrıcan marıgold, 2012. 14(2), p. 153–155.
24. Singh, V. N., et al., Effect of Gamma Irradiation on African Marigold (*Tagetes erecta* L.) Cv. Pusa Narangi Gainda. Journal of Horticultural Sciences, 2016. 4(1), p. 36–40.
25. Kumar, K., et al., Effect of spacing on growth, flowering and corm production of gladiolus (*Gladiolus* sp.) cv. American Beauty. International Journal of Environment, Agriculture and Biotechnology, 2016. 1(3), p. 550–554. doi: 10.22161/IJEAB/1.3.36.
26. Sreekanth, P., et al., Effect of planting time, spacing and nitrogen levels on yield and quality of African Marigold (*Tagetes erecta* Linn.)," Journal of Ornamental Horticulture, 2006. 9(2), p. 97–101.
27. Mohanty, C. R., A. Mohanty, and R. Parhi, Effect of planting dates and pinching on seed traits in African Marigold cv. Sirakole, Agricultural Science Digest - A Research Journal, 2015. 35(4), p. 95–99. doi: 10.18805/asd.v35i4.6860.
28. Sharma, P., et al., Evaluation of genotypes of French marigold (*Tagetes patula* L) under Nauni, Solan, Himachal Pradesh conditions. International Journal of Farm Sciences, 2019. 9(4), p. 94. doi: 10.5958/2250-0499.2019.00102.2.
29. Deepa, V. P., et al., Study on the growth and yield attributes of marigold (*Tagetes* spp.) hybrids under Dharwad condition. HortFlora Research Spectrum, 2016. 5(1), p. 43–47,
30. Srivastava, S. K., H. K. Singh, and A. K. Srivastava, Effect of spacing and pinching on growth and flowering of 'Pusa Narangi Gainda' marigold (*Tagetes erecta*). Indian Journal of Agriculture, 2021. 12(7), p. 1–8.
31. Pandey, R. K., et al., Studies on effects of planting dates and spacing in African marigold (*Tagetes erecta* L .). Progressive Horticulture, 2014. 46(1), 149-152.
32. Gaidhani, A., S. Dalal, and P. Nagre, Effect of different planting dates and pinching on growth and flowering of China aster. International Journal of Chemical Studies, 2020. 8(2), p. 1120–1124. doi: 10.22271/chemi.2020.v8.i2q.8918.
33. Dilta, B. S., Y. C. Gupta, and S. J. A. J. H. Puja, Effect of different planting dates on performance of China Aster (*Callistephus chinensis* Nees.) varieties, 2007. 2(2), p. 245–248.
34. Joshna K. and P. Pal, Effect of planting date on growth, development, aerial biomass partitioning and flower productivity of marigold (*Tagetes erecta* L.) cv. Siracole in Indo-gangetic plains of West Bengal. Journal of Applied Horticulture, 2015. 17(2), p. 151–154. doi: 10.37855/jah.2015.v17i02.28.
35. Jumar. K., R. Poonam and R. K. Dubey, Effect of planting time and spacing on zinnia. Journal of Ornamental Horticulture, 2021. Accessed: Dec. 09, 2021. [Online]. Available: https://agris.fao.org/agris-search/search.do?recordID=IN2005001208
36. Dubey, R. K., R. Kumar, and Poonam, Effect of planting time and spacing on cosmos. Journal of Ornamental Horticulture, 2021. Accessed: Dec. 09. [Online]. Available: https://agris.fao.org/agris-search/search.do?recordID=IN2005001206
37. Pratibha, C., Y.C. Gupta, S.R. Dhiman, and R.K. Gupta, Effect of planting dates and spacing on growth and flowering of French marigold Sel. FM 786. African Journal of Agricultural Research, 2018. 13(37), 1938-1941.
38. Parhi, R., A. Mohanty, and S. Harichandan, Performance of various characters in African marigold due to different pinching levels and planting dates. Biology, 2021. Accessed: Dec. 15, 2021. [Online]. Available: https://www.researchgate.net/publication/305554928_Performance_of_various_characters_in_African_marigold_due_to_different_pinching_levels_and_planting_dates

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Morphological Characterization and Diversity Analysis of Coffea Arabica Germplasm from Yirgacheffe, Ethiopia

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ABSTRACT

This study aimed to assess the genetic diversity of twenty-six Coffea Arabica landrace germplasms collected from the Yirgacheffe district of the Gedeo zone depending on morphological characteristics at the seedling stage. Collected Coffea Arabica landrace germplasms seeds and 2 standard checks were planted and raised at Wanago Tumata Chiracha nursery site in plastic bags arranged with randomized complete design in the years 2022 to 2023. Seedling characteristics variables such as seedling height, number of paired leaves, leaf length, leaf width, leaf area, petiole length, node number, inter-node length, and stem diameter were collected from one-year-old randomly selected three seedlings from each accession and each plot. The results revealed that there were significant variations ($p<0.05$) between and within collected Coffea Arabica landrace germplasms and standard checks for the above quantitative parameters. Data of five qualitative traits (young leaf color, leaf shape, leaf apex shape, leaf petiole color, and young shoot color) were recorded from three representative seedlings for each Coffea Arabica landrace germplasm accession. Frequency distribution estimation using Shannon and Weaver's diversity index results for qualitative parameters revealed the presence of genetic variability between the collected coffee germplasm accessions and standard checks. Accordingly, the highest diversity index (H) was recorded for young leaf color (1.414), followed by leaf shape (1.067) and leaf apex shape (0.908). This might be due to the oligogenic nature of gene action and slight environmental interaction. Relatively low diversity was observed in young shoot color (0.582) and leaf petiole color (0.429). Additionally, cluster analysis of qualitative parameters grouped 26 coffee landrace germplasm accessions and 2 standard checks into three clusters. Maximum numbers of coffee germplasm accessions were grouped in cluster-II (15) followed by cluster-I (9) and cluster-III (4). Consequently, Coffea Arabica landraces germplasm having high seedling height, leaf length, number of paired leaves and leaf area should get emphasis during selection for plantation. Moreover, every concerned body, such as breeders, farmers, and genetic conservationists should take action to conserve and keep the gene pool of these coffees since it paved the way for biotechnologists to characterize coffee at the molecular level and breeders consider it to release superior new coffee varieties.

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Introduction

The gene pool or natural genetic diversity of coffee plants has three well-defined categories, specifically: i) the primitive cultivars or landraces of traditional agriculture, ii) the improved cultivars released by plant breeders in the past 100 years, and iii) the wild species related to domesticated cultivars. All of the three categories of Coffea Arabica gene pool listed above were found in Ethiopia [1]. In the garden cultivation system, coffee is combined with other crops under a few shade trees or in open sunlight and accounts for 40 to 50% of national production. Usually, in the production system, coffee planting material can be referred to as coffee landraces but most of the time comprises a limited number of 'improved coffee cultivars' selected by the Jimma Agricultural Research Center (JARC) for their productivity and resistance to diseases [2]. According to Labouisse and Kotecha, (2008), constituents are contributing to the genetic erosion of Ethiopian coffee landrace genetic diversity [2]. These constituents are partial reduction of production in

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some areas for agronomic, climatic change, or economic reasons, replacement of local landraces by few improved varieties with a narrow genetic base, and deforestation due to population growth pressure in the major coffee-producing areas.

So far, the efforts that have been made to collect, conserve, and utilize Ethiopian coffee germplasm have been carried out by the national coffee collection program. During this collection program around 12,448 Arabica coffee germplasm accessions have been collected from different areas, from these collections 6,717 accessions were ex-situ conserved and maintained on research plots of the Jimma Research Center and its sub-centers. On the other hand, the Institute of Biodiversity Conservation has conserved 5,731accessions in field gene banks in Ethiopia [3, 4]. Yet, its productivity is limited due to the lack of improved varieties for biotic and abiotic stresses. To utilize the available coffee genetic resources effectively and efficiently, collection, characterization, and evaluation of germplasm is the crucial step in the improvement process succeeding different methods. Those methods that are used to characterize and evaluate rich coffee germplasm include morphological, biochemical characteristics, and molecular markers. Kebede and Bellachew, (2008) and Abdi, (2009) used morphological traits to characterize Arabica coffee collections from Hararge Ethiopia [5, 6]. Ermias, (2005) and Olika et al., (2011) also used morphological traits to characterize Arabica coffee collections from Wellega and Limmu respectively [7, 8]. Additionally, Alemu and Boke, (2017) morphologically characterized seven Coffea arabica landraces collected from seven districts of the West Guji zone of the Oromia region, and the result revealed the presence of genetic diversity among the collected coffee landraces [9]. Yirga, (2021) characterized sixty-two Coffea Arabica L. germplasms collected from the Yayu district of Illubabor zone and two commercially grown standard check varieties using phenotypic/morphological variations and revealed that the presence of enormous genetic diversity among collected germplasms and standard checks [10]. All these researchers have been showing us the presence of high genetic diversity in these coffee germplasm collections. Furthermore, Bekele, (2005), Abeyot et al., (2011), Olika et al., (2011) conducted genetic diversity analyses of cup quality and biochemical characteristics of Arabica coffee in Ethiopia and Kathurima et al., (2009) in Kenya have proved the existence of rich genetic diversity in cup quality and biochemical characteristics [8, 11, 12, 13].

Enough coffee landrace characterization for agronomic and morphological traits is necessary to facilitate the utilization of germplasm by breeders. But, except for very few observations not enough data has been recorded to enable the systematic characterization of coffee germplasm collected from Yirgacheffe of Gedeo zone [14, 15]. Though nowadays lots of coffee genetic resources are in decline due to erratic climatic and edaphic factors, little attention has been given to the collection, characterization, and sustainable utilization as our country in general and in the Gedeo zone and Yirgachaffe districts in particular [16,17]. Intensive data collection on agro-morphological traits could give sufficient information for morphological characterization, evaluation, and conservation of these coffee accessions. Such an extensive and deep study can also ensure the economic benefits of the high cost of ex-situ conservation of large coffee accessions. Therefore; the objectives of this study are to collect, morphologically characterize, evaluate, and conserve Coffea Arabica landrace germplasm accessions from the Yirgacheffe district of Gedeo zone, Southern Ethiopia Regional State.

Materials and Methods

Experimental site

A field experiment was conducted at Wanago Dilla University coffee research center nursery site. Wanago is one of the districts in the Southern Region of Ethiopia. It is part of the Gedeo Zone and bordered on the southwest by Yirgacheffe, on the northwest by the Oromia Region, on the northeast by Dila Zuria, and the southeast by Bule.

Experimental design and planting of coffee seeds

Planting materials (coffee seeds) were taken from the coffee germplasm accessions collected from the Yirgacheffe district of the Gedeo zone. Twenty-six collected Coffea Arabica germplasm accessions and two improved variety seedlings were raised in polyethylene bags at Dilla University Coffee Research Center Wanago Tumata Chiracha nursery site in April 2022- April 2023 G.C using a completely randomized design. The experiment has twenty-eight treatments (26 collected accessions + 2 standard check variety) with three replications and eighty-four total number of observations (n=28 x 3= 84). Uniform cultural practices were applied to all accessions.

Parameters for morphological characterization

Coffee seedling's quantitative and qualitative parameters were collected randomly from three selected coffee seedlings of the same accession/variety in the plot. The quantitative parameters used to determine the morphological variation of these coffees at the seedling stage were seedling height, number of paired leaves, leaf height, leaf width, leaf area, petiole length, stem diameter, number of nodes, and internode length. The qualitative parameters used to show the morphological variation of these coffee seedlings were leaf color, leaf shape, leaf apex shape, leaf petiole color, and shoot color. The data for those mentioned above parameters were collected in April 2023 before transplanting to the conservation site. After data collection, those seedlings were transplanted to the Dilla University Botanic Garden and eco-tourism center site forming a line of collected coffee germplasm accessions (one line comprises eight seedlings of single accessions) for conservation and further evaluation purposes.

Data analysis

Quantitative parameters collected were subjected to analysis of variance (ANOVA) using the GLM procedure within SAS version 9.0. A test of mean separation was done using the least significant difference at a 5 % probability level using SAS.

Shannon Index (H') was used to analyze the morphological diversity of coffee seedlings depending on the 5 qualitative traits (leaf color, leaf shape, leaf apex shape, leaf petiole color, and young shoot color) of coffee germplasm accessions. The type of diversity used here is α diversity which is the diversity of species within a community or habitat. The diversity index was calculated by using the Shannon and Weaver formula [18].

$H=-\Sigma [(pi) \times \ln (pi)]$

Where: Pi is the relative proportion of the total number of entries (N) in the ith class, which means Pi = S / N, Where S= number of individuals of one species, N = total number of all individuals in the sample, ln = logarithm to base e. In biological communities, the Shannon-Weaver diversity index varies from 0 to 5. According to this index, values less than 1 characterize low diversity; values in the range of 1 to 3 are characteristics of moderate diversity, while above 3 indicates the highest diversity [10, 19].

Cluster analysis was used to group the accessions into homogeneous forms based on qualitative traits. Hierarchical clustering was employed using the similarity coefficients among the collected coffee germplasm accessions and standard checks. The analysis was performed using SAS software by employing the method of the Ward clustering strategy of the observation [20].

Results

Quantitative parameters

The results of nine quantitative parameters (seedling height, number of paired leaves, leaf height, leaf width, leaf area, petiole length, stem diameter, number of nodes, and internode length) are as follows. The ANOVA results revealed that there were significant variations (P<0.05) in seedlings' quantitative parameters between and within 26 Coffea Arabica landraces accessions collected from the Yirgacheffe district of Gedeo zone and 2 standard check varieties as shown in Table 1. Descriptive analysis of each seedling quantitative parameters has been also assessed and the minimum, maximum, and mean values were presented as shown in Table 2.

Table1. Analysis of variance between and within Coffea Arabica germplasm collected from the Yirgacheffe district of Gedeo zone and standard checks

Parameters	Variation	DF	Sum of squares	Mean square	F	Significant level
SH	Between groups	27	1937.262381	71.750459	3.84	<.0001**
	Within groups	54	1009.026905	18.685683		
	Total	83	2967.682381			
LN	Between groups	27	54.89285714	2.03306878	5.39	<.0001**
	Within groups	54	20.35714286	0.37698413		
	Total	83	75.55952381			
LL	Between groups	27	95.09654762	3.52209436	2.52	0.0020**
	Within groups	54	75.5773810	1.3995811		
	Total	83	171.0498810			
LW	Between groups	27	32.64809524	1.20918871	4.78	<.0001**
	Within groups	54	13.65547619	0.25287919		
	Total	83	46.86809524			
LA	Between groups	27	13062.67348	483.80272	3.81	<.0001**
	Within groups	54	6853.86236	126.92338		
	Total	83	20017.25828			

PL	Between groups	27	0.70000000	0.02592593	1.78	0.0354*
	Within groups	54	0.78500000	0.01453704		
	Total	83	1.58000000			
SD	Between groups	27	26.40212857	0.97785661	3.52	<.0001**
	Within groups	54	14.98308571	0.27746455		
	Total	83	44.20579524			
NN	Between groups	27	6.32142857	0.23412698	2.19	0.0073**
	Within groups	54	5.78571429	0.10714286		
	Total	83	12.32142857			
IN	Between groups	27	72.31952381	2.67850088	2.16	0.0079**
	Within groups	54	66.8154762	1.2373236		
	Total	83	142.8395238			

Where; SH= Seedling height, LN= Leaf number, LL= Leaf length, LW= Leaf width, LA= Leaf area, PL= Petiole length, SD= Stem diameter, NN= Number of node and IN= Internode length

**indicates statistically highly significant difference implies that the value of significant level is <0.01 P value, *indicates only significant difference at least between two of the group means being compared implies that the value of significant level is <0.05 but, >0.01 P value and ns represent none significant difference.

Table 2. Summary of mean, maximum and minimum values of each seedling quantitative parameters of coffee germplasm accessions and standard checks

Parameters	Mean	St/Deviation	Variance	Minimum	Maximum
SH	29.25952	4.802	23.062	23.167	41.500
LN	7.702381	0.808	0.653	6.3333	10.3333
LL	11.75119	1.074	1.153	9.1667	13.8333
LW	5.288095	0.627	0.393	3.9333	6.5667
LA	62.94119	12,470	155.507	36.133	88.760
PL	0.500000	0.091	0.0083	0.33333	0.66667
SD	4.320238	0.561	0.314	3.5533	5.5633
NN	2.178571	0.274	0.075	2.0000	3.0000
IN	8.169048	0.928	0.861	6.3333	9.8333

Where; SH= Seedling height, LN= Leaf number, LL= Leaf length, LW= Leaf width, LA= Leaf area, PL= Petiole length, SD= Stem diameter, NN= Number of node and IN= Internode length. According to the result shown in the above table the higher variance or standard deviation value of any parameters indicates a greater dispersion/spread of data points from the mean, while a lower value indicates that the data points are clustered closer to the mean of any given parameters analyzed.

Qualitative parameters

According to the International Plant Genetic Resources Institute [21] Coffee descriptor, data of 5 qualitative traits were recorded from each accession as described in Tables 3 & 4.

Table 3. Description of collected Coffea Arabica L. germplasm accessions used in the study

Accessions	District	Name of farm owner	Specific collection site	Year of collection name
AEKN-001	Yirgacheffe	Wolde Lemo	Suke	2022
AEKN-002	Yirgacheffe	Melkamu Tadesse	Resitti	//
AEKN-003	Yirgacheffe	Jigso Kuse	Kedida	//
AEKN-004	Yirgacheffe	Ayele Kutu	Chiriku	//
AEKN-005	Yirgacheffe	Nuguse Utala	Haru	//
AEKN-006	Yirgacheffe	Amanuel Kebede	Warabi	//
AEKN-007	Yirgacheffe	Beyana Maldei	Harange	//
AEKN-008	Yirgacheffe	Alemayaw Ararso	Horu Betela	//
AEKN-009	Yirgacheffe	Tadesse Baraso	Arecha	//
AEKN-010	Yirgacheffe	Regasa Bora	Wogida	//

AEKN-011	Yirgacheffe	Markos Edema	Ela Tanacha	//
AEKN-012	Yirgacheffe	Mitiku Bori	Gerise	//
AEKN-013	Yirgacheffe	Tsegaye Demise	Adame	//
AEKN-014	Yirgacheffe	Kifle Teqabo	Buduksa	//
AEKN-015	Yirgacheffe	Tilahun Werera	Gerbota 02	//
AEKN-016	Yirgacheffe	Nugese Dama	Gerbota 01	//
AEKN-017	Yirgacheffe	Seid Dayaso	Tulise	//
AEKN-018	Yirgacheffe	Ragaso Dama	Wote	//
AEKN-022	Yirgacheffe	Bakele Badhaso	Domorso	//
AEKN-023	Yirgacheffe	Wako Legese	Cito/Deraro	//
AEKN-024	Yirgacheffe	Tadesse Bareko	Chelba	//
AEKN-025	Yirgacheffe	Samuel Wako	Tutiti	//
AEKN-026	Yirgacheffe	Dori Bedecho	Udessa	//

Table 4. Qualitative parameters of Coffea Arabica L. germplasm and its Coding [22]

Sr/N	Qualitative traits	Phenotypic class with code
1.	Young leaf color	1 (Greenish), 2 (Green), 3 (Brownish), 4 (Reddish brown), 5 (Bronze) and 6 (other)
2.	Leaf shape	1 (Obovate), 2 (Ovate), 3 (Elliptic), 4 (Lanceolate) and 5 (Other)
3.	Leaf apex shape	1 (Round), 2 (Obtuse), 3 (Acute), 4 (Acuminate), 5 (Apiculate),6 (Spatulate) and 7 (Other)
4.	Leaf petiole color	1 (Green), 2 (Dark brown) and 3 (Other)
5.	Young shoot color	1 (Green), 2 (Dark brown) and 3 (Other)

The clustering result of 26 coffee germplasm accessions and standard checks distribution pattern revealed that maximum numbers of coffee germplasm accessions were gathered together in cluster-II (15) followed by cluster-I (9)and cluster-III (4) as shown in Table 5. Cluster-II comprised 15 (53.57%) of the accessions, which are varied from other clusters by having brownish young leaf color, lanceolate leaf shape, acute leaf apex shape, green leaf petiole, and young shoot color. Cluster-I which consisted of 9 (32.14%) varies from others by having a greenish young leaf color, ovate leaf shape, acuminate leaf apex shape, dark brown leaf petiole, and young shoot color. Cluster-III comprised 4 (14.29) coffee accessions, varied from other clusters by having its bronze young leaf color, elliptic leaf shape, and apiculate leaf apex shape as shown in Table 5.

Table 5. The distribution of 26 coffee accessions and 2 standard checks in to three clusters tested at Wanago nursery site.

Cluster No.	No. acc.	Percent (%)	Accessions
I	9	32.14	74110, 74112, AEKN-5, AEKN-6, AEKN-13, AEKN-14, AEKN-19, AEKN-21 & AEKN-22
II	15	53.57	AEKN-2, AEKN-3, AEKN-4, AEKN-7, AEKN-8, AEKN-9, AEKN-12, AEKN-15, AEKN-16, AEKN-18, AEKN-20, AEKN-23, AEKN-24, AEKN-25 & AEKN-26
III	4	14.29	AEKN-1, AEKN-10, AEKN-11 & AEKN-17

Discussions

In this study, as visualized in Fig 1, there were great variations within Coffea Arabica germplasm collected from the Yirgacheffe district in seedling height, number of paired leaves, and leaf length seedling characteristics. Coffea Arabica collected from Chiriku local had the highest seedling height (41.5) whereas Wogida local had the lowest seedling height (23.2). 74110 standard checks showed the highest number of paired leaves (10.3) and the least number of paired leaves (6.3) were recorded from local landrace collected from Chelba local area. Coffea Arabica collected from Kedida local was highest in leaf length (13.8) and 74110 standard checks showed the least leaf length (9.2).

Fig 2 shows that there is a great variation within Coffea Arabica landraces collected from the Yirgacheffe district in leaf width, leaf area, and petiole length seedling characteristics. The highest leaf width (6.6) and leaf area (88.8) were recorded on Coffea Arabica landrace collected from Gerbota 01 local and the least leaf width (3.9) and leaf area (36.1) was observed on 74110 standard checks. The highest petiole length (0.7) was observed on the coffee landrace collected from Haru local and the least petiole length (0.3) was recorded on the coffee landrace collected from Buduksa local area. Fig 3 also showed us great variations within Coffea Arabica germplasm collected from the Yirgacheffe district in stem diameter, number of nodes, and internode length of seedling characteristics. The highest stem diameter (5.6) was recorded on the Coffea Arabica landrace collected from Kedida local and the least stem diameter (3.5) was observed on the coffee landrace collected from Buduksa local area. The highest node number (3) was observed on Coffea Arabica landrace collected from Resitti local and the least node number (2) was recorded on coffee landraces collected from Suke, Haru, Horu Betela, Wogida, Ela Tenacha, Gerise,Adame, Gerbota o2, Gerbota 01, Tulise, Wote, Koke, Konga, Sede, Chito, Chelba, Tutiti and Udesa locals. The highest internode length (9.8) was observed on Coffea Arabica landrace collected from Koke local and the least internode length (6.3) was recorded on 74110 standard checks.

The development of effective conservation strategies and plans for coffee breeding is only possible after understanding the extent and the distribution of genetic variation in plant species [9]. The amount of variation can be very different between species and between different populations of a species. This variation is one of the central issues in population genetic studies on the genetic structure of natural populations [23]. The difference in seedling characteristics in similar environments shows that the presence of diverse Coffea Arabica genotypes in the study area parallel to the previous study that characterized 49 coffee germplasm accessions collected from the Limu Kossa district for morphological characters and reported the presence of wide genetic variability [8]. Additionally, our study also agrees with the result of previous work done on the morphological characterization of coffee landraces at the seedling stage collected from Guji zones [9]. This opportunity could be exploited further in order to increase the genetic base of specialty coffee varieties [24]. Vegetative growth of different Coffea Arabica accessions involves significant differences in response to different environmental conditions and this variation could be attributed due to differences in climatic and edaphic conditions at different testing environments. This type of variation is further explained by the presence of the G x E interaction and these interactions are fundamental for understanding how to improve coffee production and breeding programs [25].

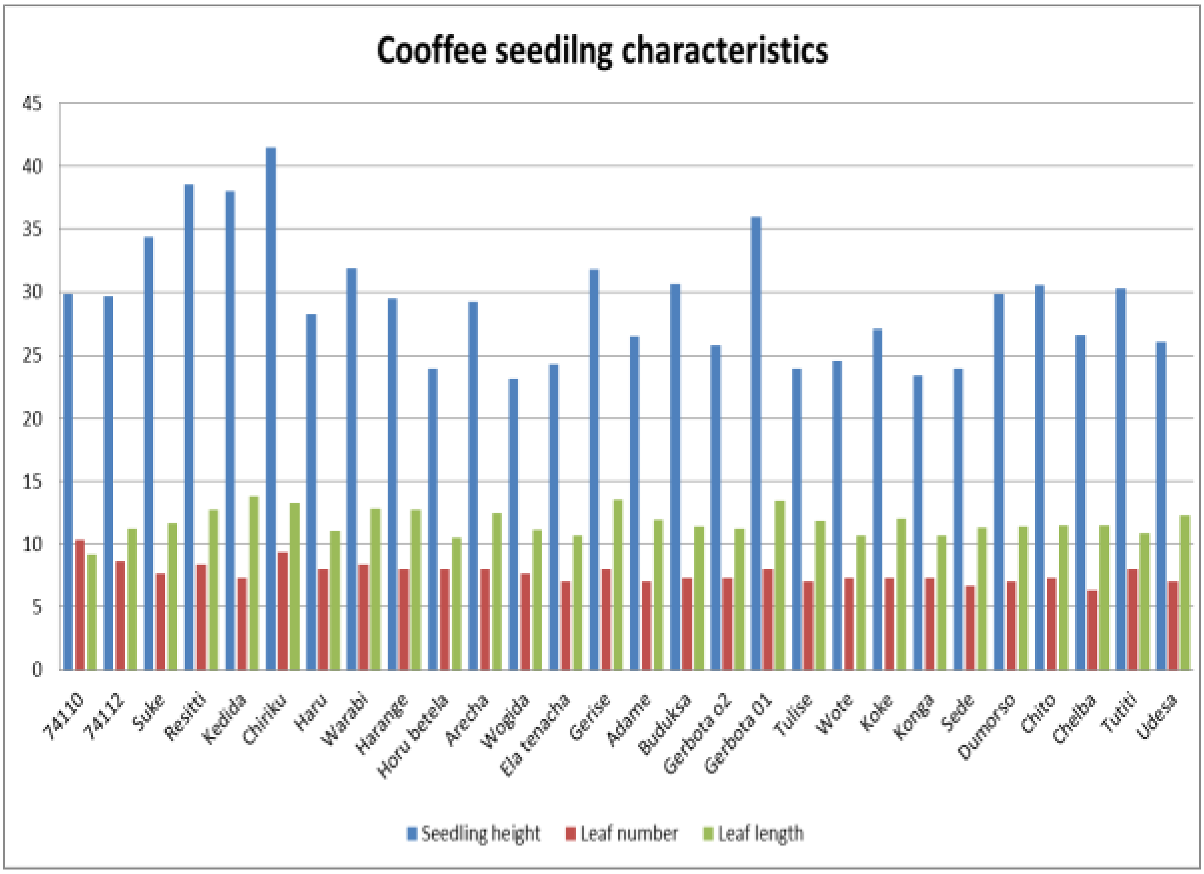

Fig 1. Graphical representation of seedling height, leaf number and leaf length for Coffee landrace accessions collected from Yirgacheffe district and two coffee variety (standard checks)

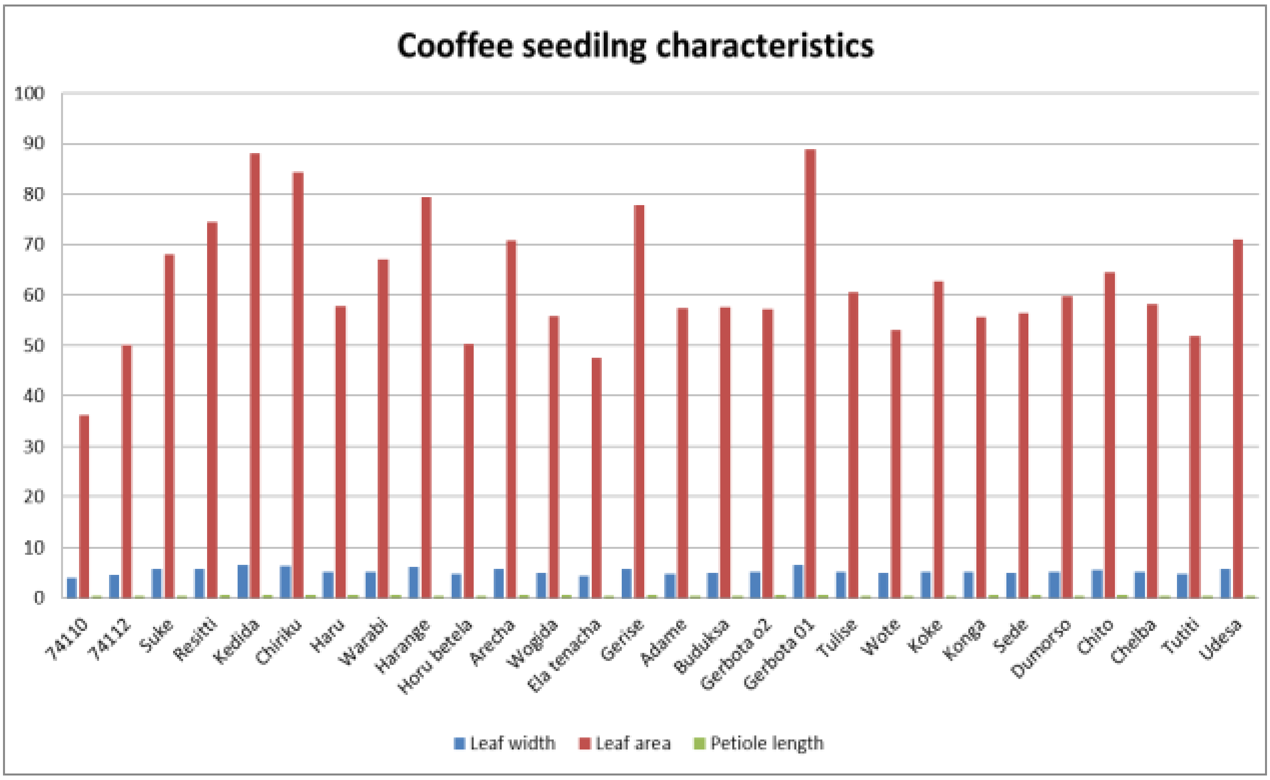

Fig 2. Graphical representation of leaf width, leaf area and petiole length for Coffee landrace accessions collected from Yirgacheffe district and two coffee variety (standard checks)

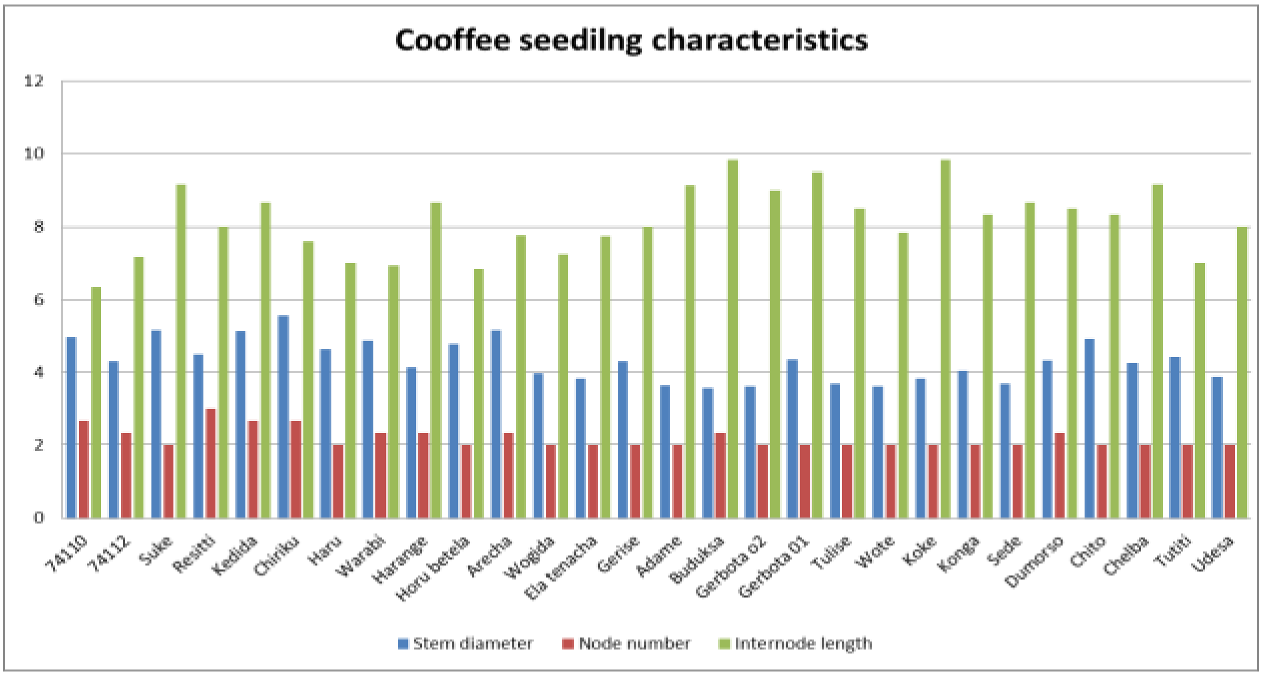

Fig 3. Graphical representation of stem diameter, node number and internode length for Coffee landrace accessions collected from Yirgacheffe district and two coffee variety (standard checks)

The diversity index (H') values range was extended from 0.429 for leaf petiole color (lowest polymorphic) up to 1.414 for young leaf color (highly polymorphic). Comparatively, the highest diversity was found for young leaf color (1.414) followed by leaf shape (1.067) and leaf apex shape (0.908) which might be due to the oligogenic essence of gene action and delicate environmental interaction. Proportionately low diversity was observed in young shoot color (0.582) and leaf petiole color (0.429). This low diversity value implicit that the majority of the population tends to fall within the same state, signifying the likelihood of a close association between coffee genotypes for these two traits [10].

Additionally, cluster analysis using SAS software by employing ward method clustering strategy clustered 26 coffee germplasm accessions and 2 standard checks into three clusters and revealed that great genetic variation between coffee germplasm accessions and standard checks as shown in Fig 4. In cluster analysis, if the classification is successful, individuals within or intra-cluster (homogenous) shall be closer, and inter-cluster (heterogeneous) shall be farther apart [10]. Hence, most of the collected germplasm accessions were grouped both from outsource and source area of collection, which stipulated that qualitative traits were highly heritable and less environmentally affected. This implies that geographic diversity is not always associated with genetic diversity [26]. Consequently, there is a possibility to develop hybrid vigor through crossing diverged parents found in different clusters. These are more highlighted by the study which suggested that morphological variation is more considerable than geographical origin as an indicator of genetic diversity in coffee [27].

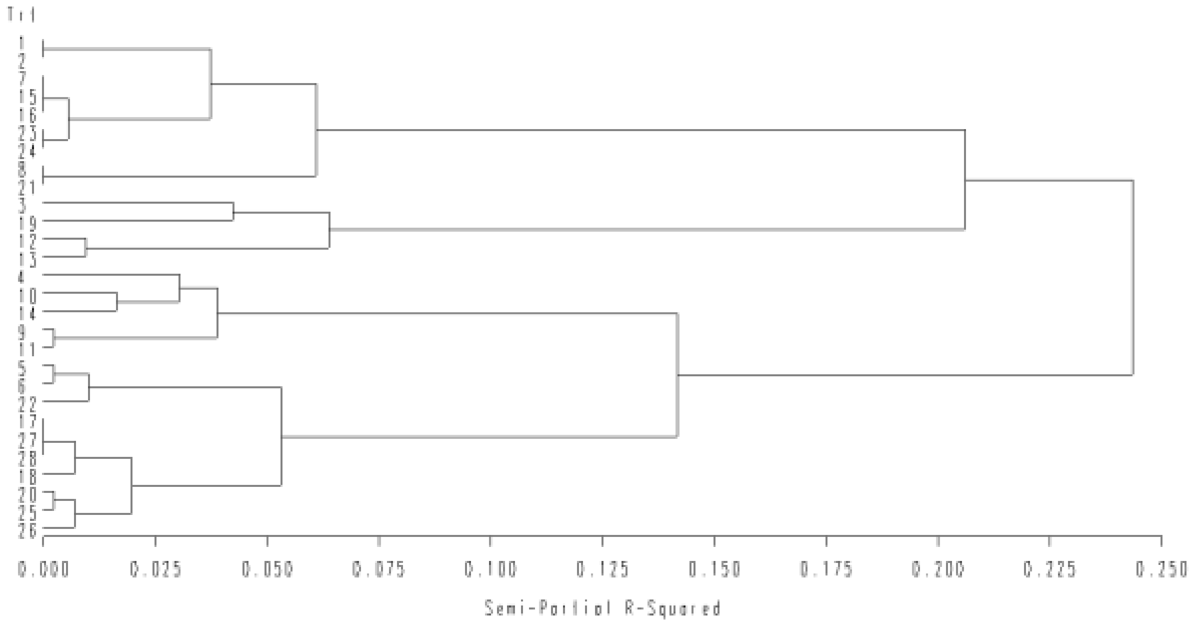

Fig 4. Dendrogram showing the clusters of 26 coffee germplasm accessions and 2 standard checks

Conclusion and Recommendations

In general terms, quantitative seedling parameters like seedling height, number of paired leaves, leaf height, leaf width, leaf area, stem diameter, number of nodes, and internode length as well as qualitative seedling parameters like young leaf color, leaf shape, leaf apex shape, leaf petiole color and young shoot color of coffee landrace germplasm accessions were evaluated at the WangoTumata Chiracha nursery site. Statistical analysis of variance at $P<0.05$ evaluated that there were differences between and within Coffea Arabica landrace germplasm accessions collected from the Yirgacheffe district of the Gedeo zone. In Yirgacheffe district Chiriku local coffee collections were higher than the others in all seedling parameters. These great variations between and within coffee seedling parameters that were collected from the Yirgacheffe district of the Gedeo zone show that there are genetic variations that can be assessed by their morphology at the seedling stage. Additionally, the results of this study have long-established the existence of enormous genetic variability among the 26 Coffea Arabica landrace germplasm accessions collected from Yirgacheffe district for most of the qualitative parameters observed. Frequency distribution analysis and diversity Index using qualitative parameters showed the existence of genetic variability between collected coffee germplasm accessions and standard checks. Cluster analysis of 26 collected coffee landrace germplasm accessions and two standard checks grouped fifteen accessions into cluster-II followed by nine into cluster-I and four into cluster-III. Moreover, these are crucial steps toward rational coffee germplasm accession use and conservation, consequently exhibiting the importance of structured data collection for individual germplasm accessions' further characterization. So the current study proved the presence of plenty of genetic differences within Yirgacheffe district Coffea Arabica landrace germplasm accessions for various morphological characteristics. Therefore, there is a chance to utilize these characteristics with the intention of developing genotypes that outstrip the existing varieties for the future coffee breeding program for this particular agro-ecology. Furthermore, based on the present result we recommend that the current findings must be further studied by breeders using coffee germplasm accessions physiological, biochemical, and quality characterization with the help of advanced molecular techniques which deliver great potential to ensure efficient and effective utilization, conservation and development of new improved varieties.

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Conflict of interest
The authors declare that no conflict of interest
Ethical standards
The study is proper with ethical standards
Author Contributions
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References

1. Tewolde, B., The importance of Ethiopian forests in the conservation of Arabica coffee gene-pools, (1990): 65-72. https://www.cabidigitallibrary.org/doi/full/10.5555/19931640244
2. Labouisse, J.-P., and S. Kotecha, Preserving diversity for specialty coffees. A focus on production systems and genetic resources of Arabica coffee in Ethiopia, 2008. https://doi.org/10.1007/s10722-008-9361-7
3. Bellachew, B., and J. P. Labouisse, Arabica coffee (Coffea arabica L.) local landrace development strategy in its center of origin and diversity, 2007. https://agritrop.cirad.fr/540084/7/ASIC2006-Communication-Bayetta-Labouisse.pdf
4. Taye, K.., Status of Arabica Coffee Germplasm in Ethiopia, In the 3rd African Coffee Sustainability Forum. AFCA Pre-Conference Event, Munyono, Uganda, 2013.
5. Abdi, A., Agro-morphological characterization of coffee (Coffea arabica L.) landrace collected from Mesela, West Harerge, Ethiopia, PhD diss., M. Sc. Thesis Submitted to Graduate Studies of Hawassa University, Hawassa, Ethiopia, 2009.
6. Kebede, M., and B. Bellachew, Phenotypic Diversity in the Hararge Coffee (Coffea arabica L) Germplasm for Quantitative Traits, East African Journal of Sciences 2, no. 1 (2008): 13-18. https://doi.org/10.4314/eajsci.v2i1.40358
7. Ermias, H., Evaluation of Wellega coffee germplasm for yield, yield component and resistant to coffee berry disease at early bearing stage, An MSc thesis submitted to school of graduate studies of Alemaya University 69 (2005).
8. Olika, K.., et al., Variability of quantitative traits in Limmu coffee (Coffea arabica L.) in Ethiopia, (2011): 482-493. https://doi.org/10.3923/ijar.2011.470.481
9. Alemu, I., and D. Boke, Morphological Characterization of Coffee (Coffea Arabica L.) Landraces at Seedling Stage Collected from Guji Zones, Bangladesh Journal of Plant Breeding and Genetics 30, no. 2 (2017): 09-18. https://doi.org/10.3329/bjpbg.v30i2.34540
10. Yirga, M., Phenotypic Characterization of Coffee (Coffea Arabica L.) Germplasm, in Ethiopia, American Journal of Bioscience 9, no. 2 (2021): 34-41. https://doi.org/10.11648/j.ajbio.20210902.11
11. Abeyot, T., et al., Genetic diversity analysis for quality attributes of some promising Coffea arabica germplasm collections in southwestern Ethiopia, (2011): 236-244. https://doi.org/10.3923/jbs.2011.236.244
12. Bekele, Y. D., Assessment of cup quality, morphological, biochemical, and molecular diversity of Coffea arabica L. genotypes of Ethiopia, PhD diss., University of the Free State, 2005. http://hdl.handle.net/11660/838
13. Kathurima, C., et al., Evaluation of beverage quality and green bean physical characteristics of selected Arabica coffee genotypes in Kenya, (2009). https://agritrop.cirad.fr/552843
14. Asner, G. P., et al., Environmental and biotic controls over aboveground biomass throughout a tropical rain forest, Ecosystems 12, no. 2 (2009): 261-278. https://doi.org/10.1007/s10021-008-9221-5
15. Senbeta, F., et al., Wild Coffea arabica L. in Afro montane rainforests of Ethiopia: distribution, ecology and conservation, SINET: Ethiopian Journal of Science 30, no. 1 (2007): 13-24. https://doi.org/10.4314/sinet.v30i1.18278
16. Ayalew N. B., The relationship between climate, disease and coffee yield: optimizing management for smallholder farmers, PhD diss., Department of Ecology, Environment and Plant Sciences, Stockholm University, 2023. http://urn.kb.se/resolve?urn=urn:nbn:se:su:diva-216221
17. Zerga K., and B. Tsegaye, Coffee Diversity and Conservation in Ethiopia, In Sustainable Agriculture Reviews 39, pp. 35-55. Cham: Springer International Publishing, 2020. https://doi.org/10.1007/978-3-030-38881-2_2
18. Shanon, C. E., and W. Weaver, The mathematical theory of communication, University of Illinois., Urbana, USA (1963).
19. Mason, C., Biology of Fresh Water Pollution, Pearson Education, 2002. https://doi.org/10.1201/9781482295368-7
20. Peeters, J.P., and J.A. Martinelli, Hierarchical cluster analysis as a tool to manage variation in germplasm collections, Theoretical and applied genetics 78, no. 1 (1989): 42-48. https://doi.org/10.1007/bf00299751
21. Chweya, J. A., and P. B. Eyzanguire, International Plant Genetic Resource Institute (IPGRI), Rome, Italy (1999).
22. International Plant Genetic Resources Institute, Descriptors for Coffee (Coffea spp. and Psilanthus spp.), Bioversity International, 1996.
23. Geletu, K. T., Genetic diversity of wild Coffea arabica populations in Ethiopia as a contribution to conservation and use planning, Vol. 44. Cuvillier Verlag, 2006. ISBN 978-3-86727-986-4
24. WeldeMichael, G., et al., Genetic diversity analysis of some Ethiopian specialty coffee (Coffea arabica L.) accessions for cup quality attributing traits, J. of Biology, Agriculture and Healthcare 5, no. 5 (2015): 88-96. https://www.academia.edu/download/102675859/21565.pdf

25. Senra J.F., et al., Initial performance and genetic diversity of coffee trees cultivated under contrasting altitude conditions, Scientia Agricola 80 (2023): e20220163. https://doi.org/10.1590/1678-992x-2022-0163
26. Ayana, A., and E. Bekele, Multivariate analysis of morphological variation in sorghum (Sorghum bicolor (L.) Moench) germplasm from Ethiopia and Eritrea, Genetic Resources and Crop Evolution 46, no. 3 (1999): 273-284. https://link.springer.com/article/10.1023/A:1008657120946
27. Belachew, B., Arabica coffee breeding for yield and resistance to coffee berry disease (Colletotrichum kahawae sp. Nov.) (2001).

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Araştırma Makale/ Research article

In vitro antisickling effect of crude stem bark extract of Ficus sycomorus on human sickled red blood cells

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ABSTRACT
Sickle cell anemia is one of a group of inherited disorders known as sickle cell disease. The *Ficus* sycomorus is used in Burkina Faso folk medicine for the management of sickle disease. This study was aimed at investigating the antisickling property of methanol and aqueous extract of *Ficus* sycomorus. Phytochemical screening was conducted using a standard method. For the antisickling test, 2% sodium metabisulphite was used to induce sickling in HbSS blood. *Ficus sycomorus* stem extractswere added after 60 minutes of incubation. Every 30 minutes for a period of 60 minutes (0 min, 30 min, and 60 min), a drop of the prepared solution was observed at (40 x) mg and percentage of reversion was calculated. The phytochemical analysis revealed the presence of alkaloid, tannins, polyphenols and flavonoids in the extracts. In methanol extract, at 0.1mg/ml, the percentage of sickled red blood cells (RBCs) is 80.00±0.414 % at 0 min, 79.00±0.414 % at 30 min and 50.000±0.414 % at 60 min. At 0.3 mg/ml, the percentage of sickled RBCs is 75.000±0.000 % at 0 min, 49.000±0.414 % at 30 min and 19.500±0.707 %. At 0.5 mg/ml the percentage of sickled RBCs is 60.000±0.243 % at 0 min, 58.500±0.121 % at 30 min and 40.000±0.707 % at 60 min. while in aqueous extract, at 0.1mg/ml, the percentage of sickled RBCs is 89.00±0.414 % at 0 min, 70.00±0.414 % at 30 min and 64.000±0.414 %at 60 min. At 0.3 mg/ml, the percentage of sickled RBCs is 66.000±0.536 % at 0 min, 51.000±0.828 % at 30 min and 18.000±0.414 %. At 0.5 mg/ml the percentage of sickled RBCs is 44.000±0.414 % at 0 min, 30.000±0.000 % at 30 min and 10.000±0.121 % at 60 min. extract of *Ficus* sycomorus have shown to be therapeutically effective in the management of sickle cell anemia.

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Introduction

Sickle cell disease (SCD) is caused by a biallelic single nucleotide substitution in the beta-globin gene resulting in an amino acid substitution HBB(Glu7Val, formally known as Glu6Val) [1]. It affects the shape of red blood cells, which carry oxygen to all parts of the body. Red blood cells are usually round and flexible, so they move easily through blood vessels. In sickle cell anemia, some red blood cells are shaped like sickles or crescent moons. These sickle cells also become rigid and sticky, which can slow or block blood flow. There's no cure for most people with sickle cell anemia. Treatments can relieve pain and help prevent complications associated with the disease. Red blood cells are usually round and flexible. In sickle cell anemia, some red blood cells look like sickles used to cut wheat. These unusually shaped cells give the disease its name which is characterized by vaso-occlusive episodes causing acute pain, as well as chronic multi organ damage, such as cerebral infarction, retinopathy, and nephropathy[21]. In recent years, there have been significant advancements in the treatment of sickle cell anemia. In 2023, the FDA approved two gene therapy products to treat sickle cell disease (SCD) in patients 12 years and older, lovotibeglogene autotemcel and exagamglogene autotemcel. Patients who receive either SCD gene therapy product took part in a long-term study to evaluate the safety and effectiveness of these

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gene therapy products [5].However, the complexity of the treatment process and high costs hinder the universal application of ex vivo gene therapy. Therefore, the development of in vivo HSC gene therapy, where gene therapy tools are directly administered to patient, is desirable to provide a more desirable to provide a more accessible, cost-effective solution that can cure SCD worldwide [10].

Sickle Cell Disease (SCD) is a major health problem worldwide.. Over 300, 000 children are usually born with hemoglobin disorders of which 83% are affected by SCD [2]. Sub-Saharan Africa is the most affected (with about 80% of the global total) [6]. In this region, 50-80% of the affected children die before the age of five years [9]. Somatic gene therapy will be one of the most exciting practices of genetic medicine in Africa and is primed to offer a “new life” for persons living with sickle cell disease (SCD). Recently, successful gene therapy trials for SCD in the USA have sparked a ray of hope within the SCD community in Africa. However, the high cost, estimated to exceed 1.5 million USD, continues to be a major concern for many stakeholders[13]. Natural products obtained from medicinal plants and used in traditional medicines are of great importance for the development of modern medicine, hence this research was conducted to evaluate the antisickling potential of *Ficus sycomorus* in the management of sickle cell anemia to provide alternative and affordable treatment for patient with sickle cell anemia.

Ficus sycomorus (FS), in Oman belongs to the Moraceae family. The plant contains different groups of biologically active compounds which are responsible for the biological activity. *Ficus sycomorus* have been reported to be used by traditional medical professionals in the management of sickle cell anemia. [20]

Methodology

Plant collection and identification

The fresh stem bark samples of *F. sycomorus* were collected from the Kasuwan Magani of Kawo, Kaduna, Kaduna State, the plant was then taken to botanist in biological science Department of Kaduna State University for authentication. The voucher specimens were packaged tightly in plastic bags after authentication and then deposited in the investigation laboratory of the Kaduna State University.

Preparation of plant sample

The *Ficus sycomorus* plant samples were air dried for 14 days under the shade and then uniformly powdered using a motor and pestle, the powder was sieved and stored in a covered sample bottle.

Fig 1. Schematic illustrating the extraction process (Methodological schematic)

Plant extraction

For methanol extraction, 50 g of dried powdered samples were dissolved in 500 mL of 70% methanol for 48 hours. Then, the mixture was filtered under pressure and dried in a rotary evaporator under reduced pressure at

22 ^{O}C to obtain the crude extract. The above method was applied to aqueous extraction in the process called exhaustic extraction. The extract of F. sycomorus was stored in a tightly stoppered container of brown glass in the refrigerator at 4 ^{O}C until required. [6, 23].with slight modification.

Preparation of the blood sample

The ethical approval for this research work was obtained from the human research ethics committee (HREC) of barau dikko teaching hospital, Kaduna, Kaduna state, with reference number BDTH/2023/126/VOL/1 And registration number NHREC/30/11/21A dated 24th August, 2023. The blood samples were collected from HbSS adult patient attending Barau Dikko Teaching Hospital by a phlebotomist, the blood samples were centrifuged at 5000 rpm for 5 minutes. The serum from each sample was removed while the red blood cell used for the analysis was washed thrice in normal saline. The washed erythrocytes was then used for the experiment,

Preliminary phytochemical screening

Phytochemical analysis of the partially purified extract of *Ficus* sycomorus was carried out using the method described by [18] with slight modifications.

Determination of Anti- sickling properties of the *Ficus sycomorus*

The ability of the plant extract to reverse the sodium metabisulphite induced sickling was estimated using sodium metabisulphite test as described by [2] with slight modifications. In this test, 2 % sodium metabisulphite was used to induce sickling in washed erythrocytes (0.5 ml). A drop of the induced erythrocyte was placed on a microscopic slide and a thin blood film was made which was then stained with giemsa stain and examined under the oil immersion light microscope (1000x). The 0.1 mg/ml, 0.2 mg/ml, and 0.3 mg/ml of the methanol and aqueous extract and fraction were tested by adding 0.5 ml to the sickled erythrocytes, and the mixture was incubated for 60 min at 37 degrees in a water bath (Grant JB series B and T Keison international Ltd, Chelmsford, Essex CM1, England). Samples were then taken and then smeared on microscopic slide after 0 min, 30 min, and 60 min, as earlier described by Emmanuel et al., (2025). Each sample smear was fixed with 98% methanol, dried and stained with giemsa stain for about 45 min and each sample was examined under the oil immersion light microscope (Herius, Kendro laboratory, Newton, USA) by focusing at x1000 the slide and counting at least 500 red blood cells in each sample from five different fields of view across the slide, while recording the total number of sickled and unsickled red blood cells. Positive control contained p-hydroxy benzoic acid (5 mg/ml). The percentage of unsickled cells was determined using the formula.

$$\% \text{ of sickling} = \frac{\text{Mean number of sickled cells}}{\text{Total number of red cells counted}} \times 100$$

Each slide was prepared and examined thrice and the result were expressed as mean standard deviation (± S.D).

Results

The table below contains the result of qualitative phytochemical analysis of methanol and aqueous extracts, all the phytochemicals tested are present in methanol extract while in aqueous extract saponins, steroids and glycosides are absent.

Table 1. Qualitative analysis result of *Ficus sycomrus*

S/N.	Phytochemicals	Methanolic extract	Aqueous extract
1.	Saponins	present	absent
2.	Tannins	present	present
3.	Flavonoids	present	present
4.	Phenols	present	present
5.	Alkaloids	present	present
6.	Anthraquinones	present	present
7.	Steroids/tarpenoids	present	absent
8.	Cardiac glycosides	present	absent

The table below is a result of quantitative phytochemical analysis Methanol extract and aqueous extract of Stem bark extract of *Ficus sycomorus;*. flavonoids, saponins and phenols are higher in methonol extract while alkaloids and tannins are higher in aqueous extract.

Table 2. **The Total Phytochemical Contents (TPC) In Each Extract**

S/N	PHYTOCHEMICALS	M (mg/G of extract)	A (mg/G of extract)
1.	Phenols	24.70 ± 0.73	14.17 ± 0.87
2.	Flavonoids	501.30 ± 0.01	245.80 ± 0.77
3.	Tannins	17.92 ± 0.67	19.18 ± 0.99
4.	Saponins	108.72 ± 0.54	1.12 ± 0.54
5.	Alkaloids	153.30 ± 0.65	390.95 ± 0.74

M: Methanol extract of *Ficus sycomorus*, A: Aqueous extract of *Ficus sycomorus*

Table 3 shows the result for the antisickling study, normal saline is the negative control which shows increase in percentage of sickling with increase in time, PABA is the positive control which shows reversal of sickling with increase in time, the extract shown reversal activity compared to the positive control at different concentrations.

Table 3. Antisickling activity (%) of methanol extracts *F. sycomorus* on 2% sodium metabisulphite induced sickled red blood cells

Sample	Concentrations	0 mins (%)	30 mins (%)	60 min (%)
N	1 mg/ml	20.00 ± 0.00^{a}	45.00 ± 0.00^{a}	$95.00 \pm 0.00_{d}$
P	1 mg/ml	60.00 ± 0.00^{b}	47.00 ± 0.00^{a}	15.00 ± 0.11^{a}
A	0.1 mg/ml	80.00 ± 0.41^{d}	79.00 ± 0.44^{d}	50.00 ± 0.14^{b}
B	0.3 mg/ml	75.00 ± 0.00^{c}	69.00 ± 0.41^{c}	50.50 ± 0.71^{b}
C	0.5 mg/ml	60.00 ± 0.24^{b}	58.50 ± 0.12^{b}	40.00 ± 0.77^{d}

N= normal saline (negative) control**,** P= PABA (positive) control**,** A, B and C= Sample. Values are Mean ± SEM for 3 determinations. Values with different superscripts down the column are statistically different at $p < 0.05$

Table 4 shows the antisickling study results, normal saline is the negative control and PABA is the positive control, the extract at higher concentration (0.5 mg/ml) at 60 min shows activity higher than that of the PABA with only 10% sickling.

Table 4. Antisickling activity (%) of aqueous extract *F. sycomorus* on 2% sodium metabisulphite induced sickled red blood cells

Sample	Concentrations	0 mins (%)	30 mins (%)	60 min (%)
N	1 mg/ml	20.00 ± 0.00^{a}	45.000 ± 0.00^{b}	95.00 ± 0.00^{d}
P	1 mg/ml	60.00 ± 0.00^{d}	47.00 ± 0.00^{b}	15.50 ± 0.12^{a}
A	0.1 mg/ml	89.00 ± 0.41^{c}	70.00 ± 0.41^{d}	64.00 ± 0.41^{c}
B	0.3 mg/ml	66.00 ± 0.53^{e}	51.00 ± 0.82^{c}	18.00 ± 0.41^{b}
C	0.5 mg/ml	44.00 ± 0.41^{b}	30.00 ± 0.00^{a}	10.00 ± 0.12^{a}

N= normal saline (negative) control, P= PABA (positive) control A, B and C= Sample. Values are Mean ± SEM for 3 determinations. Values with different superscript down the coloum are statistically different at $p < 0.05$

Discussion

The result of phytochemical screening in studied stem extracts of *Ficus sycomorus* revealed the presence of flavonoids, alkaloids, tannins, saponins and phenols (Table 1). The results revealed that the stem bark methanol extract of Ficus sycomorus is packed with varying levels of polyphenols compounds such as flavonoids, tannins, saponins, and phenols, Saponins and steroids were not detected in the aqueous extract of Ficus sycomorus, However, the two extracts contained flavonoids and alkaloids in high concentration (Table 2) which indicate solvent-based weakness. Several bodies of evidence have shown that these biofunctional ingredients decide diverse pharmacological effects which may range from antioxidant (22). The presence of this secondary metabolites contributes to the potential therapeutic effect of ficus sycomorus in antisickling activity by promoting tissue regeneration, decreased the permeability of blood capillaries and strengthen their resistance to hemolysis. Also their antioxidants activities have been demonstrated [3] and the anti-inflammatory property, anti-inflammatory properties can help in reducing the severity of sickle cell disease by mitigating the harmful effects of inflammation. Which is a major contributor to the disease's complications. Inflammation can exacerbate sickling, promote vaso-occlusion (blockage of blood vessels), and damage tissues, leading to pain and organ damage. By reducing inflammation, anti-inflammatory agents can potentially improve blood flow, reduce pain, and protect against long-term complications of sickle cell disease [17].

The medicinal plant value of plants have gained high relevance in the past decades and now medicinal plants are greatly searched (22). This is a result of many reasons such as the several beneficial offers that is enjoyed which includes minimal clinical risks, affordable nature of plant synthesized drugs, and the existence of limited and poor modern health facilities as well as poverty (22). Plants produce a very diverse group of secondary metabolites which have lifesaving and therapeutic properties. Nearly 70% of the world population most especially in the developing countries relies mostly on traditional medicine therapies as their basic way of health care. Even presently, rural population still considers herbal medicines as the most preferred therapeutic source. In recent years, plants constituent previously with unknown pharmaceuticals activities have been extensively studied as medicinal agents. This component differs from plant to plant and example includes Anthraquinones, flavonoids, glycosides, tannins, Saponins, phenols, steroids, etc (12).

Oxidative stress is one of the triggers of various degenerative diseases and metabolic syndrome. Antioxidants are compounds that exhibit the activities of neutralizing and scavenging radical molecules, which induce the process of oxidative reaction in the body. An important role is being played by the radical molecule in governing the various biological processes which are necessary for the body. This shows that free radicals are necessary but at the same time harmful to the body. Polyphenols, carotenoids, and traditional antioxidant vitamins such as vitamin C and E may all contribute to the total antioxidant activity of plant materials. Several studies demonstrated that phenolic compound are the most beneficial bioactive phytochemicals for human health. In fact, some studies have demonstrated a correlation between total phenolic content and antioxidant activity in a variety of seeds, fruits, and vegetables (22).

However, naturally occurring compounds having antisickling properties are rich in aromatic amino acids, phenolic compounds, flavonoids and antioxidant nutrient and are known to display antisickling activities either by inducing HbF (fetal hemoglobin) production, by increasing the delay time required for HbS (abnormal hemoglobin) polymerization or by affecting RBCs membrane leading to low amounts of intracellular Hb (hemoglobin) concentration and thereby inhibiting polymerization [7].

Several studies have reported antioxidant molecules as potent inhibitors of HbS polymerization and increase in the oxidant status of sickled RBCs [19, 7, 15]. The higher the levels of the of the antioxidants in the plants extracts, the higher is the possibility of them displaying antisickling properties that might be due to reduction in oxidative stress, enhanced membrane stabilizing capabilities or by donation of the free electron to the iron molecule of hemoglobin [4]. The flavonoid, alkaloid and other active compounds present in ficus sycomorus can serve as antioxidant by scavenging free radical by transferring electron to ROS to neutralize their effect by converting them into less harmful substances [7]. The use of ficus sycomorus by the traditional healers is justified due to the evidence of high level of antioxidants substances like flavonoids and alkaloids presence in both methanol and aqueous extract observed in our current study. Similar result were observed in a recent study, where the Methanolic extract displayed significant antioxidant activities that correlated with high concentrations of total flavonoids and phenolic contents [14]. The flavonoids are high in methanol extract of *Ficus sycomoru*s (501.30 mgQE/g of extract). Hence, the highest activity were observed in methanol extract of *Ficus sycomoru*s in this study.

During antisickling studies, methanol extract of *Ficus sycomorus* show the highest antisickling activity due to the high flavonoids contents by reversing the sickled red blood cells into the biconcave shape (normal shape). Flavonoid is having high antioxidants activity which contribute to its antisickling properties [7] and it is polar in nature [8].

The aqueous extract also shows antisickling activity when compared to the positive control (para amino benzoic acid). from (Table 3), the aqueous extract at different concentration shown antisickling potential, the activity of aqueous stem bark extract of Ficus sycomorus when compared with the positive result is increasing with the increase in concentration, which also indicate dosage-dependent as well as time-dependent.

The reversing rate in both extract is dosage-dependent, the highest activity was shown at the highest concentration of the extract, and also time-dependent, the highest activity was observed at 60 min.

Conclusion

This study shows that both methanol and aqueous extract of *Ficus sycomorus* contains secondary metabolites like flavonoids and alkaloids that makes the plant therapeutically effective in the management of sickle cell anemia.

Recomendations

It i**s** recommended that toxicity testing and in vivo study should also be made on this plant. The bioactive compound in *Ficus sycomorus* responsible for the antisickling activity should be isolated for the possibility of developing a drug.

References

1. Ambroise, W., et al., FLT1 and other candidatefetal haemoglobin modifying loci in sickle cell disease in African ancestries. The journal of nature communication, 2025. 16 (1) p. 42-47. https://doi.org/10.1038/s41467-02557413-5
2. Emmanuel, U., et al., Phytochemistry, Mineral Estimation, Nutritional, and the *In Vitro* Anti-Sickling Potentials of Oil Extracted from the Seeds of Mucuna Flagellipes. Journal of the Mexican chemicals society, 2024. 68(2) p. 220-233. https://doi.org/10.29356/jmcs.v68i2.1898

3. Xiang, Y., et al., Secondary metabolites and antioxidant activity of different parts from the medicinal and edible alpiniae oxyphylla Miq. Journal of Functional Foods, 2025. 128 (106803) p. https://doi.org/10.1016/j.jff.2025.106803
4. M. O. Salawu, I. O. Abdulsalam and H. O. B. Oloyede, In vitro study of anti-sickling effects of aqueous extracts of Garcinia kola (seeds), Zingiber officinale (rhizomes) and Allium sativum (bulbs) on sodium metabisulphite-treated HBSS erythrocytes. National Library of Medicine, 2025. 25(241) p.23-30. https://doi.org/10.1186/s12906-025-04943-6
5. Food and drugs administration (FDA), 2023
6. Dessy, A., Extraction techniques and optimization strategies for phytochemicals from Annona muricata leaf: A comprehensive review. International journal of Agriculture and Biology, 2025. 34(1), p. 1-13. https://doi.org/10.17957/IJAB/15.2337
7. Wang, Y., et al., Computational chemistry strategies to investigate the antioxidant activity of flavonoids. MDPI, 2024. 29(11) p. 26-27. https://doi.org/10.3390/molecules29112627
8. Tiwari, A., et al., Marine-Derived Natural Flavonoids and Their Biological Activities, Nutraceutical in Cardiac Health Management, 2025. 1(15) p. 311-325. ISBN: 9781003545644
9. Jonani B, et al., Prevalence of sickle cell anemia in Africa : A protocol for a meta-analysis of existing studies, PLOS Journal, 2025, 20 (4). https://doi.org/10.1371/journal.pone.0321535
10. Julia, B. et al., Current and future treament for sickle cell disease: From hematopoietic stem cell transplantation to in vivo gene therapy, journal of molecular therapy, 2025, 33(07): p.2172-2191. https://doi.org/10.1016/j.ymthe.2025.03.016
11. Julia, B. et al., Current and future treament for sickle cell disease: From hematopoietic stem cell transplantation to in vivo gene therapy, journal of molecular therapy, 2025, 33(07): p.2172-2191. https://doi.org/10.1016/j.ymthe.2025.03.016
12. Muhammad M.D, AbdulAzeez M.A, and Susan P.A. Phytochemical Analysis and Antibacterial Activity of Methanol and Ethyl Acetate Extracts of Detarium microcarpum Guill & Perr. Biology, Medicine & Natural product chemistry ISSN 2086-6514 (paper) Volume 12, Number 1, 2023 p281-288. https://doi.org/10.14421/biomedich.2023.121.281-288
13. Munung, N.S. et al., Looking ahead: ethical and social challenges of somatic gene therapy for sickle cell disease in Africa, Journal of gene therapy, November 2023, 31(1) p 202-208. https://doi.org/10.1038/s41434-023-00429-7
14. Messaoudi, M., et al.,Phytochemical screening, polyphenols content and antioxidant activities of some selected medicinal plants growing in Algeria, Journal of Natural Resource and Sustainable Development, 2025 15(1) p. 189-202. https://doi:10.31924/nrsd.vl5il.185
15. A Ibrahim and SA Muhammad Antioxidant-rich nutraceuticals as a therapeutic strategy for sickle cell disease, Journal of the American nutrition association, 2023. 42(6), p. 588-597. https://doi.org/10.1080/27697061.2022.2108930
16. OO Oguntibeju, T oduola, and GM Davison. Investigation of haematological, inflammatory parameters and the incidence of alloimunization in multi-transfused sickle cell disease patient. 2023, 120-126. https://doi.org/10.1016/j.htct.2025.103937
17. Camila, A., et al., Unlocking the power of polyphenols: A promising biomarker of improved metabolic health and anti-inflammatory diet in adolescents. Journal of Clinical nutrition, 2024. 43(8), p. 1865-1871. https://doi.org/10.1016/j.clnu.2024.06.023
18. Sadgrove, N.J et al., An overview of the phytochemistry of medicinal bark (trunk, stem or root) from the most popular southern African species, Phytochemistry review. 2025 1(3) p. 1-21. https://doi.org/10.1007/s11101-024-10059-5
19. J.S. Gibson, and Rees, D.C. Emmerging drug targets for sickle cell disease: shedding light on new knowledge and advances at the molecular level, Expert opinion on therapeutic targets, 2023. 27(2) p. 133-149. https://doi.org/10.1080/14728222.2023.2179484
20. S.A. Ayuba, and A. Onu, Phytochemical Analysis and Antisickling activity of some medicinal plants from Sokoto, Nigeria, UMYU Scientifica, 2024 3(3). https://doi.org/10.56919/usci.2433.015
21. Traets M, et al., Pyruvate kinase function correlates with red blood cell properties and clinical manifestations in sickle cell disease, American Journal of Hematology, 2025, 100(5) p. 785-796. https://doi.org/10.1002/ajh.27644
22. Utami I., et al., Antioxidant activity of ethanol extract of papaya leaves (Carica papaya L.) on reducing blood glucose levels of streptozotocin induced male rats. Journal Eduhealth Volume 15, Number 02, 2024, https://doi.org/10.54209/eduhealth.v15i02.
23. Soyeng, W. and Kwon, K.H. Green Technology for Extraction of Bioactive Compounds from Edible Plants, Journal of Oleo Science, 2024 73(10), p. 1249-1265. https://doi.org/10.5650/jos.ess24162

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Araştırma Makale/ Research article

Farklı Renklerde LED Işıklarının Turp Mikro Filizlerinde Vejetatif Büyüme Üzerine Etkisinin İncelenmesi

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ÖZET
Son yıllarda birçok farklı bitki türü ve çeşitte mikro filiz ticari üretimi hızla yaygınlaşmaktadır. Bu araştırmada, mikro filiz üretiminde yaygın kullanılan turp bitkisinde, farklı ürün segmentlerinde (kırmızı, beyaz ve siyah) farklı renklerde LED ışık spektrumlarının vejetatif büyüme üzerindeki etkisinin kantitatif analizlerle incelenmesi amaçlanmıştır. Çalışmada, farklı LED ışık renklerinin (beyaz, mavi, kırmızı ve mor) turp mikro filizlerinin biyokütlesi, hipokotil boyu, çapı ve renklenme gibi morfolojik özellikleri üzerindeki etkileri değerlendirilmiştir. Araştırma sonuçlarına göre kırmızı ışık, hipokotil boyu açısından en fazla büyümeyi sağlamıştır. Kırmızı ışıkta ölçülen hipokotil boyu, beyaz ışığa göre %60, mavi ışığa göre %50 ve mor ışığa göre %40 daha uzun olmuştur. En yüksek hipokotil boyu, 10.51 cm ile kırmızı ışıkta ölçülmüştür. Hipokotil çapı yönünden en yüksek değer mor ışıkta belirlenmiştir. Biyokütle analiz sonuçlarına göre, beyaz ışık bitkilerin biyokütlesini artırmada en etkili ışık kaynağı olmuştur. Beyaz ışıkta beyaz turpların kuru ağırlığı 0.54 g olarak belirlenirken, bu değer sırasıyla kırmızı ışıkta 0.49 g, mavi ışıkta 0.42 g ve mor ışıkta 0.37 g olarak ölçülmüştür. Sonuç olarak, kırmızı ışığın hipokotil boyu ve biyokütle artışı açısından en yüksek etkiye sahip olduğu, mor ışığın ise hipokotil çapını en çok artıran ışık kaynağı olduğu belirlenmiştir. Beyaz ışık ise biyokütle artışını en çok teşvik eden spektrum olarak saptanmıştır. Bu bulgular, LED ışıklarının turp mikro filiz üretiminde verimli kullanımına yönelik önemli bilgiler sunmaktadır.

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ANAHTAR KELİMELER
Raphanus sativus L.
turp
mikro filiz
LED
kantitatif

Investigation of the Effect of Different Colors of LED Light on Vegetative Growth in Radish Micro Sprouts

ABSTRACT
In recent years, the commercial production of various plant species and microgreen varieties has rapidly expanded. This study aimed to investigate the effects of different LED light spectra on the vegetative growth of radish microgreens, a commonly used species in microgreen production, across different color segments (red, white, and black). The influence of LED light colors (white, blue, red, and purple) on morphological characteristics such as biomass, hypocotyl length, diameter, and coloration were quantitatively analyzed. Results showed that red light promoted the greatest hypocotyl elongation, with measurements 60% longer than under white light, 50% longer than under blue light, and 40% longer than under purple light. The highest hypocotyl length was recorded under red light at 10.51 cm. Hypocotyl diameter was greatest under purple light. Biomass analysis revealed that white light was the most effective spectrum in promoting plant biomass. For example, the dry weight of white radishes under white light was 0.54 g, followed by 0.49 g under red light, 0.42 g under blue light, and 0.37 g under purple light. In conclusion, red light had the strongest effect on hypocotyl elongation, while purple light was most effective in increasing diameter. White light, on the other hand, was most efficient in promoting overall biomass accumulation. These findings offer valuable insights for optimizing LED light use in the production of radish microgreens, contributing to more efficient and targeted growing strategies in controlled environments.

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Giriş

Günümüzde mikro filizler, sürdürülebilir gıda sistemlerinin çeşitlendirilmesi, insan sağlığının korunması ve hızla artan kent nüfusunun taze, küçük ölçekli sebzelere erişiminin sağlanması açısından önemli bir potansiyele sahip işlevsel gıda kaynakları olarak değerlendirilmektedir [1]. Mikro filizler, yüksek oranda mineral maddeler, vitaminler, lif ve fenolik bileşikler içermeleri nedeniyle fonksiyonel gıdalar arasında sınıflandırılmakta olup [2], bağışıklık sistemini güçlendirme, destekleme ve hastalıklara karşı koruma gibi sağlık açısından önemli faydalar sunmaktadır [3,4]. Mikro filizler, tohum embriyosundaki kotiledon yapraklarının gelişimini tamamlamasının ardından hasat edilen ve bazen bir adet gerçek yaprak oluşturduktan sonra tüketilen bitkisel ürünler olarak tanımlanmaktadır [5]. Mikro filizler, farklı Türkçe kaynaklarda “mikro yeşillikler”, “bebek yeşillikleri” veya “mikro sebzeler” olarak adlandırılmaktadır [6]. Mikro filizlerin büyüme döngüsü, yetiştirme uygulamalarına göre 7 ila 28 gün arasında değişebilmektedir. Topraksız yetiştirme ortamı ve ışık gereksinimi bulunmaktadır. Kimyasal kullanımı gerektirmese de yetiştirme ortamına bağlı olarak az miktarda besin ihtiyacı duyulabilmektedir. Hasat, yetiştirme ortamı ile hipokotil başlangıcı arasından kesilerek yapılmaktadır [7]. Küresel mikro filiz pazarında en çok tüketilen sebze türleri arasında brokoli, lahana, karnabahar, roka, bezelye, turp, fesleğen ve havuç bulunmakta; ayrıca pancar, ıspanak, soğan, pırasa, kereviz, maydanoz, marul ve şalgam gibi sebzeler de mikro filiz olarak değerlendirilmektedir [1].

Mikro filizlerin gıda olarak kullanımı, Uzak Doğu orijinlidir. Asya ülkelerinde uzun yıllardır terapötik ve sağlık amaçlı olarak kullanılan mikro filizler, Batı ülkelerinde ise daha yakın bir dönemde tanınmaya başlanmıştır [8]. Amerika Birleşik Devletleri’nde 1980’li yıllardan itibaren mikro filizler üretilmeye başlanmış; yoğun aromatik tatları, çıtır dokuları ve canlı renkleri nedeniyle salatalar, sandviçler ve çorbalar gibi gıdalarda kullanımı yaygınlaşmıştır [9]. Mikro filizlerin ticari üretimi, ilk kez 1990’ların ikinci yarısında Kaliforniya'nın güney kesiminde gerçekleştirilmiştir [7]. 2000’li yıllardan itibaren, sağlığı destekleyici fonksiyonel gıdalara olan ilginin artmasıyla birlikte mikro filizlerin üretim ve tüketiminde de belirgin bir artış gözlemlenmiştir [10].

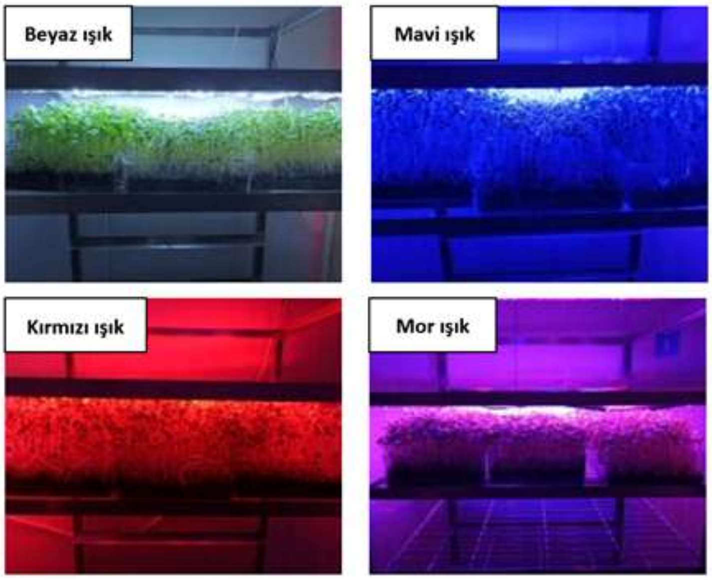
Günümüzde, çeşitli bitki türlerine ait çimlendirilmiş tohumlar ve mikro filizlerin ticari üretimi giderek yaygınlaşmaktadır. 2022 yılında küresel mikro filiz pazarının yaklaşık 1.8 milyar dolarlık bir değere ulaştığı belirtilmektedir. Pazar analizlerine göre, bu rakamın 2031 yılına kadar 2.6 milyar dolara çıkması beklenmektedir [11]. Organik sebze ve fonksiyonel gıdalara olan ilginin artmasıyla birlikte, en hızlı büyüme gösteren ticari pazar Asya-Pasifik bölgesi olmuştur. Çin ve Japonya, bu pazarda üretim ve tüketim açısından en büyük paya sahip ülkeler arasındadır. Türkiye’de ise çimlendirilmiş tohum ve mikro filiz pazarı henüz tam anlamıyla gelişmemiştir. Türkiye İstatistik Kurumu (TÜİK) tarafından bu ürünlerin ticari üretimi ve tüketimine yönelik resmi bir veri bulunmamaktadır. Ancak, bazı üreticiler özellikle büyük market zincirlerinde ve büyük şehirlerde mikro filizleri satışa sunmakta, ayrıca internet üzerinden de pazarlamaktadır [1]. Ülkemizde çimlendirilmiş tohumlar ve mikro filizlerle ilgili bilimsel çalışmalar ise oldukça sınırlı sayıdadır [12,13].

Işık, fotosentezde karbon fiksasyonu için gerekli enerjiyi sağlayan ve bitki büyümesi, morfolojisi gibi birçok fizyolojik süreci düzenleyen temel çevresel faktörlerden biridir. Işık yoğunluğu ve kalitesi, fotosentez hızını belirlemenin yanı sıra, bitkilerdeki organik bileşenlerin miktarını ve kalitesini de şekillendirmektedir. Ayrıca, sekonder bileşiklerin üretimi üzerinde de önemli bir rol oynamaktadır [14,15]. *Brassicaceae* (Lahanagiller) familyasına ait mikro filizlerde LED ışığının karotenoid içeriğine etkisini inceleyen bir araştırmada, kırmızı pak choi ve tatsoi türlerinin büyüme sürecinde LED ışığının spektral dalga boyu ve ışınım seviyeleri değerlendirilmiştir [14]. Çalışmada, yeşil ışığın karoten birikimini artırdığı, turuncu ışığın ise olumsuz bir etkiye sahip olduğu belirlenmiştir. Başka bir araştırmada ise brokoli, Brüksel lahanası, soya fasulyesi ve İran üçgülü tohumları, mikro filiz olarak tüketilmek üzere tamamen ışıklı, 12 saat ışık + 12 saat karanlık ve tamamen karanlık koşullarda, 20 ± 1°C sıcaklıkta 5 gün boyunca çimlendirilmiştir. Işığa maruz bırakılan filizlerin protein, şeker ve C vitamini içeriğinin, karanlıkta yetiştirilenlere kıyasla daha yüksek olduğu tespit edilmiştir [12].

LED ışık uygulamalarının mikro filizlerin besin değeri ve fitokimyasal bileşimi üzerinde önemli etkileri olduğu bilinmektedir. Karaağaç ve ark. [16] tarafından yürütülen “Bazı Yerel Çeşitlere Ait Tohumların Mikro Filiz Olarak Üretim Potansiyellerinin Ortaya Konulması, Yetiştirme Koşullarının Optimizasyonu ve Fonksiyonel Besin İçeriklerinin Belirlenmesi” başlıklı projede, farklı ışık tiplerinin, uygulama sürelerinin ve sıcaklık koşullarının yerel çeşitlerde mikro filiz üretimi ve besin kalitesi üzerindeki etkileri araştırılmıştır. Çalışma sonucunda, mikro filizlerde optimal hipokotil uzunluğunun mavi ve mavi + kırmızı LED ışık uygulamalarıyla sağlandığı belirlenmiştir. Demir ve ark. [17] ise LED ışıkların brokoli, lahana ve turp mikro filizlerinin büyüme süreci, besin içeriği ve glukozinolat bileşenleri üzerindeki etkisini incelemiş; LED ışık kaynaklarının ve dalga boylarının bitki türü, çeşidi, büyüme evresi ve çevresel faktörlere bağlı olarak farklı sonuçlar ortaya koyduğunu bildirmiştir. LED ışık spektrumlarının mikro filizlerin gelişimi ve besleyici özellikleri üzerindeki etkisinin tür ve çeşit bazında değişkenlik gösterdiği tespit edilmiştir. Bu araştırmada, mikro filiz üretiminde yaygın kullanılan turp bitkisinde, farklı ürün segmentlerinde (kırmızı, beyaz ve siyah) farklı renklerde LED ışık spektrumlarının vejetatif büyüme üzerindeki etkilerinin belirlenmesi amaçlanmıştır.

Materyal ve Metot

Araştırmada turp mikro filiz yetiştirme çalışmaları, Samsun Tohum Sertifikasyon ve Test Müdürlüğü çimlendirme laboratuvarında; vejetatif büyüme analizleri ise Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümü Fizyoloji Laboratuvarı'nda gerçekleştirilmiştir. Genetik materyal olarak denemede, farklı tohum firmalarından temin edilen kırmızı (Ovit-Tarmen Tohumculuk), beyaz (BT Burbeyaz - Bursa Tohum) ve siyah turp (BT Bursiyah - Bursa Tohum) ürün segmentlerine ait çeşitler kullanılmıştır.
Deneme kurulumundan önce, kullanılacak yetiştirme kaplarına (21x15x8 cm) ekilecek turp tohum miktarını tespit etmek amacıyla her bir çeşit için ISTA kurallarına uygun olarak kâğıt arası yöntemiyle (20°C'de 10 gün süreyle) 400 tohumda (100 tohum x 4 tekerrür) çimlendirme testi yapılmıştır [18]. Deneme öncesinde, tohumların başlangıç canlılığını belirlemek amacıyla yapılan ön denemede, üç turp çeşidinin tohum çimlenme oranlarının %90'nın üzerinde olduğu tespit edilmiştir. Buna göre, 21x15x8 cm ölçülerindeki yetiştirme kaplarına 5 g tohumun yeterli olduğu belirlenmiştir [16].
Mikro filiz üretimi için en yaygın kullanılan yetiştirme ortamları, ticari üretimde torf, perlit ve vermikülit gibi materyallerdir [7]. Denemede, bitki yetiştirme ortamı olarak 2 birim torf ve 1 birim perlit karışımı hazırlanmış ve her kap için 1 litre yetiştirme ortamı kullanılmıştır. Her bir yetiştirme ortamına tohumlar, hassas terazide tartılarak eşit bir şekilde dağıtılmış ve tohum ekimleri gerçekleştirilmiştir. Her bir yetiştirme kabı aynı eşit oranda sulanmıştır.
Araştırmada mikro filiz yetiştiriciliğinde, yapay ışıklandırma için 4 farklı renk (beyaz, mavi, kırmızı ve mor) LED lambaların kullanıldığı ışıklandırma sistemi kullanılmıştır (Şekil 1). Işık şiddetini stabil hale getirebilmek için yetiştirme ortamı paravanlarla izole edilmiştir. Deneme, faktöriyel deneme desenine göre 3 tekerrürlü olarak tekerrürde cm^2'de 3 bitki olacak şekilde kurulmuştur. Yetiştirme ortamına ekilen turp mikro filizleri, 20°C sabit sıcaklık ve 4 farklı LED ışıkta 10 gün süreyle büyütülmüştür. Yetiştirme ortamının yüzeyindeki ışık şiddeti, ışıkölçer (Apogee MQ-620) kullanılarak 120 µmol $m^{-2}s^{-1}$ olarak ayarlanmıştır (Şekil 1).



Şekil 1 Denemenin yürütüldüğü turp mikro filiz yetiştirme ortamının görünümü
Fig 1 The cultivation environment of radish microgreens where the experiment was conducted

Mikro filizler, ilk gerçek yaprağın ortaya çıkması aşamasında, ortam yüzeyinin 1-2 cm yukarısından makas yardımıyla hasat edilmiş [1] ve aşağıda belirtilen özellikler incelenmiştir.
Hipokotil boyu (cm): Hasat öncesi, milimetrik hassasiyetle bir cetvel yardımıyla hipokotil uzunlukları ölçülmüştür.

Hipokotil çapı (mm): Yetiştirme ortamının 1 cm üzerinden dijital kumpas yardımıyla ölçüm gerçekleştirilmiştir.
Yaprak Rengi: Yaprakların renk özellikleri, dijital renk analiz cihazı (kromametre) kullanılarak L, a, b, hue ve kroma değerleri üzerinden belirlenmiştir. Renklerin saptanması ve farklılık düzeylerinin ölçüm cihazları aracılığıyla ifade edilmesi amacıyla, Uluslararası Aydınlatma Komisyonu (CIE – International Commission on Illumination) tarafından geliştirilen ve "1976 CIE L*, a*, b*" ya da "CIE L a b üç eksenli ölçüm yöntemi" olarak adlandırılan teknik yaygın olarak tercih edilmektedir. Bu yöntemde, kromametre adı verilen dijital renk ölçüm cihazları ile numuneler üzerinden yapılan analizler sonucunda sayısal veriler elde edilmektedir. L* ışık yansıtma oranını, a* değeri kırmızı tonlarını, -a* yeşil tonlarını, b* sarı tonlarını, -b* ise mavi tonlarını göstermektedir [19]. CIE L a b renk sisteminde, a* ve b* eksenleri birbirine dik konumda yer almaktadır. L* ekseni ise açıklık-karanlık derecesini belirler ve a*-b* eksenlerinin oluşturduğu düzleme dik bir doğrultuda ilerler. Renk tonunun değişimini belirleyen açı "h" (hue) olarak adlandırılır ve h = 0° kırmızı, h = 90° sarı, h = 270° ise mavi renk tonunu ifade etmektedir. Belirli bir parlaklık seviyesinde (L* değeri) rengin doygunluğu, C* değeri (kroma) ile gösterilir. Bu yöntemde renkler, L*, a*, b* koordinatlarıyla veya L*, C* ve h değerleri kullanılarak tanımlanmaktadır. L* değeri, siyah ile beyaz arasındaki parlaklık seviyesini belirleyerek 0'dan 100'e kadar değişen sayısal değerlerle ifade edilmektedir [20,21].
Toplam bitki yaş ağırlığı (g)/yetiştirme kapı: Yetiştirme kaplarından (21x15x8 cm) hasat edilen mikro filizler, hassas terazi ile tartılmış ve yaş ağırlıkları 0.1 g hassasiyetiyle belirlenmiştir [22].
Toplam Bitki Kuru Ağırlığı (g): Bitkinin üst aksam kısımları, ayrı ayrı kese kâğıtlarına yerleştirilerek 60°C'deki etüvde 72 saat boyunca kurutulmuştur. Kurutma işleminin ardından, hassas terazi (0.001 g) kullanılarak kuru ağırlıklar belirlenmiştir [23].
Denemeden elde edilen veriler SPSS istatistik programında analiz edilmiştir. Faktörler arasındaki farklılıkları belirlemek için iki yönlü varyans analizi (ANOVA) uygulanmış ve gruplar arasındaki farklar Duncan'ın çoklu karşılaştırma testi ile %5 önem seviyesinde ($p<0.05$) değerlendirilmiştir.

Bulgular ve Tartışma

Hipokotil boyu üzerine ışık spektrumunun etkisi

Turp mikro filizlerinde ölçülen hipokotil boylarında, farklı LED ışık renklerinin istatistiksel olarak önemli düzeyde etkili olduğu tespit edilmiştir (Tablo 1). Mor (6.08 cm), beyaz (6.26 cm) ve mavi (6.91 cm) LED ışık altında tüm çeşitlerde hipokotil boyları istatistiksel olarak aynı grupta yer almıştır. Buna karşılık, kırmızı ışık altında yetiştirilen turp çeşitlerinde ortalama hipokotil boyu 10,51 cm'ye ulaşmıştır. Kırmızı ışığın, diğer ışık spektrumlarına kıyasla hipokotil boyunu yaklaşık %60 oranında artırdığı bulunmuştur. Turp mikro filizlerinde hipokotil boyunun uzun olması, hem verimi artırması hem de görsel olarak albenisinin yüksek olması nedeniyle arzu edilen bir özelliktir [1]. Ancak, mikro filiz üretiminde yetiştirme sıklığının ve ışık-sıcaklık koşullarının optimum değerlere ayarlanamaması durumunda, hipokotilin aşırı uzaması ve yere doğru kıvrılması ve yatması gibi olumsuz sonuçlar ortaya çıkabilmektedir.
Araştırmada farklı ışık koşulları altında turp çeşitleri arasındaki etkileşim incelendiğinde, çeşitlere göre hipokotil boylarının ışık türlerine bağlı olarak önemli düzeyde belirgin bir farklılık göstermediği belirlenmiştir (Tablo 1). Bu bulgular, Brazaityte ve ark. [14] tarafından yapılan bir çalışma ile uyumludur. Söz konusu çalışmada, kırmızı ışığın hipokotil uzamasını önemli ölçüde artırdığı, mavi ışığın ise daha kısa ve kompakt bitki yapısı oluşturduğu belirtilmiştir. Ayrıca, Samuoliene ve ark. [24] tarafından yapılan diğer bir araştırmada, kırmızı ışığın fotomorfogenezi (ışık etkisiyle bitkilerin morfolojik gelişim süreçleri) uyararak hipokotil uzamasını teşvik ettiği, mavi ışığın ise bu etkiyi baskıladığı bildirilmiştir. Bu sonuçlar, kırmızı ışığın mikro filizlerde hipokotil boyunu artırmada etkili olduğunu desteklemektedir. Benzer şekilde, Zhang ve ark. [25] tarafından yapılan diğer bir çalışmada, farklı ışık spektrumlarının (kırmızı, mavi, beyaz) farklı bitki türlerinde mikro filizlerin büyüme parametreleri üzerindeki etkileri incelenmiş ve kırmızı ışığın hipokotil uzamasını önemli ölçüde artırdığı, mavi ışığın ise daha kısa ve yoğun bitki yapısı oluşturduğu belirtilmiştir. Bu bulgular, kırmızı ışığın hipokotil boyu üzerindeki etkisini doğrulamaktadır.

Tablo 1 Farklı turp segmentlerinde hipokotil boyu (cm) üzerine ışık spektrumunun etkisi
Table 1 Effect of light spectrum on the hypocotyl length (cm) of different radish segments

Segment	Çeşitler	Mor Işık	Kırmızı Işık	Mavi Işık	Beyaz Işık	Ortalama
Siyah	BT Bursiyah	6.08	10.89	6.38	6.26	7.40
Beyaz	BT Burbeyaz	5.80	9.41	7.54	7.19	7.49
Kırmızı	Ovit	6.37	11.22	6.79	7.01	7.85
Ortalama		6.08 b	10.51 a	6.91 b	6.82 b	

* Aynı satır ve sütunda farklı harf taşıyan ortalamalar, %5 önem düzeyinde istatistiksel olarak farklıdır ($p<0.05$).

Hipokotil çapı üzerine ışık spektrumunun etkisi

Araştırmada üç farklı turp ürün grubunda (siyah, beyaz ve kırmızı) çeşitler arasında hipokotil çapı bakımından yapılan değerlendirmede, en iyi sonuçların mor ışık altında yetiştirilen siyah turp çeşitinde olduğu tespit edilmiştir (Tablo 2). Mor ışığın, diğer ışık kaynaklarına kıyasla siyah turpta daha güçlü bir hipokotil çapı oluşumunu teşvik ettiği belirlenmiştir. Araştırma sonucunda elde edilen bu bulgu, mor ışığın bitkilerde hücre duvarı genişlemesini teşvik ederek büyümeyi ve kütle üretimini artırdığını gösteren sonuçlar ortaya koymaktadır [26]. Ayrıca, mor ışığın etkisinin özellikle yüksek pigmentasyona sahip turp ürün segmentlerinde (örneğin siyah turpta) daha belirgin olduğu ve bu durumun hipokotil çapının artmasında etkili olabileceği bildirilmektedir [24,27].

Araştırmada üç farklı segmente ait turp çeşitleri üzerinde gerçekleştirilen iki yönlü varyans analizi, ışık kaynağı ile hipokotil çapı arasındaki etkileşimi incelemiştir. Analiz sonuçları, ışık kaynağı ile turp çeşitleri arasında hipokotil çapı bakımından istatistiksel olarak anlamlı bir etkileşim olmadığını ortaya koymuştur. Bu sonuç, hipokotil çapının ışık türüne bağlı olarak belirgin bir değişim göstermediğini ortaya koymuştur (Tablo 2). Elde edilen bulgular, mikro filizlerin büyümesi üzerinde ışık spektrumunun etkisinin çeşitlere göre değişkenlik gösterebileceğini ortaya koyan çalışmalarla benzerlik göstermiştir [28,29]. Yine benzer şekilde Garegnani ve ark. [30], ışık kaynağının bitkilerin büyüme parametreleri üzerinde belirgin bir değişiklik oluşturmadığını, ancak genetik çeşitliliğin önemli bir belirleyici etkisinin olduğunu vurgulamıştır.

Tablo 2 Farklı turp segmentlerinde ışık spektrumunun hipokotil çapı (mm) üzerine etkisi
Table 2 Effect of light spectrum on hypocotyl diameter (mm) in different radish segments

Segment	Çeşitler	Mor Işık	Kırmızı Işık	Mavi Işık	Beyaz Işık	Ortalama
Siyah	BT Bursiyah	1.27 a	1.15 ab	1.14 ab	0.96 c	1.13
Beyaz	BT Burbeyaz	1.10 b	1.13 b	1.05 bc	1.09 b	1.09
Kırmızı	Ovit	1.07 bc	1.08 bc	1.15 ab	1.10 b	1.10
Ortalama		1.15	1.12	1.11	1.052	

Turp mikro filizlerinde dijital renk ölçüm değerleri üzerine ışık spektrumunun etkisi

Araştırma sonuçları, farklı ışık kaynaklarının turp segmentlerinde mikro filizlerde dijital renk değerleri üzerinde belirgin değişikliklere neden olduğunu ortaya koymuştur. Işık spektrumunun bitkilerin renk gelişimi üzerindeki etkisi, L (aydınlık/açıklık), a (kırmızı-yeşil ekseni), b (sarı-mavi ekseni), Hue (renk tonu) ve Chroma (renk doygunluğu) değerleri ile ayrıntılı olarak aşağıda incelenmiştir (Tablo 3).

L değeri (aydınlık/açıklık)

Çalışmada genel olarak, ışık kaynakları arasında en yüksek L değerinin mor ışık (46.07) altında ortaya çıktığı gözlemlenmiştir. Bu sonuç, mor ışığın mikro filizlerde daha açık renk tonlarının gelişmesine neden olduğunu göstermiştir (Tablo 3). Kırmızı ışık altında, en düşük L değeri (42.07) kaydedilmiştir, bu sonuç bitkilerde daha koyu renklerin oluştuğunu ortaya koymuştur.

Araştırmada turp ürün gruplarına ait çeşitler birlikte karşılaştırma yapıldığında, beyaz turp çeşiti (46.75) ve kırmızı turp çeşiti (45.03) benzer L değerlerine sahipken, siyah turp çeşiti (41.03) ise en düşük değeri göstermiştir (Tablo 3). Bu bulgular, Zha ve Liu [31] tarafından yapılan bir çalışma ile uyum göstermiştir. Bu literatürde kırmızı ve mavi LED kombinasyonlarının bitkilerde açıklık değerlerini artırdığı ifade edilmiştir.

a değeri (kırmızı-yeşil ekseni)

Dijital renk ölçümü sonucunda negatif a değerleri, turpların genel olarak yeşil tonlarının baskın olduğunu göstermektedir. Siyah turp (-6.83) en düşük negatif a değerine sahipken, beyaz turp (-13.06) ve kırmızı turp (-10.76) mikro filizlerinin ise daha yüksek negatif değerlerde olduğu bulunmuştur (Tablo 3). Araştırma sonuçları, beyaz ve kırmızı turpların daha belirgin yeşil tonlara sahip olduğunu ortaya koymaktadır.

Turp mikro filizlerinde a değeri sonuçları, ışık kaynağı açısından incelendiğinde, mor ışık (-12.13) ve beyaz ışık (-10.51) en yüksek negatif a değerlerini göstermiştir (Tablo 3), bu da yeşil tonların daha belirgin hale geldiğini göstermiştir. Kong ve Zheng [32]'in yaptığı araştırmada mavi ışığın yeşil tonları artırdığını, kırmızı ışığın ise bu etkiyi azalttığı belirtilmiştir. Araştırma sonuçları belirtilen literatür ile uyum göstermiştir.

b değeri (sarı-mavi ekseni)

Dijital yaprak renk ölçümünde b değeri, bitkilerdeki sarı ve mavi tonların dengesini göstermektedir. Segmentler arasında beyaz turp (12.02) en yüksek b değerine sahip olduğu, en belirgin sarı tonlarını gösterdiği tespit edilmiştir (Tablo 3). Araştırmada kırmızı turp (9.91) ve siyah turpun (4.85) ise daha düşük b değerlerine sahip olduğu, siyah turpun daha az sarı ton içerdiği belirlenmiştir.

Çalışmada farklı ışık kaynaklarının etkisi incelendiğinde, mor ışık (10.78) ve beyaz ışık (9.71) en yüksek b değerlerini sağlamış ve bu ışıkların sarı tonlarını artırdığı tespit edilmiştir (Tablo 3). Brazaityte ve ark. [33]

tarafından yapılan bir çalışmada mor ışığın pigment sentezini teşvik ederek sarı tonları artırdığını bildirilmiştir. Bu sonuç, araştırma bulgularını destekler niteliktedir.

Hue (renk tonu)

Hue değeri, bitkinin genel renk tonunu belirlemektedir. Araştırma sonuçları, turp ürün segmentleri açısından bakıldığında, siyah turp mikro filizinin (149.79) en yüksek Hue değerine sahip bulunmuştur (Tablo 3). Beyaz turp (137.52) ve kırmızı turp (137.47) mikro filizlerinin ise daha düşük Hue değerlerine sahip olup birbirine benzer yakın sonuçlar sergilemiştir. Araştırma sonucunda ışık kaynağına göre en yüksek Hue değeri mavi ışık (148.29) altında ölçülmüştür (Tablo 3), bu bulgu mavi ışığın yeşil tonlarını artırdığını göstermektedir. Yorio ve ark. [34] tarafından yapılan bir çalışmada, mavi ışığın klorofil sentezini artırarak yeşil tonları öne çıkardığı bildirilmiştir.

Chroma (renk doygunluğu)

Chroma değeri, bitki renklerinin canlılık düzeyini yansıtmaktadır. Araştırmada en yüksek Chroma değeri beyaz turp (17.76) mikro filizinde belirlenmiştir (Tablo 3). Kırmızı turp mikro filizi (14.66) orta seviyede bir doygunluk sergilemiştir. Siyah turp mikro filizi (8.53) ise en düşük Chroma değerine sahip olup daha soluk renk tonları göstermiştir. Çalışmada ışık kaynakları açısından incelendiğinde, mor ışık (16.24) ve beyaz ışık (14.39) en yüksek Chroma değerlerini göstermiştir (Tablo 3). Pennisi ve ark. [35], beyaz LED'lerin renk doygunluğunu artırarak ticari üretimde tercih edildiğini vurgulamıştır.

Tablo 3 Farklı turp segmentlerinde dijital renk değerleri üzerine ışık spektrumunun etkisi
Table 3 Effect of light spectrum on digital color values in different radish segments

Renk Değeri	Segment	Çeşitler	Mor Işık	Kırmızı Işık	Mavi Işık	Beyaz Işık	Ortalama
L	Siyah	BT Bursiyah	44.88	39.25	39.14	40.86	41.03 b
	Beyaz	BT Burbeyaz	46.87	44.63	48.75	46.74	46.75 a
	Kırmızı	Ovit	46.44	42.32	45.62	45.75	45.03 a
	Ortalama		46.07 a	42.07 b	44.50 ab	44.45 ab	
a	Siyah	BT Bursiyah	-10.50	-6.05	-4.58	-6.18	-6.83 a
	Beyaz	BT Burbeyaz	-13.53	-10.91	-14.36	-13.44	-13.06 c
	Kırmızı	Ovit	-12.37	-8.18	-10.56	-11.93	-10.76 b
	Ortalama		-12.13 c	-8.38 a	-9.83 ab	-10.51 b	
b	Siyah	BT Bursiyah	9.00 ce	4.74 fg	1.21 h	4.44 gh	4.85 c
	Beyaz	BT Burbeyaz	12.25 ac	9.57 be	13.38 a	12.90 ab	12.02 a
	Kırmızı	Ovit	11.10 ae	7.98 ef	8.80 de	11.80 ad	9.91 b
	Ortalama		10.78 a	7.43 c	7.79 bc	9.71 ab	
Hue	Siyah	BT Bursiyah	139.52 bc	144.15 bc	167.15 a	148.35 b	149.79 a
	Beyaz	BT Burbeyaz	137.84 c	138.82 bc	137.14 c	136.28 c	137.52 b
	Kırmızı	Ovit	138.34 bc	135.64 c	140.57 bc	135.33 c	137.47 b
	Ortalama		138.57 b	139.54 b	148.29 a	139.99 b	
Chroma	Siyah	BT Bursiyah	13.83 cd	7.73 ef	4.77 f	7.78 ef	8.53 c
	Beyaz	BT Burbeyaz	18.25 ab	14.52 bcd	19.63 a	18.63 ab	17.76 a
	Kırmızı	Ovit	16.64 abc	11.43 de	13.79 cd	16.77 abc	14.66 b
	Ortalama		16.24 a	11.23 c	12.73 bc	14.39 ab	

Turp mikro filizlerinde bitki yaş ağırlığı üzerine ışık spektrumunun etkisi

Araştırma varyans analizi sonuçlarına göre, incelenen beyaz, kırmızı ve siyah turp segmentlerinde ışık kaynağının mikro filiz bitki yaş ağırlığı üzerinde istatistiksel olarak önemli düzeyde bir etkisinin olmadığı belirlenmiştir (Tablo 4). Basit ana etki analizi, bitki yaş ağırlığının spektral değişikliklerden anlamlı düzeyde etkilenmediğini göstermiştir ($p>0.05$). Bu bulgu, mikro filizler üzerine yapılan önceki araştırmalarla tutarlılık göstermektedir. Örneğin, Kopsell ve ark. [36], ışık türlerinin mikro filizlerin biyokütlesi üzerindeki etkilerinin çeşitlere göre değişkenlik gösterebileceğini, ancak genel olarak ışık türünün etkisinin sınırlı olabileceğini ifade etmiştir. Ayrıca, Jones-Baumgardt ve ark. [26] mikro filizlerde ışık türlerinin bitki biyokütlesi üzerindeki etkilerinin türler arası farklılıklar gösterdiğini ve beyaz ışığın biyokütleyi artırıcı etkiler sağladığını vurgulamaktadır. Bununla birlikte, araştırmada beyaz turp ve kırmızı turp mikro filiz bitkilerinin, uygulanan dört farklı ışık kaynağı (mor, kırmızı, mavi ve beyaz ışık) altında en yüksek bitki yaş ağırlığı değerlerine ulaştığı görülmektedir (Tablo 4). Bu sonuçlar, Zhang ve ark. [25] tarafından yapılan bir çalışma ile uyum göstermekte olup söz konusu çalışmada, beyaz ve kırmızı turp çeşitlerinin genel olarak daha yüksek biyokütle üretimine sahip olduğu ve farklı ışık koşullarına daha iyi adapte olabildiği bildirilmiştir.

Siyah turp çeşidinde mikro filizlerde en yüksek bitki yaş ağırlığı (190.5 g), diğer ışık uygulamalarına kıyasla kırmızı ışık altında yetiştirilen bitkilerde gözlemlenmiştir (Tablo 4). Bu sonuç, Samuoliene ve ark. [24] tarafından yapılan bir çalışma ile uyum göstermiştir. İlgili çalışmada, kırmızı ışığın özellikle siyah turp gibi pigmentasyonu yüksek türlerde biyokütle artışını teşvik ettiği belirtilmiştir. Ayrıca, kırmızı ışığın fotosentez verimliliğini artırarak bitki büyümesini desteklediği vurgulanmaktadır. Bu sonuç, kırmızı ışığın mikro filizlerin büyümesini artırmada etkili olduğunu belirten bir diğer çalışma ile de tutarlılık göstermektedir [27].

Tablo 4 Farklı turp segmentlerinde bitki yaş ağırlığı (g) üzerine ışık spektrumunun etkisi
Table 4 Effect of light spectrum on fresh plant weight (g) in different radish segments

Segment	Çeşitler	Mor Işık	Kırmızı Işık	Mavi Işık	Beyaz Işık	Ortalama
Siyah	BT Bursiyah	174.14	190.50	111.12	105.32	145.27 b
Beyaz	BT Burbeyaz	205.59	229.65	208.58	242.33	221.54 a
Kırmızı	Ovit	193.40	231.34	190.58	212.14	206.87 a
Ortalama		191.04	217.16	170.10	186.56	

Turp mikro filizlerinde bitki kuru ağırlığı üzerine ışık spektrumunun etkisi

Araştırma sonuçlarına göre, Farklı turp segmentlerine ait mikro filizlerde LED ışık uygulamalarının bitki kuru ağırlığı üzerindeki etkisi istatistiksel olarak anlamlı bulunmuştur ($p<0.05$; Tablo 5). Ortalama değerlere göre, mor (11.22 g) ve beyaz ışık (11.34 g) uygulamaları, bitki kuru ağırlığını artırmada kırmızı (9.33 g) ve mavi ışık (9.75 g) uygulamalarına kıyasla istatistiksel olarak anlamlı düzeyde daha yüksek değerler sağlamıştır. Bu bulgular, mor ve beyaz LED ışık spektrumlarının turp mikro filizlerinin biyokütle gelişimini desteklediğini göstermektedir.

Segment bazında değerlendirildiğinde, en yüksek ortalama kuru ağırlık 11.63 g ile beyaz turp (BT Burbeyaz) çeşidinde belirlenmiş ve bu segment, istatistiksel olarak diğer iki segmente göre anlamlı farklılık göstermiştir. Siyah (BT Bursiyah) ve kırmızı (Ovit) turp çeşitleri ise sırasıyla 9.47 g ve 10.14 g ortalama kuru ağırlık değerleri ile benzer istatistiksel grupta yer almıştır. Bu durum, çeşitler arasında ışık spektrumlarına verilen tepkilerin genotipik farklılıklara bağlı olarak değiştiğini ortaya koymaktadır.

Elde edilen bulgular, Liu ve ark. [37] ile Jones-Baumgardt ve ark. [26] tarafından yapılan çalışmalarla paralellik göstermekte olup, söz konusu çalışmalarda da mor ve beyaz ışığın mikro filizlerin biyokütle gelişimi üzerinde olumlu etkiler sağladığı belirtilmiştir.

Çalışmada ışık kaynakları açısından sonuçlar değerlendirildiğinde, mor (11.22 g) ve beyaz ışık (11.34 g) uygulamaları, kırmızı (9.33 g) ve mavi (9.75 g) ışık uygulamalarına kıyasla daha yüksek kuru ağırlık değerleri sağlamıştır (Tablo 5). Bu bulgular, ışık renginin bitki türüne bağlı olarak farklı etkiler gösterebileceğini ortaya koymaktadır. Yapılan bilimsel çalışmalarda özellikle mor ve beyaz ışık, mikro filizlerin hücresel gelişimini ve biyokütle üretimini teşvik ederken, kırmızı ve mavi ışığın daha az etki gösterdiği belirlenmiştir [26]. Araştırma sonuçları, bu bulguyu destekler nitelikte olmuştur.

Tablo 5 Farklı turp segmentlerinde bitki kuru ağırlığı (g) üzerine ışık spektrumunun etkisi
Table 5 Effect of light spectrum on dry plant weight (g) in different radish segments

Segment	Çeşitler	Mor Işık	Kırmızı Işık	Mavi Işık	Beyaz Işık	Ortalama
Siyah	BT Bursiyah	10.95	8.92	8.06	9.94	9.47 b
Beyaz	BT Burbeyaz	12.49	9.87	11.04	13.11	11.63 a
Kırmızı	Ovit	10.23	9.20	10.16	10.96	10.14 b
Ortalama		11.22 a	9.33 b	9.75 b	11.34 a	

Turp mikro filizlerinde bitki kuru ağırlığı üzerine ışık spektrumunun etkisi

Araştırma sonuçlarına göre, Farklı turp segmentlerine ait mikro filizlerde LED ışık uygulamalarının bitki kuru ağırlığı üzerindeki etkisi istatistiksel olarak anlamlı bulunmuştur ($p<0.05$; Tablo 5). Ortalama değerlere göre, mor (11.22 g) ve beyaz ışık (11.34 g) uygulamaları, bitki kuru ağırlığını artırmada kırmızı (9.33 g) ve mavi ışık (9.75 g) uygulamalarına kıyasla istatistiksel olarak anlamlı düzeyde daha yüksek değerler sağlamıştır. Bu bulgular, mor ve beyaz LED ışık spektrumlarının turp mikro filizlerinin biyokütle gelişimini desteklediğini göstermektedir.

Segment bazında değerlendirildiğinde, en yüksek ortalama kuru ağırlık 11.63 g ile beyaz turp (BT Burbeyaz) çeşidinde belirlenmiş ve bu segment, istatistiksel olarak diğer iki segmente göre anlamlı farklılık göstermiştir. Siyah (BT Bursiyah) ve kırmızı (Ovit) turp çeşitleri ise sırasıyla 9.47 g ve 10.14 g ortalama kuru ağırlık değerleri ile benzer istatistiksel grupta yer almıştır. Bu durum, çeşitler arasında ışık spektrumlarına verilen tepkilerin genotipik farklılıklara bağlı olarak değiştiğini ortaya koymaktadır.

Elde edilen bulgular, Liu ve ark. [37] ile Jones-Baumgardt ve ark. [26] tarafından yapılan çalışmalarla paralellik göstermekte olup, söz konusu çalışmalarda da mor ve beyaz ışığın mikro filizlerin biyokütle gelişimi üzerinde olumlu etkiler sağladığı belirtilmiştir.

Çalışmada ışık kaynakları açısından sonuçlar değerlendirildiğinde, mor (11.22 g) ve beyaz ışık (11.34 g) uygulamaları, kırmızı (9.33 g) ve mavi (9.75 g) ışık uygulamalarına kıyasla daha yüksek kuru ağırlık değerleri sağlamıştır (Tablo 5). Bu bulgular, ışık renginin bitki türüne bağlı olarak farklı etkiler gösterebileceğini ortaya koymaktadır. Yapılan bilimsel çalışmalarda özellikle mor ve beyaz ışık, mikro filizlerin hücresel gelişimini ve biyokütle üretimini teşvik ederken, kırmızı ve mavi ışığın daha az etki gösterdiği belirlenmiştir [26]. Araştırma sonuçları, bu bulguyu destekler nitelikte olmuştur.

Sonuçlar

Mikro filizler, yüksek besin değeri ve biyoaktif bileşen içeriğiyle fonksiyonel gıdalar arasında önemli bir yer tutmaktadır. Özellikle vitaminler, mineraller, lif ve fenolik bileşikler açısından zengin olmaları, bağışıklık sistemini destekleme, iltihaplanmayı azaltma ve kronik hastalıklara karşı koruyucu etki sağlama potansiyellerini artırmaktadır [2]. Örneğin, brokoli filizleri yüksek sülforafan içeriğiyle kanser riskini azaltıcı etkiler gösterebilirken, diğer mikro filiz türleri de farklı koruyucu bileşenleriyle sağlık üzerinde olumlu katkılar sunmaktadır [38]. Bu özellikleriyle mikro filizler, sağlıklı beslenmenin yanı sıra hastalıklardan korunmada da önemli bir besin kaynağı olarak değerlendirilmektedir.

Günümüzde birçok bitki türü ticari mikro filiz üretiminde kullanılmaktadır. Bunlar içerisinde özellikle Brassicaceae ve Amaranthaceae familyaları, bu üretimde yaygın olarak tercih edilen tür ve çeşitleri içermektedir [1,10,39]. Küresel mikro yeşillik pazarında en fazla yer bulan sebzeler arasında brokoli, lahana, karnabahar, roka, bezelye, turp, fesleğen ve havuç bulunmaktadır [11]. Türkiye'de ise çimlendirilmiş tohum ve mikro filiz pazarı henüz yeterince gelişmemiştir [1]. Mikro filiz konusu, toplumumuz için yeni bir alan olup, bu konuda yapılan bilimsel araştırmalar ve mevcut veri kaynakları oldukça sınırlıdır [12,13].

Bu araştırma ile farklı ürün segmentlerinde yer alan turp çeşitlerinde, LED ışık renklerinin vejetatif büyüme sürecine olan etkileri detaylı olarak incelenmiştir. Araştırma sonuçları, farklı ışık kaynaklarının üç turp segmentine ait çeşitlerde dijital renk özellikleri üzerindeki etkilerini inceleyerek, ışık spektrumlarının mikro filiz üretiminde renk gelişimini nasıl şekillendirdiğine dair önemli bulgular sunmaktadır. Elde edilen bulgular, ışık kaynağının turp mikro filizinde çeşitler arası farklılıklara bağlı olarak bitkilerin renk gelişimine nasıl etki gösterdiği ve bu bilgilerin hem bilimsel hem de ticari uygulamalara katkı sağlayabileceğini ortaya koymaktadır. Ayrıca elde edilen araştırma bulguları, ışık spektrumlarının turp mikro filiz üretiminde hedeflenen renk özelliklerine göre bilinçli bir şekilde kullanılabileceğini, bu sayede daha kaliteli mikro filizler elde edilebileceğini göstermektedir.

Beyaz turp mikro filizi genel olarak daha açık ve doygun renklere sahipken, siyah turp mikro filizi daha koyu renk tonları sergilemiştir. Kırmızı turp mikro filizi ise bu iki tür arasında renk tonu ve doygunluk açısından bir geçiş sunmuştur. Bu bulgular, çeşitlerin genetik özelliklerinin ışık spektrumlarına karşı farklı tepkiler verebileceğini ve bu tepkilerin renk gelişimini etkilediğini göstermiştir. Mor ve beyaz ışık, bitkilerde daha açık ve canlı renklerin gelişmesini sağlamış, özellikle yeşil ve sarı tonlarında artış gözlemlenmiştir. Kırmızı ışık, yeşil tonlarını azaltarak daha koyu ve doygun olmayan renklerin oluşmasına yol açarken, mavi ışık Hue değerinde artışa neden olarak daha belirgin yeşil tonlarının ortaya çıkmasını sağlamıştır. Bu sonuçlar, turp mikro filiz üretiminde ışık kaynaklarının, ürünlerin görsel kalitesini artırmak için dikkatle seçilmesi gerektiğini göstermektedir. Özellikle mor ve beyaz ışık uygulamalarının, ticari üretimde daha canlı ve estetik olarak çekici bitkiler elde edilmesini sağlayacağı tespit edilmiştir. Ayrıca, hedeflenen renk özelliklerine göre uygun ışık kaynağının seçilmesinin, tüketici tercihlerinin olumlu yönde şekillenmesine katkı sağlayacağı öngörülmektedir. Üreticilerin ise ışık kaynaklarının çeşitler arası farklı etkilerini göz önünde bulundurarak ticari pazarlarda rekabet avantajı elde edebilmesi söz konusudur.

Gelecekte yapılacak bilimsel çalışmalarla, ışık kaynaklarının pigment sentezi üzerindeki etkilerinin biyokimyasal analizlerle desteklenmesi, bu süreçlerin daha iyi anlaşılmasına katkı sağlayacaktır. Bu tür bilimsel araştırmalarla birlikte ışık spektrumlarının bitki büyümesi ve renk gelişimi üzerindeki etkileri diğer sebze türlerinde de ayrıntılı şekilde ele alınarak, ticari mikro filiz üretiminin verimliliğini artırmaya yönelik stratejilerin geliştirilmesine yardımcı olabilecektir.

Abbreviations/Kısaltmalar

LED: Light Emitting Diode/Işık Yayan Diyot, g: gram/gram, °C: degree Celsius/Santigrat derece, ISTA: International Seed Testing Association/Uluslararası Tohum Test Derneği, $\mu mol\ m^{-2}\ s^{-1}$: micromoles per square meter per second/metrekare saniyede mikromol (ışık yoğunluğu birimi), cm: centimeter/santimetre, mm: millimeter/milimetre

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Kaynaklar

1. Atasoy, S. and A. Balkaya, Sebze türlerinin çimlendirilmiş tohum ve mikro filiz olarak değerlendirilmesi, Sebze tohum üretimi ve teknolojisinde güncel ve yenilikçi uygulamalar, A. Balkaya and L. Arın, Editors. 2024, İksad Yayıncılık. Türkiye. p. 91–118.
2. Tuncer, B., Sebze mikroyeşillikleri: Brassicaceae familyası türleri, Ziraat, Orman ve Su Ürünlerinde Güncel Araştırmalar, T. Akar, İ. Daşdemir, and İ. Cengizler, Editors. 2022, Gece Kitaplığı. Türkiye. p. 65–88.
3. Delian, E., et al., Insights into microgreens physiology. Scientific Papers. Series B, Horticulture, 2015. 59: p. 447–454.
4. Xiao, Z., et al., Assessment of vitamin and carotenoid concentrations of emerging food products: Edible microgreens. Journal of Agricultural and Food Chemistry, 2012. 60: p. 7644–7651.
5. Çolak, C., Mikro yeşillikler (microgreens) nedir? Bebek yeşillikler ve sebze filizlerinden farkı nedir? Evrim Ağacı, 2020. Erişim Adresi: https://evrimagaci.org/mikro-yesillikler-microgreens-nedir-bebek-yesillikler-ve-sebze-filizlerinden-farki-nedir-8825
6. Işık, S., et al., Mikroyeşillikler: Besinsel içeriği, sağlık üzerine etkisi, üretimi ve gıda güvenliği. Gıda, 2022. 47(4): p. 630–649.
7. Di Gioia, F., C. Mininni, and P. Santamaria, Come coltivare microortaggi, Microgreens, F. Di Gioia and P. Santamaria, Editors. 2015, ECO-logica. Italy. p. 51–80.
8. Lobiuc, A., et al., Blue and red LED illumination improves growth and bioactive compounds contents in acyanic and cyanic *Ocimum basilicum* L. microgreens. Molecules, 2017. 22(12): p. 2111.
9. Kyriacou, M.C., et al., Functional quality in novel food sources: Genotypic variation in the nutritive and phytochemical composition of thirteen microgreens species. Food Chemistry, 2019. 277: p. 107–118.
10. Kyriacou, M.C., et al., Micro-scale vegetable production and the rise of microgreens. Trends in Food Science & Technology, 2016. 57: p. 103–115.
11. Anonim, Microgreens market. 2023, Straits Research. [Erişim Tarihi: 14 Ekim 2023]
12. Arın, L., et al., Bazı lahanagil (*Cruciferae*) ve baklagil (*Fabaceae*) türlerinin tohum filizlerindeki kimyasal özelliklerin değişimi ve beslenme değeri. Journal of Agricultural Sciences, 2014. 20(3): s. 230–238.
13. Orhun, G.E., and A. Levent, Determining the best sprouting conditions for germination of radish (*Raphanus sativus*) seeds consumed as vegetables. Journal of Food, Agriculture & Environment, 2008. 6(1): p. 123–127.
14. Brazaityte, A., et al., Effect of supplemental UV-A irradiation in solid-state lighting on the growth and phytochemical content of microgreens. International Agrophysics, 2015. 29(1): p. 13–22.
15. Murchie, E.H., and K.K. Niyogi, Manipulation of photoprotection to improve plant photosynthesis. Plant Physiology, 2011. 155(1): p. 86–92.
16. Karaağaç, O., et al., Bazı yerel çeşitlere ait tohumların mikrofiliz olarak üretim potansiyellerinin ortaya konulması, yetiştirme koşullarının optimizasyonu ve besin içeriklerinin belirlenmesi. BÜGEM Projesi, 2022.
17. Demir, K., G. Sarıkamış, and G.Ç. Seyrek, Effect of LED lights on the growth, nutritional quality and glucosinolate content of broccoli, cabbage and radish microgreens. Food Chemistry, 2023. 401: p. 134088.
18. International Seed Testing Association, ISTA rules: International rules for seed testing. 2021.
19. Batu, A., et al., Minolta ve Hunter renk ölçüm aletleri ile domates, elma ve muzun renk değerlerinin karşılaştırılması. Gıda, 1997. 22(4): p. 301–307.

20. Acar, K., Fluoresans Renkler İçeren Boyama Reçetesi Tahmin Algoritmalarında Başarının Artırılmasına Yönelik Yeni Bir Yöntem. Doktora Tezi. 2009, Marmara Üniversitesi, Fen Bilimleri Enstitüsü: İstanbul.
21. Bağcı, A., Soğan (*Allium cepa* L.) Genetik Kaynaklarının Morfolojik ve Moleküler Karakterizasyonu. Yüksek Lisans Tezi. 2023, Ondokuz Mayıs Üniversitesi, Lisansüstü Eğitim Enstitüsü: Samsun.
22. Kim, J., H.S. Lee, and S. Park, Biomass accumulation under different light environments. Journal of Plant Growth Regulation, 2015. 62(1): p. 45–52.
23. Kandemir, D., et al., Lahanagiller familyasına ait bazı sebze türlerinin vejetatif büyüme özelliklerinin incelenmesi. Black Sea Journal of Engineering and Science, 2023. 6(4): p. 624–632.
24. Samuoliene, G., et al., LED lighting and seasonality effects antioxidant properties of baby leaf lettuce. Food Chemistry, 2012. 134(3): p. 1494–1499.
25. Zhang, X., et al., A review on the effects of light-emitting diode (LED) light on the nutrients of sprouts and microgreens. Trends in Food Science & Technology, 2020. 99: p. 193–204.
26. Jones-Baumgardt, et al., The growth and morphology of microgreens is associated with modified ascorbate and anthocyanin profiles in response to the intensity of sole-source light-emitting diodes. Canadian Journal of Plant Science, 2021. 101(2): p. 212–228.
27. Tuan, P.A., et al., Effects of white, blue, and red light-emitting diodes on carotenoid biosynthetic gene expression levels and carotenoid accumulation in sprouts of tartary buckwheat (*Fagopyrum tataricum* Gaertn.). Journal of Agricultural and Food Chemistry, 2013. 61(50): p. 12356–12361.
28. Choe, U., L.L. Yu, and T.T. Wang, The science behind microgreens as an exciting new food for the 21st century. Journal of Agricultural and Food Chemistry, 2018. 66(44): p. 11519–11530.
29. Kurina, A.B., et al., Genetic diversity of phenotypic and biochemical traits in VIR radish (*Raphanus sativus* L.) germplasm collection. Plants, 2021. 10(9): p. 1799.
30. Garegnani, M., et al., Non-destructive real-time analysis of plant metabolite accumulation in radish microgreens under different LED light recipes. Frontiers in Plant Science, 2024. 14: p. 1289208.
31. Zha, L., and W. Liu, Effects of light quality, light intensity, and photoperiod on growth and yield of cherry radish grown under red plus blue LEDs. Horticulture, Environment and Biotechnology, 2018. 59: p. 511–518.
32. Kong, Y., and Y. Zheng, Phototropin is partly involved in blue-light-mediated stem elongation, flower initiation, and leaf expansion: A comparison of phenotypic responses between wild Arabidopsis and its phototropin mutants. Environmental and Experimental Botany, 2020. 171: p. 103967.
33. Brazaityte, A., et al., Effect of different ratios of blue and red LED light on *Brassicaceae* microgreens under a controlled environment. Plants, 2021. 10(4): p. 801.
34. Yorio, N.C., et al., Improving spinach, radish, and lettuce growth under red LEDs with blue light supplementation. HortScience, 2001. 36: p. 380–383.
35. Pennisi, G., et al., Modelling environmental burdens of indoor-grown vegetables and herbs as affected by red and blue LED lighting. Sustainability, 2019. 11(15): p. 4063.
36. Kopsell, D.A., et al., Shoot tissue pigment levels increase in ‘Florida Broadleaf’ mustard (*Brassica juncea* L.) microgreens following high light treatment. Scientia Horticulturae, 2012. 140: p. 96–99.
37. Liu, H., et al., The influence of light-emitting diodes on the phenolic compounds and antioxidant activities in pea sprouts. Journal of Functional Foods, 2016. 25: p. 459–465.
38. Fahey, J.W., Y. Zhang, and P. Talalay, Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proceedings of the National Academy of Sciences, 1997. 94(19): p. 10367–10372.
39. Xiao, Z., et al., Microgreens of *Brassicaceae*: Mineral composition and content of 30 varieties. Journal of Food Composition and Analysis, 2016. 49: p. 87–93.

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Odunsu Bitkilerin İstilasına Maruz Kalan Bir Meranın Islahı İçin Öneriler: Hacıosmanoğlu Köyü Merası Örneği, Bartın

Ferat Uzun[1]

ÖZET
Bartın ili, Hacıosmanoğlu köyünün merasında 2024 tarihinde yürütülmüş olan bu çalışmanın amacı, meranın verimliliğini artırmaya yönelik yapılabilecek amenajman ve ıslah çalışmaları için gerekli verilerin elde edilmesidir. Mera vejetasyonunda yer alan türleri belirlemede tekerlekli lup metodu kullanılmıştır. Meranın bazı kısımları düz, bazı kısımları ise yer yer % 3 ila 5 arasında değişen eğim derecesine sahiptir. Toprağın bitki ile kaplılık oranının ise % 100 olduğu belirlenmiştir. Vejetasyonda tespit edilen azalıcı, çoğalıcı ve istilacı bitki türlerinin toplam oranları sırasıyla % 1.12, % 5.32 ve % 94.68'dir. Bu verilere göre mera durumu "Zayıf" sınıftadır. Toprak yüzeyinin % 89.40'ının çalı ve ağaçlar, kalan % 10.60'ının ise otsu bitkiler ile örtülü olduğu görülmüştür. Köyde uzun yıllardır çoğunluğu simental ırk başta olmak üzere sadece büyükbaş hayvan yetiştiriciliği yapılmaktadır. Otlamada çalı-ağaç türlerini öncelikle tercih eden keçi yetiştiriciliğinin hiç olmaması, mera bitki örtüsündeki otsu türlerin azalması, odunsu türlerin ise artmasına sebebiyet verdiği söylenilebilir. İncelenen birçok araştırma sonucundan elde edilen verilere ve literatüre göre, bu meranın bitki örtüsü tamamen yenilenmelidir. Mevcut bitki örtüsü uzaklaştırıldıktan sonra alanın yapay mera için hazırlanması amacıyla en az 2 yıl süreyle ön bitki tarımı yapılmalıdır. Üçüncü yıl çok yıllık yem bitkileri ile oluşturulacak yeni vejetasyon için, merada görülen *Lotus corniculatus, Lotus tenuis, Trifolium repens, Dactylis glomerata, Poa pratensis)* ve onlar ile benzer ekolojik isteklere sahip olan *Lolium perenne ve Festuca pratensis* gibi türler kullanılabilir.

MAKALE GEÇMİŞİ
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ANAHTAR KELİMELER
Meralarda bozulma,
mera durumu,
mera gübrelemesi,
mera ıslahı,
vejetasyon yenileme

The Recommendations for Rehabilitation of the Rangeland Exposed to Woody Plant Encroachment: The Case of Hacıosmanoğlu Rangeland, Bartın

ABSTRACT
The aim of this study, which was carried out in the rangeland of Hacıosmanoğlu village, Bartın province, in 2024, was to obtain the necessary data for management and improvement studies to increase the productivity of the rangeland. Wheel loop method was used to determine the species in the rangeland vegetation. Some parts of the rangeland were flat, while some parts had a slope degree varying between 3 and 5%. The rate of soil cover with plants was 100%. The total rates of decreaser, increaser and invader plant species detected in the vegetation were 1.12%, 5.32% and 94.68%, respectively. According to these data, the rangeland was in the poor condition class. It was observed that 89.40% of the soil surface was covered with bushes and trees, and the remaining 10.60% was covered with herbaceous plants. Only cattle breeding has been carried out in the village for long years, mostly Simmental breeds. It can be said that the absence of goat breeding, which primarily prefers shrub-tree species in grazing caused the herbaceous species in the rangeland vegetation to decrease rapidly and the woody species to increase. According to the data and literatures were collected from many research results reviewed, the vegetation of this rangeland should be completely renewed. After the existing vegetation is removed, pre-crop should be cultivated to control weeds at least 2 years in order to improve the area for pasture. For the new vegetation to be created with perennial forage plants in the third year, species such as *Lotus corniculatus, Lotus tenuis, Trifolium repens, Dactylis glomerata, Poa pratensis* seen in the rangeland and *Medicago sativa, Lolium perenne* and *Festuca pratensis*, which have similar ecological requirements, should be used.

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Giriş

Verim ve kalite anlamında mera vejetasyonlarının zirve noktası olarak tanımlanan klimaks bitki varlığından en yüksek oranda faydalanma yoluna gidilirken, vejetasyonun muhafazası da ihmal edilmemesi gereken en öncelikli konudur. Hayvanların meralardan besin maddeleri ihtiyaçlarını karşılama oranları, mera bitki örtüsünün klimaks yapılarını koruma derecesiyle birebir ilgilidir. Bu nedenle mera vejetasyonları sürekli takip edilmeli, herhangi bir sebeple meydana gelebilecek olumsuzluklar erkenden tespit edilmeli ve yapılacak doğru müdahaleler ile mera vejetasyonlarının kalitesi muhafaza edilmelidir. Bu bakımdan üreticinin bir gözü hayvanda ise bir gözü de meradaki bitkilerde olmalıdır. Mera vejetasyonlarının üretkenliğini ve kalitesinde azalmaya neden olabilecek zamansız ve ağır otlatma, yangınlar ve kuraklıklar gibi birçok sebepler mevcuttur. Bunlardan biri de sadece ekonomik getirileri dikkate alınarak meraların tek tür hayvan ile otlatılmasıdır. Başta sığırlar olmak üzere tek bir hayvan türü ile otlatılan meralarda hayvanların doğal fıtratlarına bağlı olarak bazı bitki türleri tercihen daha fazla otlanılmaktadır. Normal hayat döngülerine daha fazla müdahale edilmesi anlamına gelen bu otlama şeklinin devamlılık göstermesi, bu türlerin daha fazla yıpranması ve vejetasyondan çekilmesi ile son bulmaktadır. Vejetasyondan çekilen bu bitkilerin yerini öncelikle vejetasyonda yer alan, ancak hayvanlar tarafından ikinci derecede tercih edilen veya otlatmaya daha dayanıklı olan diğer klimaks türler ve akabinde de istilacı türler doldurmaktadır [1]. Dünya genelinde son 100-150 yıldır bu tür değişimlerden biri de otsu mera bitkilerinin, odunsu türler ile yer değiştirmesi olarak ifade edilmektedir [2, 3]. Bu değişimin en önemli sebeplerinden birisi, meraların otlatılmaması [4] veya kapasitelerinin altında otlatılması [5], diğeri meralardaki çalı ve diğer odunsu bitki türlerini tercihen otlayan keçilerin azalmasıdır [6, 7]. Bartın ilinin 15 farklı köy merasında yapılan çalışma verilerine göre tüm köylerde büyükbaş hayvan mevcutken, sadece 5 köyde küçükbaş hayvan olduğu ve hayvan birimi olarak ta toplam varlığın sadece % 1.16'sını oluşturduğu ifade edilmiştir [8]. Zonguldak ilinde yapılan diğer bir çalışmada, meralarda tüm hayvan türlerinin olması gerektiği, aksi takdirde meralardaki otsu türlerin hızla çalı-ağaç topluluklarına evrilmesine katkı sunulacağına dikkat çekilmiştir [9]. Bartın il genelinde gerçekleşen yıllık yağış miktarının da görece fazla olması, doğal bitki süksesyonunun odunsu bitkilere doğru dönüşümünü hızlandırdığı söylenilebilir [10]. Sonuç itibarıyla bitki örtüsündeki odunsular lehine meydana gelen bu dönüşüm, klimaks meradan uzaklaşmayı ifade etmekte, vejetasyondaki tür sayısı azalmakta [11] ve özellikle büyükbaş hayvanların meralardan faydalanmasını azaltmaktadır. İfade edilen dönüşüm, diğer yandan Orman Müdürlüğü'nün mera olarak tescilli de olsa bu alanları orman kayıtlarına alma gibi bir isteği ortaya çıkarmakta, akabinde bu istek hukuksal mücadeleye evrilebilmekte ve neticede meralarda alan kayıplarına sebep olabilmektedir [12, 13].

Bu çalışma ile bitki örtüsü içerindeki odunsu bitkilerin zaman içerisinde arttığı gözlemlenen Hacıosmanoğlu köyü merasının, bitki örtüsü ve dolayısıyla mera durumunun belirlenerek mera üretkenliğini artırma çalışmaları için gerekli verilerin elde edilmesi amaçlanmıştır.

Materyal ve Metot

Çalışma, Bartın ili, Merkez ilçe, Hacıosmanoğlu köyünün 43122.36 da'lık alana sahip 102 ada, 8 numaralı mera parselinde 25.06.2024 tarihinde yapılmıştır. Hacıosmanoğlu köyünün 10 parselde, büyüklükleri 6.34 ile 43.12 da arasında değişen toplam 144.20 da tahsisli merası mevcuttur. Diğer parsellerin bitki örtüsü de çalışılan parsel ile oldukça benzerdir.

Hacıosmanoğlu köyünün hayvan varlığı, 2025 yılı itibarıyla 2'si yerli ve geri kalan 324'ü büyük çoğunluğu simental olmak üzere tamamı kültür ırkı büyükbaş hayvanlardan oluşmaktadır [14]. Tarım ve Orman Bakanlığı'nın 2021 yılı kayıtları [15] ve köy sakinlerinin ifadelerine göre geriye dönük 15 yıllık süreçte köyde küçükbaş hayvan mevcut değildir.

Çalışılan mera parselinin Tapu ve Kadastro Genel Müdürlüğü'nün Parsel Sorgu uygulamasından elde edilen bilgileri Şekil 1'de [16], QGİS programı ile elde edilen harita çıktısı ise Şekil 2'de görülmektedir.

TAPU VE KADASTRO GENEL MÜDÜRLÜĞÜ

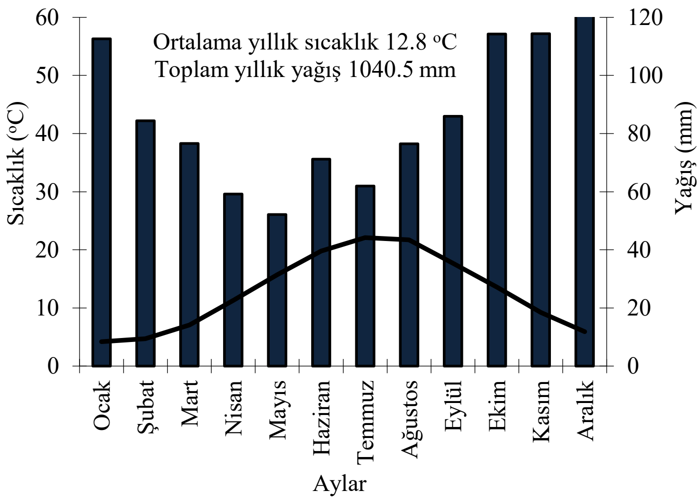
PARSEL SORGULAMA BİLGİLERİ

İl	İlçe				Mahalle
Bartın	Merkez				Hacıosmanoğlu
Ada	Parsel	Tapu Alanı (m2)	Nitelik	Mevki	Pafta
102	8	43.122,36	Mera	-	E28-D-24-A-2-B

Şekil 1 Hacıosmanoğlu köyü 102/8 numaralı mera parseli bilgileri
Fig 1 Information about rangeland parcel number 102/8 in Hacıosmanoğlu village

Şekil 2 Mera parselinin uydu görüntüsü
Fig 2 Satellite image of the rangeland parcel

Tekerlekli lup yöntemiyle yapılan vejetasyon etüdü çalışmasında "yaprak alanı" esas alınarak kuzey-güney istikametinde tüm alanı en iyi temsil edecek şekilde yaklaşık 433 m uzunluğa sahip hatta 1 m arayla 400 okuma yapılmıştır. Bitki teşhislerinde Serin [17]'den faydalanılmıştır. Tespit edilen bitki türleri azalıcı, çoğalıcı ve istilacılar olarak sınıflandırılmışlardır. Azalıcılar ve çoğalıcıların toplam oranı dikkate alınarak mera durumu belirlenmiştir. Bitki örtüsünün toprağı kaplama oranı, vejetasyon etüdü sırasında bitkiye rastlanan nokta sayısının ölçülen toplam nokta sayısına oranlanması ile belirlenmiştir. Okunan her bir bitki türüne ait değerler, toplam bitki sayısına oranlanarak türlerin vejetasyondaki oranları tespit edilmiştir. Mera durumunun belirlenmesinde Holechek ve ark. [18]'dan yararlanılmıştır (Tablo 1).
Karadeniz Bölgesi'nde yer alan ve tipik deniz iklimi hakim olan Bartın'da uzun yıllara ait (1950-2023) ortalama yıllık yağış toplamı 1040.5 mm, ortalama sıcaklık ise 12.8 ºC'dir [10]; Şekil 3). Thornthwaite iklim sınıflandırmasına göre Bartın, nemli-mezotermal iklim sınıfına girmektedir [19]. İklim diyagramından da anlaşılacağı üzere, yağışın yıl içerisindeki dağılımı, ülkemizin diğer birçok iline göre daha düzenlidir. En kurak aylarda bile yağış miktarı 50 mm'nin üzerindedir. Ancak, sıcaklık değerlerinin daha yüksek olduğu yaz aylarında gerçekleşen yağış miktarları, yılın en düşük değerlerine sahiptir. Bu nedenle tüm ülkemiz genelinde olduğu gibi bölgedeki meraların ana kaba yem kaynağı olan serin iklim yem bitkileri türlerinin bu dönemde üretkenlikleri azalmaktadır.



Şekil 3 Bartın ilinin uzun yıllara ait sıcaklık (─) ve yağış (▌) diyagramı
Fig 3 Long-term temperature (─) and precipitation (▌) diagram of Bartın province

Sonuçlar ve Tartışma

Yapılan etüt çalışmasından elde edilen verilere göre, meranın bazı kısımları düz, bazı kısımlarıysa yer yer % 3 ila 5 arasında bir eğime sahiptir. Bu eğim dereceleri her türlü hayvanın bu alanda rahatlıkla otlayabileceği anlamına gelmektedir [20]. Mera yüzeyinin tamamının bitki ile örtülü olduğu belirlenmiştir. Mera bitki varlığının % 89.40'ını çalı ve ağaçlar, % 10.60'lık kısmını ise otsu bitkiler oluşturmuştur. Otsu bitkilerin yoğun olduğu kısımda futbol kaleleri dikkat çekmiş, bu alanda futbol oynamak için çalı-ağaç temizliği yapıldığı kanısına varılmıştır. Alanın bitki örtüsüne ait ayrıntılı bilgiler Tablo 1 ve 2'de verilmiştir.

Tablo 1 Azalıcı ve çoğalıcı bitki türlerinin mera vejetasyonundaki oranları
Table 1 The ratios of decreaser and increaser plant species in the rangeland vegetation

Türler /Species	Oran/Ratio (%)	Türler/Species	Oran/Ratio (%)
Azalıcılar/Decreaser			
Lotus corniculatus L.	0.41	*Poa pratensis* L.	0.10
Lotus tenuis Waldst. & Kit.	0.21	*Sanguiosorba minor* Scop.	0.06
Paspalum distichum L.	0.20	*Trifolium pratense* L.	0.01
Dactylis glomerata L.	0.12	*Trifolium repens* L.	0.01
Toplam / Total			1.12
Çoğalıcılar / Increaser			
Brachypodium pinnatum L.	2.07	*Plantago lanceolata* L.	0.06
Dorycnium graecum L.	2.07		
Toplam / Total			4.20
Genel toplam / Grand total			5.32

Tablo 1 ve 2'de verilen bilgilere göre mera bitki örtüsü içerisindeki kaliteli bitkileri oluşturan, azalıcı ve çoğalıcı bitki türleri sırasıyla % 1.12 ve % 4.20 olmak üzere toplamda % 5.32'lik bir orana sahiptir. Buna göre parselin mera durumu "Zayıf" sınıfta yer almaktadır. Hacıosmanoğlu köyündeki hayvan varlığının tamamının büyükbaş hayvanlardan oluşmasına binaen meranın mevcut ot üretim potansiyelinden de faydalanılamadığı söylenilebilir. Küçükbaş hayvan yetiştiriciliğine olan ilgi, Bartın'ın diğer birçok köyünde de oldukça düşüktür. Bartın'ın 15 farklı köyünde yapılan çalışma kapsamında Bartın İl Tarım ve Orman Müdürlüğü'nden temin edilen verilere göre, tüm köylerde büyükbaş hayvan mevcutken, sadece 5 köyde küçükbaş hayvan olduğu ve hayvan birimi olarak ta toplam varlığın sadece % 1.16'sını oluşturduğu ifade edilmiştir [8]. Bilindiği üzere her hayvanın fıtri özelliklerinden mütevellid, gerek otlama şekli ve gerekse bitkisel tercih anlamında farklılıklar vardır. Bu bakımdan koyun, keçi, manda ve sığırlardan oluşan karma bir hayvan varlığı meralardan en yüksek oranda faydalanma ve bitki tür dengesinin istikrarı bakımından çok önemlidir. Bu bakımdan her ne kadar kaliteli bitkiler sınıfına girseler de vejetasyonda zaten çok düşük oranda bulunan *Paspalum distichum* ve *Plantago lanceolata* gibi kısa boylu bitki türlerini sığırların otlayabilme kabiliyeti de sınırlıdır.

Tablo 2 İstilacı bitki türlerinin botanik kompozisyondaki oranları
Table 2 The ratios of invasive plant species in the rangeland vegetation

Türler /Species	Oran/Ratio (%)	Türler /Species	Oran/Ratio (%)
Otsu istilacı türler / Herbaceous invasive species			
Carex flacca Schreb.	1.04	*Trifolium setiforum* Boiss.	0.02
Astrodaucus orientalis L.	0.10	*Convolvulus lineatus* L.	0.02
Dryopteris filix-mas L.	1.04	*Trifolium subterraneum* L.	0.06
Medicago polymorpha L.	0.10	*Dryopteris filix-mas* (L.) Schott.	1.04
Crepis vesicaria L.	0.06	*Iris sintenisii* Janka.	0.01
Oenanthe pimpinelloides L.	0.01	*Centaurea virgata* Lam.	0.06
Euphorbia helioscopia L.	0.04	*Scabiosa crinita* Kotschy&Boiss.	0.06
Taraxacum aleppicum Dahlst.	0.02	*Galega officinalis* L.	0.62
Centaurium erythraea Rafn.	0.02	*Galium verum* L.	0.62
Trifolium dubium Sibth.	0.12	*Plantago lagopus* L.	0.04
Cerinthe minor L.	0.01	*Anthemis cretica* L.	0.05
Mentha longifolia L.	0.04	*Prunella vulgaris* L.	0.04
Polygala major Jacq	0.02	*Clinopodium vulgare* L.	0.02
Toplam / Total			5.28
Odunsu istilacı bitkiler / Woody invasive plants			
Rubus discolour Weihe&Ness	25.20	*Smilax excelsa* L.	6.30
Cornus nuttallii Audubon.	13.50	*Crataegus orientalis* M. Bieb	5.40
Carpinus betulus L.	13.50	*Genista lydia* Boiss.	3.60
Rosa canina Bechst.	10.80	*Laurus nobilis* L.	1.00
Prunus spinosa L.	10.10		
Toplam / Total			89.40
İstilacı toplamı / Total invasive plants			94.68

Çalı ve ağaç türü bitkiler de özellikle kültür ırkı sığırların faydalanabileceği bitkiler değildir. Hâlbuki bu merada çalı ve ağaç türü istilacıların % 25.20'sini oluşturan *Rubus discolor*, % 10.80'ini oluşturan *Rosa canina* ve % 3.60'ını oluşturan *Genista lydia*, keçilerin yüksek derecede lezzetli buldukları ve tercihen otladıkları bitkilerden oldukları ifade edilmektedir [21]. Diğer bir araştırıcı ise merada % 10.10 oranında bulunan *Prunus spinosa* çalısının taze yapraklarının ve filizlerinin keçiler tarafından tercih edildiğini belirtmiştir [22]. Diğer yandan Almanya'nın orta bölgesinde yapılan çalışmada içlerinde *Crataegus* ve *Rosa* türlerinin de bulunduğu çalıların istila ettiği meralarda keçilerle otlatmanın meralardaki çalı varlığını % 69.8'den 37.4'e düşürdüğünü [24], diğer bir araştırma sonuçları ise odunsu bitkilerin kontrolünde diğer yöntemlere kıyasla keçi otlatmanın maliyet açısından daha uygun ve sürdürülebilir olduğunu işaret etmiştir [25]. Bu bilgilerden hareketle köyde yeterince keçi olmuş olsaydı hem çalı-ağaç türü istilacılar otlanılmak suretiyle baskı altına alındığı için merayı bu derecede istila edemez ve hem de bu bitkiler kaba yem olarak hayvansal ürünlere dönüşebilirdi. Sonuç itibarıyla otlamada çalı-ağaç türlerini öncelikle tercih eden keçi yetiştiriciliğinin hiç olmaması ve aldığı yıllık yağışın bitki süksesyonu sistematiği içinde odunsu bitkiler safhasına geçişini hızlandırması nedeniyle Hacıosmanoğlu köyünün mera vejetasyonundaki otsu bitki türlerin azaldığı, odunsuların ise arttığı söylenilebilir.

Öneriler

1. Çalı-ağaç ve otsu istilacı yabancı bitki türlerinin meradan temizliği

Hacıosmanoğlu köyünün vejetasyon etüdü yapılan 102/8 ada/parsel numaralı alanın bitki örtüsü içerisindeki odunsu türlerin oranı % 89.40'dır. Bu oran, meranın amenajman düzenlemeleri ve bazı bakım çalışmaları ile ıslah edilemeyecek derecede yabancı bitkilerin istilası ile karşı karşıya olduğunun ifadesidir. Bu nedenle odunsu ve otsu istilacı bitki türlerinin alandan uzaklaştırılması gerekmektedir. Teksas meralarında odunsu bitki türleri ile mücadelede genel olarak mekanik, Oklahoma'da mekanik ve kimyasal, Kansas'ta ise kontrollü yakma yöntemlerinin kullanıldığını fakat uzun vadede ağaç varlığını hiçbir yöntemin azaltmadığını, çalı varlığını azaltmada ise minimum fayda sağlandığı ifade edilmiştir [26]. Bu noktada odunsu bitkileri tercihen otlamak suretiyle sürekli baskılayabilen keçilerin meralarda bulundurulmasını sağlayacak tarımsal politikalar geliştirilmesi faydalı olacaktır [7, 27].

2. Otlatmayı kolaylaştırıcı yapılar

Gölgelik: Vejetasyonda yer alan gürgen (*Carpinus* sp.) ve yabani erik (*Prunus* sp.) gibi bazı ağaçlar kesilmeyip, sıcak yaz günlerinde gölgelik amaçlı kullanılabilir. Yapılan bir çalışmada sıcak yaz günlerinde gölgelikli meralarda sıcaktan korunan hayvanların daha verimli olduğunu ifade edilmiştir [28]. Aynı araştırıcılara göre, hayvanlar genellikle yapay gölgeliklerden ziyade canlı ağaçların altlarını tercih etmektedir. Çünkü ağaçlar altlarında daha iyi hava akışına olanak sağlamakta ve yaprakları vasıtasıyla evapotranspirasyon yaptıkları için de ortamı serinletici etkide bulunmaktadırlar. Ağaç gölgelikler ayrıca az da olsa meradaki biyolojik çeşitliliği desteklemek için ek yaşam alanı oluşturmakta ve hatta hayvanlar için gerektiğinde yaprakları ve meyvelerinden ek besin kaynağı olarak faydalanılmaktadır [29].

İçme suyu tesisi (Sıvat): Mera parselinin en uzun mesafesi olan kuzey-güney uçları arasındaki mesafe 400 m kadardır. Bu nedenle meranın ortasına yapılacak bir adet sıvat, otlayan hayvanların mera alanını terk etmeden su ihtiyaçlarını karşılayabilmeleri için yeterli olacaktır [21].

3. Yapay mera tesisine hazırlık amacıyla ön bitki ekimi

Mera alanındaki istilacı bitki türlerinin toprak üstü aksamının temizlenmesini müteakip alandaki yoğun ve güçlü kök kalıntılarının da temizlenmesi gerekmektedir. Bunu yaparken de öncelikle riperli iş makinesi ve sonrasında da toprak sürülürken klasik soklu pulluğun topraktaki kök kalıntılarına takılarak traktöre zarar vermemesi adına diskli pulluk kullanımı yerinde olacaktır.

Toprak hazırlığını müteakip en az 2 yıllık süreyle ön bitki yetiştiriciliği yapıldıktan sonra alanda bölge ekolojisine uygun çok yıllık yem bitkilerinden karışım oluşturarak yapay mera tesis edilmelidir. Bartın ilinin uzun yılların ortalaması olarak yıllık 1040 mm civarında olan yağış miktarı ve bu yağışın yıl çerisindeki dağılımı dikkate alınarak alanda ilk 2 yıl sonbaharda tek yıllık çim bitkisi ekimi yapılabilir. Ekilen tek yıllık çim bitkisinin hasadı köy sakinlerinin imkân ve istekleri dikkate alınarak 2 şekilde yapılabilir; 1) Bahar aylarında bitkiler başaklanma başlangıcı evresine ulaştığı dönemde biçilmelidir [29]. Biçilen bitki yeniden sürdüğü ve aynı biçim evresine ulaştığında yeniden biçilmelidir. Elde edilen ot hayvancılık yapan köy sakinlerine dağıtılabilir. İkinci biçim sonrasında bitkinin yeniden gelişimi izlenmeli tekrar biçime gelebilirse 3. kez biçilmeli yok eğer yaz sıcakları bastırıp bitki gelişimi istenildiği gibi olmazsa, hayvanlar meraya salınarak son hasat hayvan otlatılarak gerçekleştirilebilir. 2) Tek yıllık çim otlatmaya uygun olmakla birlikte buna dayanımı pek de iyi değildir. Ancak yine de duruma göre münavebeli bir şekilde hayvan otlatmak suretiyle de değerlendirilebilir. Otlatmada çiğneme kaybını en aza indirmek adına münavebeli otlatma hususu çok önemlidir. Gerek biçilerek ve gerekse hayvan otlatılarak yapılan hasatlar neticesinde bitki yaz sıcakları bastırınca yeniden sürme gücünü kaybedip ömrünü tamamlar. Alandaki tek yıllık çim bitkileri yaz sıcakları başlayıp yeniden sürme kabiliyetlerini yitirince, sulama imkânının sağlanabilmesi mümkün olabilirse alana; 1) Eğer hayvan otlatılarak faydalanılacaksa sorgum-sudan otu veya şeker sorgumu ekimi yapılarak yaz döneminde de ön bitki ekimi devam ettirilebilir [30]. Bu bitkiler hayvan sağlığı da düşünülerek bitkiler en az 1 m boya ulaşınca otlatılmaya başlanılmalıdır [31]. 2) Silaj yapılması düşünülürse aynı türler olabileceği gibi, mısır da ekilebilir [30]. Bu şekilde yapılacak 2 yıllık ön bitki tarımı neticesinde, mera alanı öncelikle yabancı otların varlığı ve toprağın fiziksel özellikleri bakımından daha uygun hale gelebilecek ve kalıcı yapay mera tesisi için daha uygun bir ortam hazırlanmış olacaktır.

4. Yapay mera tesisi

Yapay mera tesisi için alanda yeniden toprak işlemesi yapılarak, alanın ekolojisine uygun çok yıllık yem bitkileri seçilerek bir karışım oluşturulmalı ve ekimi sonbaharda gerçekleştirilmelidir. Tesisin sürekliliği ve hayvanların da ihtiyaçlarına binaen yem bitkisi karışımının yaklaşık olarak % 60'ı buğdaygil, % 40'ı ise baklagiller ve diğer familyalara ait türlerden teşkil edilmelidir [32]. Karışıma alınacak yem bitkisi türleri seçilirken, çalışma alanı ve çalışma alanına mesafe ve ekolojik bakımdan benzer mera alanlarında yapılan vejetasyon etüdü çalışmalarında [33, 34] belirlenen azalıcı türler dikkate alınmalıdır. Bu anlamda buğdaygil yem bitkilerinden *Lolium perenne, Dactylis glomerata, Poa pratensis, Festuca arundinacea;* baklagil yem bitkilerinden *Trifolium repens, Medicago sativa, Lotus corniculatus,* diğer familyalardan ise *Sangiosorba minor* oluşturulacak karışımda yer alabilecek bitkiler olarak tavsiye edilebilir. Yapay mera tesislerinde ilk yıl çok yıllık yem bitkileri kök gelişimini öncelemekte bu nedenle toprak üstü gelişimleri genel olarak zayıf olmaktadır. Bu nedenle yabancı otlarla olan rekabette geri kalmaktadır. Bu nedenle ilk yıl hızlı gelişerek yabancı otların tesisi istila etmesini engelleyen tek yıllık çim (*Lolium multiflorum*) bitkisinden 300 g kadar karışıma ilave edilmesi, çok yıllık bitki türlerinin verimde eksik kaldığı süre zarfında tatmin edici miktarda ot üretimine katkı sağlayabilecektir.

5. Gübreleme

Ön bitki olarak ekilen tek yıllık çimin gübrelemesi, ürünün biçilerek mi yoksa hayvan otlatılarak mı hasat edileceğine bağlıdır. Ön bitkinin hasadı biçme şeklinde yapılırsa, tahmini kuru ot verimleri dikkate alınarak her bir ton verim için saf madde olarak yaklaşık 4.5 kg azot ve 2.5 kg kadar da fosforlu gübre verilebilir (35, 36, 37]. Ön bitkiden faydalanma hayvan otlatma şeklinde yapılacaksa alandan kaldırılan besin maddelerinin tamamına yakınının hayvan gübreleri ile meraya geri döndüğü dikkate alınmalıdır [35, 38].

Tek yıllık çimin ömrünü doldurup alandan çekilmesini müteakiben yaz döneminde ikinci ürün olarak ekilecek mısır veya sorgum gibi buğdaygiller familyasından olan yem bitkileri için de aynı şekilde tahmini kuru ot verimleri dikkate alınarak, yukarıda ifade edildiği şekilde verilecek gübre miktarı hesaplanabilir.

Yapay mera tesisine verilecek gübre miktarı, yine tesisin ot verimi tahmini üzerinden yapılmalıdır. Ancak yapay merayı oluşturan yem bitkileri karışımında baklagiller familyasına ait türler de olduğu için azotlu gübre dozunu belirlemede daha dikkatli olunmalıdır. Aksi taktirde mera vejetasyonunda buğdaygiller, baklagilleri baskılamayabilmekte ve oranlarının azalmasına neden olabilmektedir [37, 39]. Tersi durumda yani azotun hiç verilmemesi veya az verilmesi de bu kez baklagillerin buğdaygil yem bitkilerini baskılamasına neden olabilir. Buğdaygiller familyalarına ait yem bitkisi türlerinden oluşan suni mera tesislerinde kaldırılan besin elementlerinin % 85-90'ının otlayan hayvan gübreleriyle meralara geri döndüğü belirtilmiştir [35, 38]. Ancak meralara geri dönen bu hayvansal gübrenin bir miktarının gaz halinde uçtuğu, diğer kısmı ise organik halde olduğu ve bitki tarafından alıma hazır olmadığı ifade edilmektedir. Ayrıca bu gübrelerin meraya dağılımının da üniform olmadığı ve gübrelerin bırakıldığı yerlerde yoğunlaştığı ve azotlu ve diğer besin elementlerine ihtiyaç duyulduğu bildirilmektedir. Bu nedenle hayvan gübrelerinin tırmık yardımıyla mera yüzeyine dağıtılması faydalı olacaktır. Aynı araştırıcılar meralarda baklagillerin oranının % 50'nin üzerinde olması halinde ise azotlu gübrelemeye gerek olmadığını ifade etmişlerdir. Vejetasyonlarındaki yem bitkilerinin % 40 kadarını baklagillerin oluşturduğu meralarda; her bir ton kuru ot verimi için yıllık ihtiyaç olarak dekara 1 kg azot, 2 kg kadar da fosforlu gübre tavsiye edilmiştir [35, 40]. Hacıosmanoğlu köyünün iklim değerleri ve yapay merada yer alması planlanan yem bitkisi türleri dikkate alındığında, yapılacak planlı bir otlatma ile hayvanlar tarafından kuru ot hesabıyla 1 ton kadar otun otlanılacağı söylenilebilir. Son olarak mera topraklarındaki besin maddelerinin kolay kolay değişmeyeceği gerçeğinden hareketle üç yılda bir toprak analizi ile meranın gübre ihtiyacının takibi faydalı olacağı ifade edilmiştir [35].

6. Diğer faaliyetler

Kalıcı mera tesisinin kurulmasından sonra, meranın kapasitesinde otlatılması, merada otlatmaya başlama ve bitiş tarihlerine dikkat edilmesi, meranın bölümlere ayrılarak münavebeli otlatılması, alanda yapılacak ıslah çalışmaları için yapılan masrafların karşılığını tam olarak alma anlamında çok önemlidir [18]. Mera bitki örtüsünün devamlılığının sağlanması amacıyla da, her 3-4 yılda bir bitkilerin tohum bağlayıp doğal tohumlama yapmalarına izin verilmelidir.

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Please contact the corresponding author for any data request.

Kaynaklar

1. Lacey, J. and J.E. Taylor, Montana Guide to Range Site, Condition and Initial Stocking Rates. Montana State University Extension Service, MT 198515 AG, http://store.msuextension.org/publications/AgandNaturalResources/MT198515AG.pdf, 2005. (Access: 19.June.2015).
2. Archer, S.R., Rangeland conservation and shrub encroachment: New perspectives on an old problem, Wild Rangelands: Conserving Wildlife While Maintaining Livestock in Semi-Arid Ecosystems, J.T. Toit, R. Kock and J.C. Deutsch, Editors. 2010. John Wiley &Sons. Chichester, UK. P. 53–97. ISBN (Print): 9781405177856

3. Fuhlendorf, S.D., Ecological considerations for woody plant management. Rangeland, 1999. 21(1): 12-15.
4. Preiss, E., J.L. Martin, and M. Debussche, Rural depopulation and recent landscape changes in a Mediterranean region: consequences to the breeding avifauna. Landscape Ecology, 1997. 12: 51-61.
5. Conti, G., and L. Fagarazzi, Sustainable mountain development and the key-issue of abandonment of marginal rural areas. Planum, 2004. 11: 1-20.
6. Naito, A.T., and D.M. Cairns, Patterns and processes of global shrub expansion. Progress in Physical Geography, 2011. 35(4): 423–442.
7. Uzun, F., A.V. Garipoğlu, ve H.B. Dönmez, Mera yabancı otlarının kontrolünde keçilerin kullanımı. Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi, 2015. 1(1): 40-50.
8. Uzun, F., F. Alay, ve K. İspirli, Bartın ili meralarının bazı özellikleri. Türkiye Tarımsal Araştırmalar Dergisi, 2016. 3(2): 174-183.
9. Uzun, F. ve N. Ocak, Some vegetation characteristics of rangelands subjected to different grazing pressures with single-or multispecies of animals for a long time (A case of Zonguldak province, Turkey). Anadolu Tarım Bilimleri Dergisi, 2019. 34(3): 360-370.
10. Anonim, İllere ait mevsim normalleri. Bartın (1991-2020). TC. Çevre, Şehircilik ve İklim Değişikliği Bakanlığı Meteoroloji Genel Müdürlüğü. https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?m=BARTIN, 2024. (Erişim: 25.01.2025).
11. Pornaro, C., M.K. Schneider, and S. Macolino, Plant species loss due to forest succession in alpine pastures depends on site conditions and observation scale. Biological Conservation, 2013. 161: 213-222.
12. Anonim, Helvacılar Köyü Kadim Merasının Orman Kayıtlarına Alınması Davası. Tapu İptali ve Tescil. Zonguldak, TC. Çaycuma, 2. Asliye Hukuk Mahkemesi. Gerekçeli Karar, 2013. Esas No: 2013/419, Karar No: 2013/577.
13. Uzun, F., N. Akgün, ve Ö.F. Uzun, Kurucaşile ilçesi, Ömerler mahallesi merasının tahsis kararının iptali isteminin irdelenmesi, IV. Bilsel Uluslararası Sur Bilimsel Araştırmalar Kongresi. 2025: Diyarbakır-Türkiye. p. 263-275.
14. Anonim, Hayvan Ara. TC. Tarım ve Orman Bakanlığı. https://hbsapp.tarbil.gov.tr./Modüles /TURKVET/Pages/Animal/CM/BSearchAnimal.aspx, 2025. (Erişim: 25.01.2025).
15. Anonim, Hayvan Ara. TC. Tarım ve Orman Bakanlığı. https://hbsapp.tarbil.gov.tr./Modüles /TURKVET/Pages/Animal/CM/BSearchAnimal.aspx, 2021. (Erişim: 25.01.2025).
16. Anonim, Tapu ve Kadastro Genel Müdürlüğü parsel sorgu uygulaması. https://parselsorgu.tkgm.gov.tr/#ara/ idari/130907/ 102/8/1738070712694, 2025. (Erişim: 25.01.2025).
17. Serin, Y., Türkiye'nin Çayır Mera Bitkileri. 2008, Ankara, T.C. Tarım ve Köyişleri Bakanlığı. Tarımsal Üretim ve Geliştirme Genel Müdürlüğü-In Turkish. ISBN: 9789944077613.
18. Holechek, J.L., R.D. Pieper, and C.H. Herbel, Range Management: Principles and Practices (6th Edition). 2010, Amsterdam: Prentice Hall, one Lake Street, Upper Saddle River. ISBN: 9780137527915.
19. Bölük, E., Thornthwaite İklim Sınıflandırmasına Göre Türkiye İklimi. 2016, Ankara, Araştırma Dairesi Başkanlığı Klimatoloji Şube Müdürlüğü https://www.mgm.gov.tr/FILES/iklim/iklim_siniflandirmalari/koppen.pdf. (Erişim: 02.04.2025).
20. Lyons, R.K. and R.V. Machen, Livestock grazing distribution: considerations and management. Texas Farmer Collection. Agrilife Extension. https:/hdl.handle.net/1969.1/87089, 2002. (Access: 28.01.2025).
21. Holst, P.J. and H.E. Simmonds, Palatability of weeds to goats in Australia, in Proceedings 7th, International Conference on Goats. 2000: France. p. 111-113.
22. Rahmann, G., Biotoppflege als neue Funktion und Leistung der Tierhaltung: dargestellt am Beispiel der Entbuschung von Kalkmagerrasen durch Ziegenbeweidung. Schriftenreihe Agraria: Studien zur Agrarökologie, 2000. 28: XVIII-384.
23. Elias, D. and S. Tischew, Goat pasturing—A biological solution to counteract shrub encroachment on abandoned dry grasslands in Central Europe? Agriculture, Ecosystems & Environment, 2016. 234: 98-106.
24. Hart, S.P., Recent perspectives in using goats for vegetation management in the USA. Journal of Dairy Science, 2001. 84: E170-E176.
25. Scholtz, R., et al., Challenges of brush management treatment effectiveness in southern Great Plains, United States. Rangeland Ecology & Management, 2021. 77: 57-65.
26. Dellow, J.J., et al., Large area blackberry (*Rubus fruticosus* Agg.) control using grazing goats. Plant Protection Quarterly, 1988. 3(2):83-84.
27. Higgins, S.F., C.T. Agouridis, and S.J. Wightman, Shade options for grazing cattle (AEN-99). University of Kentucky Cooperative Extension Service, https://publications.ca.uky.edu/files/aen99.pdf, 2024. (Access: 12.02.2024).
28. Anonim, Grazing Management and Foraging Behaviour. Scottish Forestry. https://www.forestry.gov.scot/woodland-grazing-toolbox/grazing-management/foraging/ palatability-and-resilience-of-native-trees, (2021). (Erişim: 02.12.2025).
29. Aydın, F., ve F. Uzun, Çayır-Mera Amenajmanı ve Islahı. 2002, Ondokuz Mayıs Üniversitesi, Ziraat Fakültesi, Ders Kitabı No:9. Samsun. ISBN: 9786056141324.
30. Çiğdem, İ. ve F. Uzun, Samsun ili taban alanlarında ikinci ürün olarak yetiştirilebilecek bazı silajlık sorgum ve mısır çeşitleri üzerine bir araştırma. OMÜ, Ziraat Fakültesi Dergisi, 2006. 21(1): 14-19.
31. Uzun, F. ve İ. Çiğdem, Yemlik kocadarı ve kocadarı-sudan otu melezleri. OMÜ, Ziraat Fakültesi Dergisi, 2005. 20(2): 66-72.
32. Sanderson, M.A., et al., Grass–legume proportions in forage seed mixtures and effects on herbage yield and weed abundance. Agronomy Journal, 2013. 105(5): 1289-1297.
33. İspirli, K., et al., Nemli-mezotermal iklim kuşağında yer alan ve ağır otlanılan Hasanlar köyü merasının bazı özellikleri, Anadolu Tarım Bilimleri Dergisi, 2022. 37(3): 487-504.
34. Uzun, F., Nemli-mezotermal iklimli Serdar köyü merası vejetasyon yapısı ve uygulanabilecek ıslah yöntemlerinin irdelenmesi. IV. Bilsel Uluslararası Sur Bilimsel Araştırmalar Kongresi. 2025: Diyarbakır, Türkiye. p. 276-287.

35. Koenig, R., M. Nelson, and J. Barnhill, Fertilizer Management for Grass and Grass-Legume Mixtures. Utah University Extension. AG-FG-003. https://digitalcommons.usu.edu/cgi/viewcontent.cgi?article=1008&context =extension_histall, 2002. (Access: 08.12.2024).
36. Heckman, J., Soil Fertility Recommendations for Producing Grass Hay. Cooperative Extension Fact Sheet. FS1295. New Jersey Agricultural Experiment Station. https://njaes.rutgers.edu/fs1295/#:~:text=The%20 most %20commonly%20grown %20species,ton%20of%20expected%20hay%20yield, 2018. (Access: 08.12.2024).
37. Kaiser, D.E., Fertilizing Hay and Pasture Grasses. University of Minnesota Extension. https://extension.umn.edu/crop-specific-needs/grasses-hay-pasture-fertilizer, 2023. (Access: 08.12.2024).
38. Brummer, J.E., J.G. Davis, and M.R. Booher, Fertilizing Cool Season Grass and Grass/Legume Mixtures. Colorado State University Extension. Crop Series/Soil, Fact Sheet No: 0522. https://scholar.google.com/scholar?hl=tr&as_sdt=0%2C5&q=Brummer%2C+J.E.%2C+J.G.+Davis%2C+and+M.R.+Booher%2C+Fertilizing+Cool+Season+Grasss+and+Grass%2FLegume+Mixtures.+Colorado+State+University+Extension.++&btnG=, 2011. (Access: 08.12.2024).
39. Aydin, I. ve F. Uzun, Nitrogen and phosphorus fertilization of rangelands affects yield, forage quality and the botanical composition. European Journal of Agronomy, 2005. 23(1): 8-14.
40. Anderson, B., J.D. Volesky, and C.A. Shapiro. Fertilizing Grass Pastures and Hayland. University of Nebraska-Lincoln Extension, Institute of Agriculture and Natural Resources. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://extensionpubs.unl.edu/publication/g1977/2009/pdf/view/g1977-2009.pdf, 2010. (Access: 08.12.2024).

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A Review on Industrial Application of Lactic Acid Bacteria in Food Microbiology

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ABSTRACT
Lactic acid bacteria (LAB) are a diverse group of microorganisms primarily known for their pivotal role in food fermentation, enhancing the flavor, texture, and preservation of various food products. This review explores the industrial applications of LAB in food microbiology, emphasizing their significance in fermentation processes, probiotic development, and food preservation. LAB are integral to the production of fermented foods such as yogurt, cheese, sauerkraut, and kimchi, where they metabolize sugars to produce lactic acid, imparting unique sensory characteristics while inhibiting pathogenic bacteria. This acidification process not only enhances food safety but also contributes to improved shelf life. The probiotic potential of LAB is another critical aspect of their industrial application. Certain strains have demonstrated health benefits, including improved gut health, enhanced immune response, and reduced risk of gastrointestinal diseases. As consumer interest in functional foods rises, the incorporation of LAB into various food products has become increasingly prevalent, leading to the development of innovative probiotic formulations. Moreover, LAB possess antimicrobial properties that make them suitable as biopreservatives in food systems. This natural preservation approach aligns with the growing demand for clean label products, minimizing the use of synthetic preservatives. The review also addresses the challenges faced in industrial applications, such as strain selection, fermentation control, and scalability. Lactic acid bacteria enhance food safety, sensory qualities, and health benefits through probiotics. This review highlights their vital role in food microbiology, emphasizing ongoing research to optimize their applications for advancing food technology and meeting health-conscious consumer demands.
The purpose of this review is to provide a comprehensive analysis of the industrial applications of lactic acid bacteria (LAB) in food microbiology. It aims to highlight their roles in fermentation, food preservation, probiotic development, and enhancement of food safety and quality. The review also seeks to explore recent advancements, challenges, and future prospects in the utilization of LAB to promote sustainable and innovative food processing solutions.

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Introduction

Lactic acid bacteria (LAB) play a pivotal role in the fermentation of various foods, contributing not only to preservation but also to the enhancement of nutritional value and health benefits [1, 2]. Recent studies have highlighted the significance of LAB, particularly *Lactobacillus* species, in the fermentation of traditional products like cassava, showcasing their probiotic potential [3]. These microorganisms are recognized for their ability to produce lactic acid, which acts as a natural preservative, inhibiting the growth of spoilage and pathogenic microorganisms [4]. The health benefits of LAB are well-documented. They are known to contribute to gut health by improving intestinal microbiota balance, enhancing immune response, and preventing gastrointestinal disorders [5]. Probiotics derived from LAB have been linked to various health improvements, including the alleviation of lactose intolerance, reduction of cholesterol levels, and even potential anti-cancer properties [6, 7]. The safety of these bacteria in fermented foods has also been

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emphasized, ensuring that they can be consumed without adverse effects while providing functional benefits [8]. In terms of technological advancements, the application of LAB in food technology has evolved significantly. Innovations in fermentation processes have led to enhanced preservation methods that extend shelf life while maintaining sensory qualities [9]. Additionally, the use of LAB as biopreservatives is gaining traction, offering a natural alternative to chemical preservatives [10]. Recent findings suggest that LAB can effectively suppress foodborne pathogens, thereby improving food safety [11, 12]. Fermented foods, rich in LAB, are increasingly recognized for their bioactive compounds that contribute to health benefits. These compounds have antioxidant, anti-inflammatory, and antimicrobial properties, promoting overall health [13]. Studies have shown that incorporating fermented products into diets can lead to significant health improvements, including better metabolic health and reduced risk of chronic diseases [14].

Furthermore, the increasing consumer interest in functional foods has propelled research into the probiotic capabilities of various LAB strains. Recent reviews indicate that fermented foods can serve as excellent vehicles for delivering probiotics, making them an integral part of modern diets [15, 16]. The exploration of new LAB strains from diverse sources, such as traditional fermented foods, holds promise for discovering strains with superior probiotic properties [17, 18]. Overall, the integration of lactic acid bacteria in food production offers numerous advantages, from enhancing food safety to providing health benefits. As research continues to unfold, the role of LAB in functional foods will likely expand, catering to the growing demand for health-promoting dietary options [19, 20]. The continued exploration of these beneficial microorganisms will contribute to the development of innovative food products that align with consumer health trends, emphasizing the importance of fermented foods in contemporary diets.

The purpose of this review is to provide a comprehensive analysis of the industrial applications of lactic acid bacteria (LAB) in food microbiology. It aims to highlight their roles in fermentation, food preservation, probiotic development, and enhancement of food safety and quality. The review also seeks to explore recent advancements, challenges, and future prospects in the utilization of LAB to promote sustainable and innovative food processing solutions.

Characteristics of Lactic Acid Bacteria

Lactic acid bacteria (LAB) are a diverse group of gram-positive, non-spore-forming bacteria that play a crucial role in food fermentation and preservation. One of the defining characteristics of LAB is their ability to ferment sugars primarily into lactic acid, which serves as their primary metabolic end product [21, 22]. This metabolic pathway not only contributes to the preservation of fermented foods but also influences the flavor and texture of products such as yogurt, cheese, and fermented vegetables. LAB are typically categorized into two groups based on their fermentation type: homofermentative and heterofermentative. Homofermentative LAB, such as *Lactobacillus* species, convert sugars mainly into lactic acid, resulting in a more acidic environment that inhibits the growth of spoilage organisms. In contrast, heterofermentative LAB, like *Leuconostoc*, produce lactic acid, carbon dioxide, and ethanol, leading to a more complex flavor profile and gas production during fermentation. Another characteristic of LAB is their ability to thrive in low pH environments, which is essential for their role in food preservation. They can grow in acidic conditions that would be detrimental to many other microorganisms, allowing them to dominate in fermented foods. Additionally, LAB are known for their probiotic properties, contributing positively to human health by enhancing gut microbiota balance, boosting immune function, and preventing gastrointestinal disorders. LAB also exhibit a range of biochemical capabilities, including the production of bacteriocins—proteinaceous compounds with antimicrobial activity against pathogenic bacteria. This characteristic further enhances their utility in food preservation and safety. Overall, the unique metabolic, ecological, and health-promoting characteristics of lactic acid bacteria make them indispensable in the food industry and human nutrition. The biochemical pathways employed by lactic acid bacteria (LAB) play a central role in food fermentation, preservation, and flavor development. LAB exhibit two primary metabolic pathways, the homofermentative and heterofermentative pathways, which determine their end products and influence the sensory characteristics of fermented foods [3].

Homofermentative Pathway

The homofermentative pathway is a highly efficient process in which LAB species, such as *Lactobacillus* and *Lactococcus*, convert glucose almost exclusively into lactic acid via glycolysis [23]. This pathway is advantageous in food preservation, as the lactic acid produced rapidly lowers the pH of the food environment, creating an acidic setting unfavorable for spoilage organisms and pathogens. The production of lactic acid also stabilizes food by inhibiting proteolytic and lipolytic activity, which can cause spoilage [24]. By creating an inhospitable environment for many microorganisms, the homofermentative pathway is essential in extending the shelf life of foods like yogurt, cheese, and pickled vegetables, allowing these products to retain nutritional and organoleptic qualities for prolonged periods [4].

Heterofermentative Pathway

In contrast, heterofermentative LAB, including certain strains of *Lactobacillus* and *Leuconostoc*, employ the pentose phosphate pathway to metabolize glucose, yielding lactic acid, carbon dioxide, ethanol, and acetic acid [19]. This metabolic diversity contributes to the distinct sensory properties of fermented foods by producing multiple end-products that enhance flavor complexity [25]. Carbon dioxide generated in this pathway results in the effervescence seen in products like sauerkraut, kimchi, and certain sourdough breads [16]. The presence of acetic acid and ethanol not only contributes to the characteristic taste but also adds antimicrobial properties, further protecting the food from spoilage and pathogenic bacteria [26].

Bacteriocins Produced by Lactic Acid Bacteria

In addition to their acidifying capabilities, certain LAB strains produce bacteriocins, which are proteinaceous compounds with targeted antibacterial activity [27]. Bacteriocins act as biopreservatives by inhibiting the growth of other bacteria, adding a valuable layer of defense against foodborne pathogens. For example, *Lactobacillus acidophilus* synthesizes acidophilin, a bacteriocin effective against pathogens like *Salmonella* and *Listeria* [7]. These antimicrobial peptides provide an advantage in food preservation by extending shelf life and enhancing food safety without the need for chemical additives [15]. LAB's production of lactic acid, diverse metabolites, and bacteriocins underscores their multifaceted role in fermentation processes, where they contribute both to food preservation and flavor enhancement. This metabolic versatility has made LAB indispensable in the food industry [28], supporting the development of safe, nutritious, and flavorful fermented products.

Classification and Properties of LAB Bacteriocins

LAB bacteriocins are classified based on their molecular weight, heat stability, and activity spectrum. This classification generally falls into several types [28]:

a) **Class I**: Small, heat-stable lantibiotics.
b) **Class II**: Heat-stable, non-lantibiotic peptides with broad antimicrobial spectra.
c) **Class III**: Large, heat-labile proteins.
d) **Class IV**: Complex bacteriocins associated with other molecules (lipids, carbohydrates).

Each class has distinct properties such as pH tolerance and effectiveness against various pathogens.

Lactic Acid Bacteria against Foodborne Pathogens in the Food Industry

LAB strains like *Lactobacillus*, *Pediococcus*, and *Enterococcus* are applied in food preservation due to their bacteriocin production, which inhibits harmful bacteria like *Listeria*, *Salmonella*, and *E. coli*. These bacteriocins help reduce spoilage, enhance food safety, and extend shelf life, thus playing a critical role in food safety management systems [28].

Classification and Sources of Lactic Acid Bacteria (LAB)

Lactic acid bacteria (LAB) are generally classified into several genera, primarily based on their morphological, metabolic, and physiological characteristics [28]. The major groups include:

i. **Lactobacillus**: This is one of the largest LAB genera, widely known for its probiotic qualities and found in various fermented foods.
ii. **Lactococcus**: Commonly sourced from milk and dairy products, *Lactococcus* species are integral in cheese and buttermilk production.
iii. **Streptococcus**: Specifically, *Streptococcus thermophilus* is used in yogurt production.
iv. **Pediococcus**: Often found in fermented plant-based foods and alcoholic beverages.
v. **Leuconostoc**: Found in vegetables, dairy products, and even on meat surfaces, *Leuconostoc* species are essential in sauerkraut and kimchi production.

vi. **Enterococcus**: Though some species can be opportunistic pathogens, *Enterococcus* species are used in specific food fermentations.

LAB are sourced from diverse environments, such as dairy products, fermented vegetables, meat, and various plant materials. Their role in food fermentation is significant due to their ability to inhibit spoilage organisms, enhancing the safety and shelf-life of food products [28].

Industrial Applications of LAB in Food Microbiology

Dairy Fermentation

In dairy products, LAB play an essential role in fermenting lactose into lactic acid, which lowers pH and creates a favorable environment that prevents the growth of spoilage organisms and pathogens [29]. The production of lactic acid also enhances the nutritional value and digestibility of dairy products, making them more suitable for lactose-intolerant individuals [25]. *Lactobacillus delbrueckii* and *Streptococcus thermophilus* are frequently used in yogurt production, where they convert lactose into lactic acid, producing a distinctive sour taste while thickening the texture [17]. Additionally, these bacteria produce exopolysaccharides (EPS), which improve the viscosity and mouthfeel of yogurt [10]. EPS also improve the shelf life and texture stability of fermented dairy products, such as yogurt and kefir, providing a smooth and creamy texture that appeals to consumers [23]. Flavor development is another critical function of LAB in dairy fermentation. *Lactobacillus delbrueckii* and *Streptococcus thermophilus* generate various flavor compounds, such as acetaldehyde and diacetyl, which contribute to the characteristic aroma and taste of yogurt [26]. These metabolic activities create a rich sensory profile in products like cheese, where LAB fermentation leads to a broader spectrum of volatile compounds, giving each cheese variety its unique flavor. LAB's influence on texture, taste, and digestibility significantly impacts the dairy industry by making fermented dairy products more appealing and accessible to a wide range of consumers [30].

Meat Fermentation

In meat products, LAB play a vital role in enhancing food safety, texture, and flavor. Fermented meats, such as sausages and salami, rely on LAB's acidification process to reduce pH, which helps inhibit the growth of spoilage organisms and pathogenic bacteria, including *Listeria monocytogenes* [31]. Acidification by LAB also contributes to the characteristic tangy taste of cured meats, making LAB an essential component of starter cultures used in fermented meat production [32]. In addition to acid production, some LAB strains produce bacteriocins, proteinaceous substances with antimicrobial properties that provide additional protection against harmful bacteria. *Pediococcus* and *Lactobacillus* species, commonly used in meat fermentation, produce bacteriocins that inhibit pathogens, thereby enhancing product safety and extending shelf life [33]. These LAB not only improve microbial safety but also contribute to the desired sensory attributes of cured meats, making LAB starter cultures highly valuable in meat fermentation [29].

Vegetable and Fruit Fermentation

LAB are also essential in vegetable and fruit fermentations, such as in sauerkraut, kimchi, and pickles. By fermenting sugars present in vegetables, LAB lower the pH and create acidic environments that inhibit the growth of spoilage organisms and pathogens [30]. *Leuconostoc mesenteroides*, often involved in these fermentations, initiates lactic acid production, setting the stage for subsequent LAB activity that enhances flavor profiles and creates desirable textures [32]. Fermented vegetables benefit from LAB's production of bacteriocins, which extend the shelf life of products by preventing spoilage [33]. LAB's metabolic activities in vegetable fermentation contribute to the development of complex, tangy flavors characteristic of products like kimchi and sauerkraut. Moreover, the acidification and bacteriocin production by LAB enhance the safety of these fermented foods by controlling pathogenic microorganisms and adding unique flavors that appeal to consumers worldwide [31].

Cereal-Based Fermentation

In cereal-based foods, LAB are integral to sourdough fermentation, where they improve both the shelf life and sensory properties of bread. *Lactobacillus sanfranciscensis*, a key LAB species in sourdough, produces lactic acid and acetic acid, which add distinct sour notes to bread while enhancing its overall flavor [21]. The acids produced also strengthen gluten structure, resulting in improved dough elasticity and better crumb texture, making LAB-fermented breads more resilient to staling and spoilage [25]. Moreover, LAB contribute to the formation of various volatile compounds, which enhance the bread's aroma and flavor profile. These characteristics are especially valued in artisanal and traditional bread production, where natural sourdough fermentation imparts unique sensory qualities that distinguish these products from commercially leavened bread [30]. The contribution of LAB to texture, flavor, and preservation in cereal-based products has driven the popularity of sourdough products and highlighted LAB's role in extending the shelf life of bakery items [33]. Figure 1 illustrates the traditional method of preparation of *Khorisa*, a fermented bamboo shoot product. It showcases the key steps involved, including harvesting, chopping, fermenting, and preservation, emphasizing the role of indigenous fermentation practices. Figure 2 presents a generalized flow chart for the production of lactic acid-fermented pickles. The diagram highlights the primary stages, including raw material selection, brining, fermentation, and packaging, demonstrating the critical role of lactic acid bacteria in the fermentation process. Figure 3 depicts the flow chart for the preparation of prebiotic- and probiotic-enriched *Shrikhand*. It outlines the sequential process steps, including milk preparation, curd formation, whey removal, incorporation of probiotics and prebiotics, flavoring, and final packaging, highlighting the integration of functional ingredients.

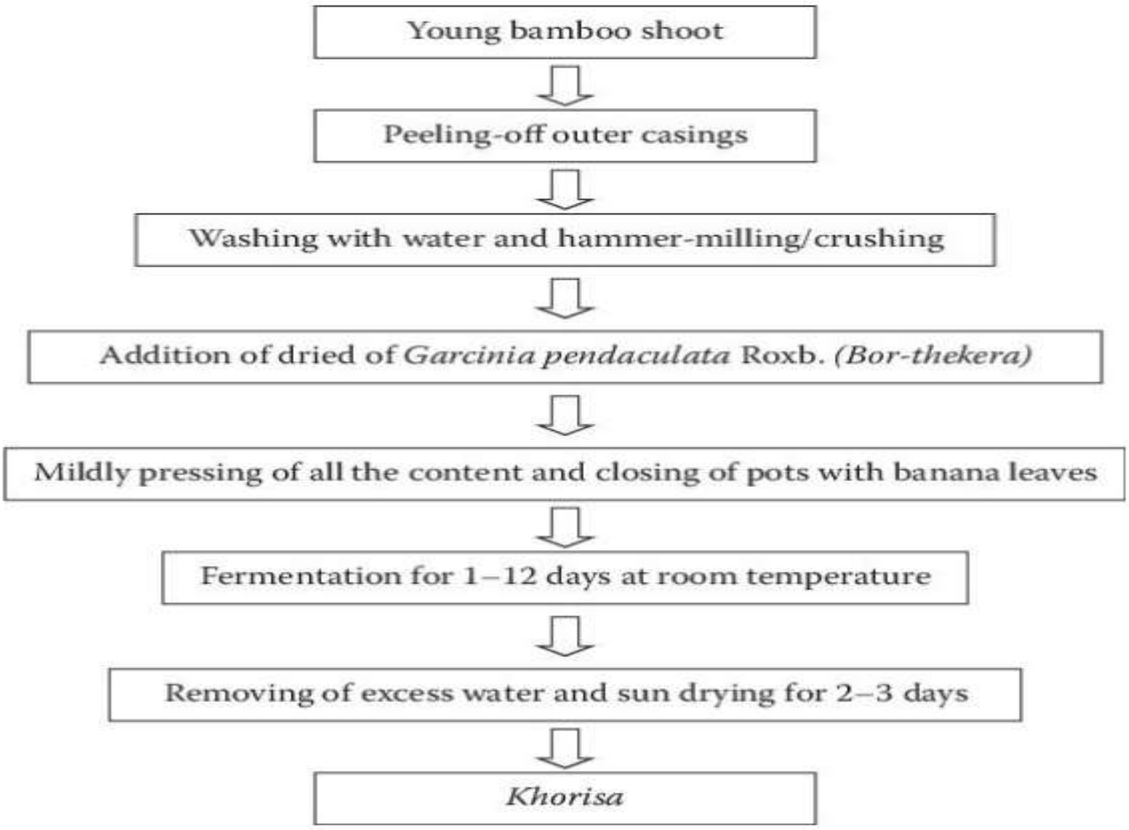

Fig 1. Method of preparation of khorisa [18, 23, 33].

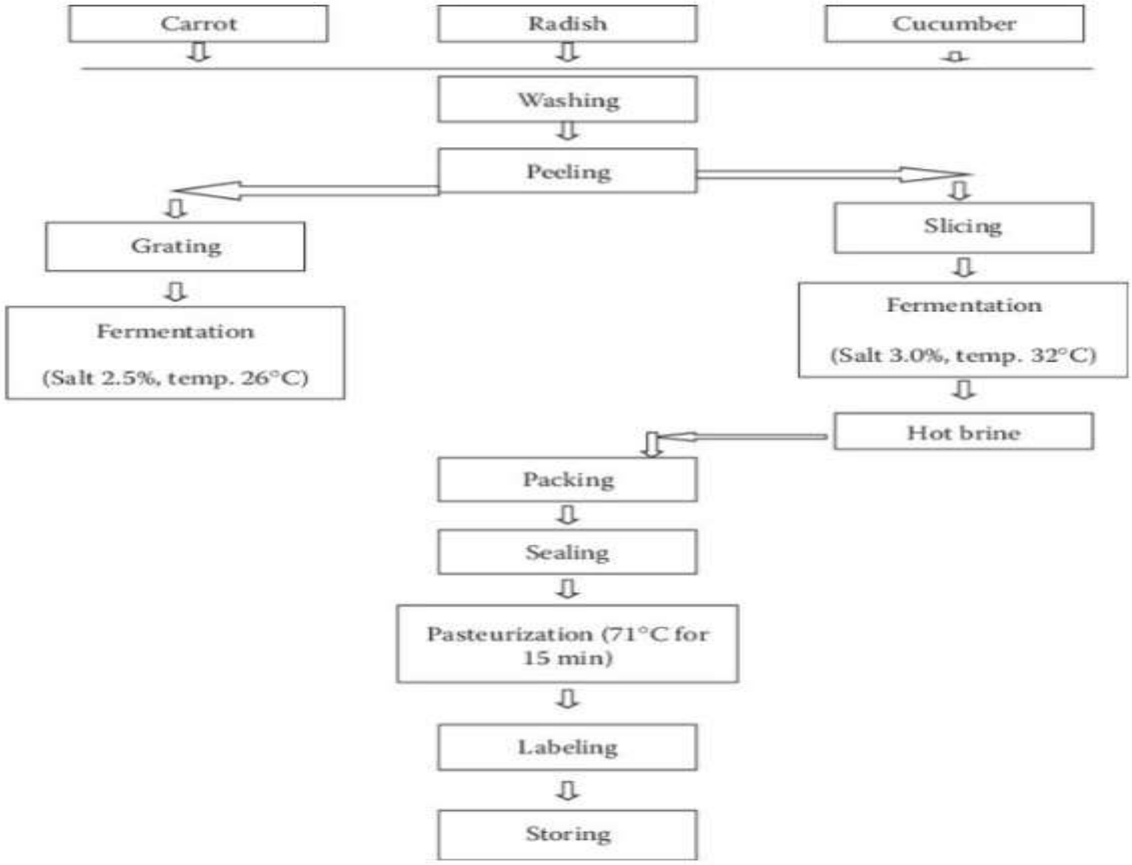

Fig 2. Generalized flow chart of the production of lactic acid-fermented pickles [25].

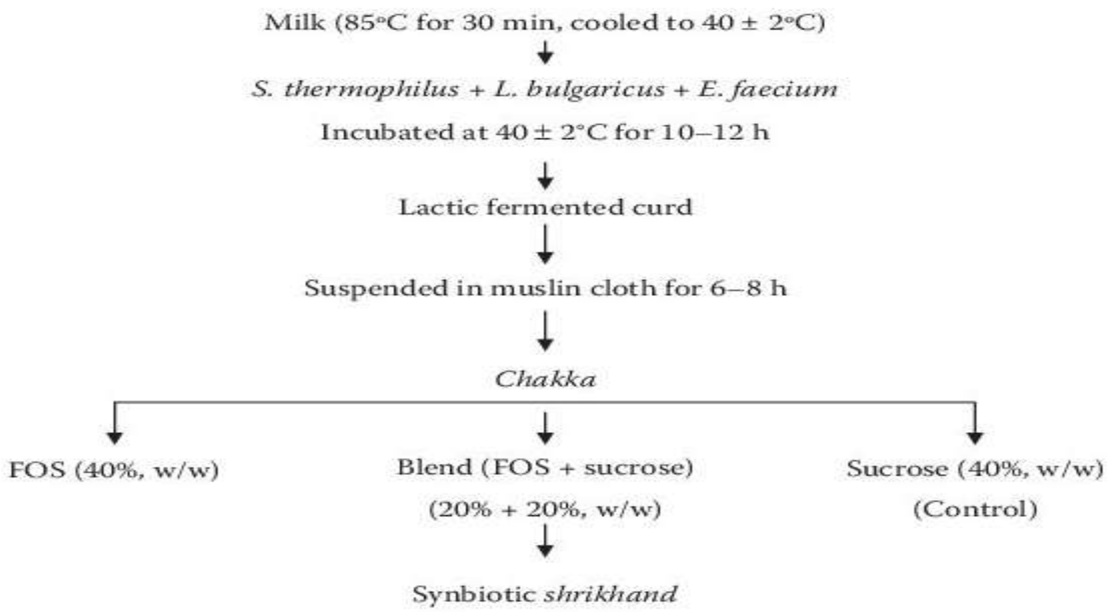

Fig 3. Flow chart of the process for the preparation of prebiotic-and probiotic-enriched shrik hand [22].

Health Benefits and Probiotic Properties

Lactic acid bacteria (LAB) have been widely studied for their health benefits, especially as probiotics that support gut health, immune function, cardiovascular health, and protection against harmful pathogens. Their metabolic activities not only contribute to food preservation but also support various aspects of human health.

Gut Health and Microbiota Balance

LAB play a critical role in maintaining gut health by modulating the balance of the intestinal microbiota, supporting nutrient absorption, and enhancing immune function. Strains like *Lactobacillus acidophilus* help prevent harmful bacteria from colonizing the gut, promoting a healthier microbial balance [20]. This is particularly beneficial in managing conditions such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), where disruptions in gut microbiota are common 18]. Fermented foods rich in LAB, such as yogurt and kefir, have shown positive effects on gut health, providing a source of live bacteria that improve digestion and nutrient absorption [28].

Immunomodulatory and Anti-inflammatory Properties

LAB have significant immunomodulatory properties, meaning they can influence immune responses to reduce inflammation and enhance immune defenses. Studies indicate that LAB stimulate the production of cytokines and regulate immune cells, which helps manage inflammation in the gut [31]. For example, *Lactobacillus rhamnosus* has been associated with reduced inflammatory markers in patients with Irritable Bowel Syndrome (IBS), showing a potential therapeutic effect for conditions linked to gut inflammation [30]. Additionally, LAB enhance the body's defense against infections, with some strains boosting the activity of immune cells such as macrophages, which protect against pathogens [4].

Cholesterol Reduction and Cardiovascular Health

Some LAB strains have demonstrated potential for improving cardiovascular health by lowering cholesterol levels. Certain strains produce bile salt hydrolase, an enzyme that deconjugates bile acids, allowing them to be excreted rather than reabsorbed, which indirectly reduces cholesterol levels in the blood [6]. Clinical studies have shown that consuming fermented milk products containing LAB, such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, can contribute to lower serum cholesterol levels, supporting cardiovascular health and reducing the risk of atherosclerosis [14].

Antimicrobial Properties

LAB exhibit antimicrobial properties that enhance both food safety and human health by producing organic acids, hydrogen peroxide, and bacteriocins—natural antimicrobial compounds effective against pathogenic bacteria. *Lactobacillus plantarum* and *Lactobacillus sakei*, for example, produce bacteriocins that inhibit harmful microbes like *Listeria monocytogenes*, reducing foodborne illness risks [17]. This antimicrobial activity is also beneficial in the gut, as LAB inhibit the growth of pathogens such as *Salmonella* and *Escherichia coli*, contributing to a healthier intestinal environment [28].

LAB and Food Safety Enhancement

Lactic acid bacteria (LAB) are increasingly valued in food safety due to their ability to produce natural antimicrobial agents, especially bacteriocins, which inhibit a variety of spoilage organisms and foodborne pathogens. This capability is particularly beneficial as food manufacturers seek alternatives to chemical preservatives, aligning with the clean-label trend that emphasizes natural ingredients [25]. Bacteriocins, such as nisin and pediocin, are proteinaceous compounds produced by specific LAB strains that exhibit strong antibacterial effects against pathogens like *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* spp. [26]. By incorporating bacteriocin-producing LAB into food products, manufacturers can reduce reliance on synthetic additives, which appeals to consumers who favor minimally processed and naturally preserved foods.

In addition to bacteriocin production, LAB contribute to food safety through organic acid production, particularly lactic and acetic acids, which lower the pH and create unfavorable conditions for pathogen growth [29]. This acidification process is crucial for preserving foods such as dairy, fermented vegetables, and ready-to-eat products, helping to extend shelf life and prevent spoilage. LAB can thus stabilize foods prone to spoilage and enhance the safety of ready-to-eat items by naturally inhibiting pathogens, without compromising sensory qualities [31]. Using LAB as bio-preservatives in minimally processed foods meets both safety and quality demands by maintaining the food's integrity while ensuring microbial safety. As consumer preference for naturally preserved, clean-label products continue to rise, LAB offer a sustainable and effective means of enhancing food safety without synthetic preservatives.

Advances and Emerging Trends in LAB Research

Research on lactic acid bacteria (LAB) has made substantial progress, especially in genomic and metabolic engineering, as well as in encapsulation technologies, each paving the way for expanded applications in food and health.

Genomic and Metabolic Engineering

Genomic and metabolic engineering have revolutionized LAB research by enabling the development of genetically modified strains with enhanced functionalities. Genomic advancements, such as CRISPR-Cas9, allow for precise gene modifications to optimize metabolic pathways in LAB, boosting fermentation efficiency and facilitating the production of beneficial compounds [24]. These engineered LAB strains can produce enhanced flavors and aromas, which are valuable for food applications, particularly for plant-based and non-dairy foods where flavor can be challenging to replicate. Enhanced LAB strains also demonstrate improved tolerance to environmental stressors, including temperature fluctuations and pH variations, which makes them more viable for broader applications across diverse food matrices [17]. As consumer interest in plant-based diets grows, engineered LAB strains offer promising solutions for non-dairy fermentation, providing not only unique flavors but also functional benefits, such as improved digestion and nutrient availability [22].

Encapsulation and Viability Enhancement

Encapsulation techniques, especially microencapsulation and spray drying, are gaining traction in LAB research to protect probiotic strains from environmental stressors, thereby enhancing their stability and viability. Microencapsulation involves coating LAB cells in a protective layer, typically using polymers like alginate or chitosan, which shields them from heat, moisture, and oxygen during storage [13]. This method ensures higher cell viability, which is particularly advantageous for probiotic LAB, as it helps them survive both food processing and the acidic conditions of the gastrointestinal tract. Similarly, spray drying—where LAB are rapidly dried under controlled conditions—ensures the stable delivery of viable cells in powdered forms, useful for supplement and food product applications [12].

These encapsulation advancements are crucial for the probiotic efficacy of LAB, ensuring they reach the intestines in sufficient numbers to confer health benefits. This technological progress not only enhances product shelf life and consumer safety but also allows for the incorporation of LAB into a broader range of food products, including those with higher thermal processing requirements. Consequently, encapsulation technologies represent a pivotal advancement, bridging the gap between LAB's functional benefits and their real-world applications [33].

Challenges and Future Directions

Although lactic acid bacteria (LAB) hold vast potential in food, health, and biotechnology, several challenges impede their full exploitation. Addressing these hurdles will require continued innovation and an adaptable regulatory landscape.

Fermentation Efficiency and Environmental Sensitivity

LAB strains exhibit variability in fermentation efficiency, influenced by species, environmental conditions, and substrate availability. For instance, certain LAB strains struggle in acidic or high-salt conditions, which are common in fermented foods, thus affecting their fermentation performance [25]. Additionally, LAB's sensitivity to temperature, pH, and oxygen levels can reduce their viability in end products, especially those requiring long shelf lives or transport. To tackle these issues, researchers are exploring genetic and environmental optimizations, but consistent strain stability remains challenging.

Regulatory Concerns for Genetically Modified LAB

With advances in genomic engineering, genetically modified (GM) LAB strains are increasingly explored to enhance flavor, nutritional quality, and preservation. However, regulatory frameworks around GM organisms remain stringent, especially for food applications, leading to potential delays in adoption [18]. Public perception of GM organisms also raises consumer acceptance issues. To integrate GM LAB effectively, regulatory bodies may need to adopt clearer guidelines balancing innovation and safety, possibly through labeling and transparency requirements.

Antibiotic Resistance Concerns

LAB can carry antibiotic resistance genes, which poses a risk of horizontal gene transfer to pathogenic bacteria, a concern particularly in probiotic and therapeutic applications [23]. Regulatory agencies often require screening for antibiotic resistance in LAB strains intended for human consumption, yet ensuring compliance across all strains and products remains challenging. Mitigating this issue will involve screening for resistance markers and selecting non-resistant strains for applications, as well as ongoing research into LAB's interactions with other microbial populations in the gut.

Future Prospects of LAB and Bacteriocins in the Food Industry

The potential of lactic acid bacteria (LAB) and their bacteriocins in the food industry continues to grow, driven by the demand for natural preservatives and natural products. Future applications focus on enhancing food safety, extending shelf life, and improving consumer health without synthetic additives [28]. Key prospects include:

i. Advanced Food Preservation Techniques: The use of LAB and bacteriocins as bio-preservatives is expected to expand, offering an alternative to chemical preservatives. Bacteriocins, such as nisin and pediocin, are likely to see increased integration in fresh produce, dairy, and meat packaging.

ii. Targeted Pathogen Control: Advances in genomics and biotechnology could allow the development of customized bacteriocins targeting specific foodborne pathogens, like *Listeria monocytogenes*, *Salmonella*, and *E. coli*, making food products safer without affecting beneficial microflora.

iii. Encapsulation and Controlled Release: Innovations in encapsulation could improve the stability and effectiveness of bacteriocins, allowing their controlled release in food matrices over time. This could lead to better preservation outcomes in minimally processed or fresh foods.

iv. Enhanced Probiotic Functionality: As probiotics, LAB species can contribute both to gut health and food preservation. Research into the health benefits of LAB-derived bacteriocins may lead to the development of functional foods with enhanced health-promoting properties.

v. Synergistic Use with Other Preservation Methods: Combining LAB bacteriocins with other mild preservation techniques, such as high-pressure processing and natural extracts, could improve food quality and shelf life while maintaining a clean label.

vi. Regulatory and Commercial Expansion: As regulatory bodies increasingly recognize LAB and bacteriocins as safe, more industries may adopt them, boosting commercial applications globally. Expansion into sectors like plant-based foods and ready-to-eat meals is also anticipated.

Conclusion

In conclusion, lactic acid bacteria (LAB) hold significant potential across various sectors, particularly in food safety, preservation, and health. Through their unique metabolic pathways, LAB contribute to flavor development, nutrient enhancement, and shelf-life extension in fermented foods. Their probiotic properties further support human health by promoting gut microbiota balance, immune modulation, and cholesterol reduction. Advances in genetic engineering and encapsulation techniques are paving the way for broader and more efficient applications, especially in non-dairy and plant-based food markets. However, challenges such as fermentation efficiency, environmental sensitivity, and regulatory concerns around genetically modified strains and antibiotic resistance persist. Addressing these issues will require continued research, robust regulatory frameworks, and consumer education. Overall, the future of LAB remains promising, with ongoing innovations set to unlock further applications in food technology and human health.

Conflict of interest

The authors declare no conflict of interest.

Ethical standards

The study is proper with ethical standards.

Authors' contributions

The review article was entirely written by Olodu Blessing Adoh and Prof. Stephen Amadin Enabulele. Both authors contributed to the extensive literature search, analysis, and synthesis of findings across relevant studies. They collaborated on structuring the article and interpreting the research insights, aiming to provide a comprehensive overview of the topic

References

1. Aladeboyeje, O., and Şanlı, N. Ö. Fermented traditional probiotic beverages of Turkish origin: A concise review. *International Journal of Life Science and Biotechnology,* 2021. *4*(3): p. 546–564. https://doi.org/10.38001/ijlsb.936982
2. Aguirre-Garcia, Y. L., et al., Lactic acid fermentation in the food industry and bio-preservation of food. *Fermentation,* 2024. 10(3): p. 168. https://doi.org/10.3390/fermentation10030168
3. Azeta, I. and Olubukola, O. Probiotic *Lactobacillus* species isolated from fermented beverages for antibacterial activity and shelf life extension against spoilage bacteria. *IEEE SEB4SDG Conference Proceedings*, 2024. 2: p. 58–67. https://doi.org/10.1109/seb4sdg60871.2024.10629730
4. Nikoleta, N. and Alexandra, L. Isolation and in vitro screening of the probiotic potential of microorganisms from fermented food products. *Frontiers in Microbiology*, 2023; p. 14, Article 1257483. https://doi.org/10.3389/finmi.2023.1257483
5. Divyashree, S., Ramu, R., and Sreenivasa, M. Y. Evaluation of new candidate probiotic *Lactobacillus* strains isolated from a traditional fermented food: Multigrain-millet dosa batter. *Food Bioscience*, 2024; p. 57, Article 103450. https://doi.org/10.1016/j.fbio.2023.103450
6. Akpa, E., et al., Selection of lactic acid bacteria isolated from cocoa and cassava fermentation as potential probiotics for pathogenic microorganism control in poultry farming. *Annual Research & Review in Biology*, 2022. 37(3): p. 125–134. https://doi.org/10.9734/arrb/2022/v37i330495
7. Amenu, D., and Bacha, K. Probiotic potential and safety analysis of lactic acid bacteria isolated from Ethiopian traditional fermented foods and beverages. *Annals of Microbiology*, 2023. p. 73, Article 1740. https://doi.org/10.1186/s13213-023-01740-9
8. Wejinya, A. O., et al., Probiotic potentials of lactic acid bacteria isolated from fermented foods. *European Journal of Nutrition & Food Safety*, 2022. 14(6): p. 251–262. https://doi.org/10.9734/ejnfs/2022/v14i630509
9. Adenike O., et al.,. Isolation, identification, and characterization of potential probiotics from fermented food products. *Asian Food Science Journal*, 2022. 21(5): p. 14–25. https://doi.org/10.9734/afsj/2022/v21i530427
10. Kho, H. M., et al., An overview of the role of lactic acid bacteria in fermented foods and their potential probiotic properties. *Borneo International Journal of Biotechnology*, 2023. 2: p. 18–29. https://doi.org/10.51200/bijb.v2i.4186
11. Yulita, V., Suprapti, B., and Amin, M. The characteristics of lactic acid bacteria isolated from fermented food as potential probiotics. *Journal of Basic and Clinical Physiology and Pharmacology*, 2021. 32(3): p. 341–350. https://doi.org/10.1515/jbcpp-2020-0482
12. Jeyagowri, N., et al., Phenotypic characterization and molecular identification of potentially probiotic *Lactobacillus* sp. isolated from fermented rice. *Preprints*, 2023, p. 3, Article 2094. https://doi.org/10.20944/preprints202307.2094.v1
13. Diaz, M., et al., *Lactobacillus garii* sp. nov., isolated from a fermented cassava product. *International Journal of Systematic and Evolutionary Microbiology*, 2020. 70(9): p. 4121–4129. https://doi.org/10.1099/ijsem.0.004121
14. Paillin, W. B. Probiotic potential of *Lactobacillus* strains isolated from fermented vegetables in Shaanxi, China. *Frontiers in Microbiology*, 2022, p. 13, Article 774903. https://doi.org/10.3389/fmicb.2021.774903
15. Sornsenee, P., et al., Probiotic properties of *Lactobacillus* species isolated from fermented palm sap in Thailand. *Probiotics and Antimicrobial Proteins*, 2021. 13(3): p. 684–695. https://doi.org/10.1007/s12602-021-09754-y
16. Zhang, T., Wang, J., and Chen, L. *Lactobacillus* species as potential probiotics for gut health improvement in fermented foods. *Journal of Applied Microbiology*, 2022. 132(8): p. 2336–2345. https://doi.org/10.1111/jam.15158
17. Soni, P., and Bhattacharya, S. Probiotic potential of *Lactobacillus* isolates from traditional Indian fermented foods. *BMC Microbiology*, 2023. 23(1): Article 215. https://doi.org/10.1186/s12866-023-02453-z
18. Abubakar, I., et al., Isolation, characterization, and probiotic potential of lactic acid bacteria from fermented fish. *African Journal of Microbiology Research*, 2022. 17(10): p. 1982–1993. https://doi.org/10.5897/ajmr2022.9872
19. Rai, K., Pandey, A., and Prakash, M. Probiotic *Lactobacillus* strains from fermented soybean products: Characterization and health benefits. *Frontiers in Nutrition*, 2023, 10, Article 1031786. https://doi.org/10.3389/fnut.2023.1031786
20. Elsaadany, K., EL-Sayed, A. I. M., and Awad, S. Identification, safety assessment, and antimicrobial characteristics of cocci lactic acid bacteria isolated from traditional Egyptian dairy products. *Foods,* 2024. 13(19): p. 3059. https://doi.org/10.3390/foods13193059
21. Costa, S., et al., Lactic acid production by *Lactobacillus casei* using a sequence of seasonally available fruit wastes as sustainable carbon sources. *Frontiers in Bioengineering and Biotechnology,* 2024, 12. https://doi.org/10.3389/fbioe.2024.1447278
22. Sharma, P., Mishra, S. S., and Kumari, P. *Lactobacillus* species and their potential for probiotic applications in the dairy industry. *International Journal of Dairy Technology*, 2022. 75(2): p. 153–165. https://doi.org/10.1111/1471-0307.12965
23. Banerjee, S., and Saha, S. Isolation and characterization of *Lactobacillus* strains from fermented foods for their probiotic potential. *Food Science and Biotechnology*, 2021. 30(4): p. 957–965. https://doi.org/10.1007/s10068-021-00858-7
24. Tripathi, D., Singh, M., and Gupta, R. Characterization of *Lactobacillus* isolates from fermented millets as novel probiotics. *Journal of Food Science and Technology*, 2024. 61(1): p. 212–220. https://doi.org/10.1007/s11483-023-08442-w
25. Xu, F., Liu, Y., and Zhang, R. Isolation and identification of *Lactobacillus* strains with probiotic potential from Chinese fermented vegetables. *Food Control*, 2023, 144, Article 109281. https://doi.org/10.1016/j.foodcont.2023.109281

26. Kim, T. H., and Lim, Y. J. Probiotic properties of *Lactobacillus* strains from traditional fermented foods in Korea. *Probiotics and Antimicrobial Proteins*, 2023. 15(6): p. 2149–2157. https://doi.org/10.1007/s12602-023-09956-w
27. Kiran, S., and Raghavendra, S. Isolation of *Lactobacillus* species from fermented foods and their potential for application as probiotics. *Journal of Microbiological Methods*, 2023, 183, Article 106178. https://doi.org/10.1016/j.mimet.2022.106178
28. Mokoena, M. P., Omatola, C. A., and Olaniran, A. O. Applications of lactic acid bacteria and their bacteriocins against food spoilage microorganisms and foodborne pathogens. *Molecules,* 2021. *26*(22): p. 7055. https://doi.org/10.3390/molecules26227055
29. Radhakrishnan, G., and Ravi, P. Antibacterial and probiotic characteristics of *Lactobacillus* species isolated from fermented foods. *Journal of Food Biochemistry*, 2023. 46(2): e13800. https://doi.org/10.1111/jfbc.13800
30. Hamid, T. H. T. A., and Amysya, N. F. (2021). Lactic acid bacterium with antimicrobial properties from selected Malay traditional fermented foods. *International Journal of Life Science and Biotechnology,* 2021. 4(1): p. 13–24. https://doi.org/10.38001/ijlsb.781522
31. Pateiro, M., and Dominguez, R. *Lactobacillus* species isolated from fermented meats: Safety, characterization, and their functional roles. *International Journal of Food Science & Technology*, 2024. 59(5): p. 2328–2339. https://doi.org/10.1111/ijfs.15101
32. Mohideen, N. H. R. H., et al., The co-isolation of lactic acid bacteria (LAB) and a related pathogenic strain from *Pangasius nasutus*. *International Journal of Life Science and Biotechnology,* 2023. 6(2): p. 143–154. https://doi.org/10.38001/ijlsb.1268388
33. Repally, A., et al.,. *Lactobacillus fermentum* SNR1: Probiotic efficiency and characterization of the antimicrobial peptide against dental caries and foodborne pathogens. *The Microbe,* 2024, 5: 100190. https://doi.org/10.1016/j.microb.2024.100190

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Araştırma Makale/ Research article

Cultural and Medicinal Uses of *Ferula foetida*: From Kyrgyzstan to Global Perspectives

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ABSTRACT

Ferula foetida holds significant botanical, medicinal, and economic importance in Kyrgyzstan. This literature review provides an in-depth exploration of medicinal Ferula, focusing on its chemical contents, traditional uses, pharmacological activities, and future considerations within the context of Kyrgyzstan. The morphological diversity of Ferula foetida is examined alongside their distribution patterns across the varied landscapes of Kyrgyzstan. Chemical constituents, including secondary metabolites, essential oils, and bioactive compounds, are elucidated, highlighting their potential therapeutic significance. Traditional uses of Ferula foetida in Kyrgyzstan encompass a wide range of applications in folk medicine. Pharmacological activities, such as antioxidant, anti-inflammatory, and antimicrobial properties, are discussed based on scientific evidence and traditional knowledge. Additionally, future considerations address the sustainable utilization and conservation of Ferula foetida. In Kyrgyzstan, particularly in the face of increasing export demands. Excessive exploitation of resources forces agriculturists and biologists in our country to urgently search for ways to balance the exploitation of natural populations, protect reserves, and cultivate valuable plants. The burgeoning export market underscores the economic value of Ferula foetida, prompting the need for responsible harvesting practices and conservation efforts to safeguard this valuable botanical resource for future generations.

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Introduction

Due to intense anthropogenic load and climate change, the Earth's vegetation cover is changing rapidly. These changes and trends are almost always undesirable, which challenges states with a predominant agricultural sector of the economy, prompting them to adapt to changing living conditions or move to resource-saving environmental management technologies [1]. Meadows turn into unnatural steppes, and the remains of natural steppes into lifeless wastelands; fresh water becomes a strategic resource, and the unprecedented amplitude of the intensity of surface runoff becomes an element of emergencies [2]. The present value of competitive agricultural technology for many crops has increased during the period of independence by 3–8 times, depending on the specific crop and region; this has already greatly influenced the structure of crop production in Kyrgyzstan and in the future will undoubtedly require corrective actions in response to the destabilization of plant ecosystems, regardless of what it is called [3]. The time has come to change agricultural tactics and switch to new crops that, in our specific conditions, require lower costs for irrigation and chemicals in the production cycle [4]. Conducting field expeditions and environmental monitoring throughout Kyrgyzstan, agronomists and researchers see the prerequisites for seeking to increase the profitability of crop production enterprises in the introduction into cultivation and industrial cultivation of some valuable species of medicinal plants that naturally grow in the Tien Shan and Pamir-Alai [5]. Nowadays, the irregular export of the cheapest and randomly harvested raw materials from the wild should be transformed into stable export items, ensuring profitability and protection from market surprises posed by competitors.

Kyrgyzstan occupies a central position within the Eurasian continent, situated in the northeastern region of Central Asia. The total land area spans approximately 199.95 thousand square kilometers [6]. It stretches

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from west to east - 900 km, from north to south – 450 km. Kyrgyzstan shares its borders with four neighboring countries: Kazakhstan, the western section of China (Xinjiang), Tajikistan, and Uzbekistan [42]. Situated amidst the Tien-Shan and Pamir-Alai mountain ranges, Kyrgyzstan encapsulates a diverse array of landscapes and climatic conditions, which can be classified into four distinct natural and climatic zones: valley-foothill (up to 1,200 meters), mid-mountain (ranging from 1,200 to 2,200 meters), high-mountain (spanning from 2,200 to 3,500 meters), and naval (elevations exceeding 3,500 meters) [7]. The climate of Kyrgyzstan is highly continental, primarily arid, and slightly smoothed by increased cloudiness and precipitation due to the high-mountainous relief. The climate peculiarities are determined by the location of the country in the Northern Hemisphere in the center of the Eurasian continent, as well as the distance from significant water bodies and proximity to deserts [5]. The flora of Kyrgyzstan is characterized by taxonomic diversity: the 4100 species mentioned above of vascular plants belong to 870 genera from 140 families [8]. Among these species, many are officially classified as useful wild plants: spice, medicinal, essential oils, etc. [9]. Among these diverse species, *Ferula foetida* stands out not only for its wide range of traditional uses but also for its potential in contributing to sustainable agricultural practices and economic development, which this review aims to explore comprehensively. This article offers a thorough and current analysis of *Ferula foetida*, examining its classification, physical traits, geographical range, varied applications, active compounds, health benefits, and potential for technological innovation. Moreover, it will consider the preservation challenges facing this species and its natural habitat. The overarching objective is to deepen the appreciation of *Ferula foetida*'s varied uses and importance, ranging from its role in traditional remedies to its possibilities in commercial sectors.

Morphological structure

The plant's height can exceed 1.5 m. Every year, there is a brief period of fast expansion and development. The generative shoots grow at a pace of 15 cm per day. A huge napiform root usually characterizes the plant's underground portion. *Ferula foetida* (Bunge) Regel grows a thick, tapering stem between the fourth and seventh years [10]. During this time, the plant forms a basal rosette. The leaves are triangular, long-petiolate, and pinnately divided in many places, with oblong-lanceolate end segments. The number of leaves varies with the plant's age. Apical leaves have a shorter petiole and are often seen with a single sheath. The plant's flowers are pale yellow, clustered in umbels, without wrappers, and form a vast inflorescence. The plant's fruit is a cremocarp that splits into two one-seeded pericarps with extensive border lines when ripe [10].

It is a perennial plant that can grow as tall as 12 feet in the wild, with a circular mass of leaves measuring 30-40 centimeters in diameter. The basal leaves feature broad sheathing petioles. Flowering stems, towering at 2.5–3 meters in height and 10 centimeters in thickness, possess a hollow structure. Within the cortex of these flowering stems lie schizogenous ducts that generate resinous gum. The blooms are tiny, yellowish, and grow in enormous compound umbels. This plant's fruits are round, thin, flat, and reddish-brown, containing a milky fluid inside. The roots are thick, massive, and pulpy. The distinctive scent is caused by the resin-like gum collected from the stems and roots [11].

Geographical distribution of *Ferula foetida*

Ferula foetida, a member of the Ferula genus, is indigenous to Central Asia, Eastern Iran, and western regions of Afghanistan and Pakistan. It stands as the most prevalent species responsible for the production of asafoetida (resin). Ferula L. includes about 200 species of flowering plants of the Apiaceae family in the world; some of these species are medicinal, nutritious, fodder, essential oil, and resinous plants. About 185 species of Ferula are found worldwide [12] and are mainly located in Central Asia, South Asia, the Middle East, and Siberia [13]. There are 114 species in Central Asia and more than 40 in Kyrgyzstan. 10 species are known as of endemics: *Ferula ferganensis* Lipsky ex Korovin, *Ferula inciso-serrata* Pimenov & J.V. Baranova, *Ferula kirialovii* Pimenov, *Ferula pallida* Korovin, *Ferula pimenovii* Lazkov, *Ferula prangifolia* Korovin, *Ferula renardii* (Regel & Schmalh.) Pimenov, *Ferula tenuisecta* Korovin, *Ferula tschimaganica* Lipsky ex Korovin, *Ferula ugamica* Korovin.

Species of the genus Ferula, mainly mountain plants, are found relatively high - at 300 to 3600 m above sea level, both on fine soils, variegated soils, and on gravelly slopes, screes, and pebbles. Like most plants of the Umbelliferae family, species of this genus contain essential oils or resinous substances, coumarins, flavonoids, and, less commonly, saponins in all their parts. In recent years, resins have begun to be produced in our country from the roots of *Ferula foetida* (Bunge) Regel. Over the past decade, most natural populations in Kyrgyzstan have been subject to increased exploitation due to the collection of resin from underground organs, mainly from adult virginal individuals. As a result, many plants, without reaching the generative stage of development, were exhausted and lost their viability.

Fig 1 Illustration of 3-4 years old *Ferula inciso-serrata* from Toktogul, Djalal-Abad

Chemical content

Ferula has various chemical components, including coumarins, particularly sesquiterpene coumarins, volatile oils, sulfur-containing chemicals, and aromatic compounds, all having different biological functions [14].
Coumarins: It belongs to the category of beta-D-glucosiduronic acid. Coumarins have been identified within the Ferula genus, with a significant portion deriving from 7-hydroxycoumarin as the primary nucleus [15]. Within the Ferula genus, coumarins can be categorized based on their substituents, primarily featuring sesquiterpene coumarins and monoterpene coumarins. Sesquiterpene coumarins are classified as bicyclic, monocyclic, straight-chain, and some furan and other coumarins [14].
Volatile oils: Ferula's volatile oils comprise terpenoids and polysulfide chemicals [16]. The volatile oils often contain a rich array of terpene components, comprising over 80% of their composition, predominantly monoterpenes and sesquiterpenes. Conversely, polysulfide compounds are chiefly characterized by disulfides, trisulfides, bis-disulfides, and thio-disulfides [14].

Fig 2 Distribution areas of *Ferula foetida* in the world

Anticancer activity: The main chemical components of Ferula plants that exert anti-tumor effects are ferulic acids [17], sesquiterpenoids [18], and volatile oils [19].
According to an analysis, resin comprises carbohydrates - 67.8% per 100 gms, moisture 16.0%, minerals 7.0%, protein 4.0%, fat 1.1%, and fiber 4.1%. In addition to phosphorus, iron, carotene, riboflavin, and niacin, it contains a high calcium concentration. The calorific value is 297, and it contains 40-64% resinous material composed of ferulic acid, asaresinotannols, umbel-liferone, farnesiferols A, B, and C, about 25% gum composed of glucose, l-arabinose, rhamnose, and glucuronic acid, galactose and volatile oil (3-17%) consisting of disulfides as its major components, notably 2-butyl propenyl disulfide (E- and Z-isomers), with monoterpenes (α- and β-pinene, etc.). The unpleasant odor of the oil is attributed primarily to the disulphide $C_{11}H_{20}S_2$ [20].

Traditional uses

F. foetida has been utilized in Central Asia for an extended period and in Ayurvedic and herbal medicine traditions. It is valuable in abdominal pain-relieving and reducing vascular disorders, in cases of anxiety disorders, liver troubles, and indigestion. The root of *F. foetida* is one of drugs in practice not only by physicians, but also as a home remedy.
Use in Kyrgyzstan: *F. foetida,* known colloquially as the "Sasyk chair" or "Uuljan", holds a storied heritage deeply ingrained in Kyrgyzstan's cultural and medicinal tapestry. Thriving primarily in the Alai, Fergana, and Ketmen-Tube valleys, this versatile plant has been integral to Kyrgyz traditional medicine for centuries. Revered for its roots, which form a cornerstone in countless remedies, it symbolizes the resilience of Kyrgyz culture and its enduring connection to nature's bounty. One prominent use of *F. foetida* in medicine is its utilization in treating whooping cough. Additionally, it has anticoagulant properties and reduces blood pressure. In the polluted climate of Bishkek, respiratory illnesses are prevalent, and *F. foetida* contributes to addressing these health issues.
Traditional remedies frequently incorporate *F. foetida* to alleviate symptoms associated with common respiratory infections like asthma, whooping cough, and bronchitis.
This is credited to its expectorant qualities, potential anti-inflammatory effects, and antimicrobial properties, all of which are advantageous for respiratory well-being. *F. foetida* is highly esteemed in Kyrgyz traditional medicine for its capacity to address gastrointestinal tract problems, particularly stomach-related issues such as flatulence and distension. Local people in Toktogul, Djalal-Abad, use *Ferula inciso-serrata*'s roots for women's reproductive concerns, teenage skin issues like acne, etc. They cut the root into pieces, dry it out for several days under dark conditions, and then dissolve the piece of root in hot water. When the root is dissolved, locals drink a warm infusion every evening for ten days, rest for one month, and drink again for another ten days (Figure 3). In addition to its medicinal applications, *F. foetida* has also been incorporated into commercial markets. The properties of ferulic acid render it a fitting component in skincare cosmetics, including serums, creams, and anti-aging products. Its anti-inflammatory properties may prevent pimples and reduce the appearance of discoloration. Cell damage can make skin look loose or saggy, while ferulic acid can preserve skin firmness. It also prevents the appearance of new blood vessels under the skin, which leads to reduced skin redness.
Presently, certain areas in Central Asia, including Kyrgyzstan, are witnessing a surge in the sale of resin to neighboring nations, leading to the depletion of stocks. This situation raises concerns regarding the sustainability of plant utilization. Hence, innovative approaches need to be devised to look after the resources of Ferula species. To tackle these concerns, various initiatives have been implemented to encourage the sustainable cultivation and conservation of *F. foetida* in Kyrgyzstan. For instance, a moratorium on special permittance of the cultivation of *F. foetida* by the Ministry of Natural Resources, Ecology, and Technical Supervision of Kyrgyz Republic.


Fig 3 a) Root of Ferula inciso-serrata, b) traditional use of its roots in Toktogul, Kyrgyzstan

Use in Xinjiang Uyghur Region (China): Ferula species serve as a medicinal herb in traditional medicine in Uyghur, boasting a lengthy history of usage [14]. Ferula has already been integrated into the Uyghur Medicine Criteria [21]. Additionally, Ferula has been included in the calendar version of the Chinese Pharmacopoeia [22]. It stands as a potent remedy for various ailments and possesses a long history of usage in numerous traditional medicines. For instance, Nepali people include Ferula as spice in their everyday meals (Chinese Pharmacopoeia Commission, Beijing, China, 2020). Ferula's traditional uses include healing against indigestion, flatulence, stomach pain, intestinal parasites, asthma, and flu [23].

The documented advantages of Ferula encompass lump elimination, deworming, antibacterial properties, and other traditional applications [14].

Traditional Chinese medicines (TCMs) significantly impact China's contemporary healthcare system and have demonstrated effectiveness in clinical settings. Even with the lack of standardized criteria for selecting quality control measures has led to uncertainty regarding the certification and effectiveness of herbal medicines. Several studies have documented Q-marker research for the genus Ferula. Therefore, using the Q-marker method to identify various markers for the genus Ferula is crucial for ensuring its authenticity and conserving plant resources [14]. Moreover, *F. foetida* extends its healing potential to treating intestinal parasites, such as helminths [24]. Broadening its scope, *F. foetida* is prominent in addressing stomach issues within TCM.

Use in Iranian traditional medicine: *F. foetida* is a rich source of resin and is much utilized in folklore medicine [25]. A study published in the European Review for Medical and Pharmacological Sciences found that *F. foetida'*s stem, flower, and leaf extracts have potent antihaemolytic and antioxidant properties, which are very promising for biochemical experiments. The widespread use of traditional medicine continues, and plants remain a significant source of natural antioxidants; ayurvedic texts have categorized ferula as an appetizer and a restorer of consciousness [20]. These antioxidants could serve as starting points for developing new pharmaceuticals [21]. The presence of phenols and flavonoids in the extract could be responsible for its biological effects [25]. A study published in the Iranian Journal of Basic Medical Sciences found that *F. foetida*'s extracts have anti-inflammatory antioxidant and immunomodulatory activities that may support its traditional use in these conditions [26].

Use in traditional Indian medicine: *F. foetida* boasts a lengthy history of utilization in traditional Indian medicine - Ayurveda. Below are instances of its traditional application accompanied by references to supporting research: *F. foetida* has been traditionally used as a nervine stimulant, digestive agent, and a sedative [20]. The dried resin is extracted with hot water and taken orally as an emmenagogue [27], and hot water extract of dried gum is given orally as a carminative, antispasmodic, and expectorant in chronic bronchitis. Dried gum resin exudates are eaten to avoid guinea worm disease. *F. foetida* is extensively utilized globally as a flavoring spice in various meals. Traditionally, it has been employed to address a spectrum of ailments like asthma, epilepsy, stomach discomfort, gas, intestinal parasites, poor digestion, and flu. Recent pharmacological and biological research has additionally revealed multiple properties associated with resin, including antioxidant, antiviral, antifungal, cancer prevention, antidiabetic, antispasmodic, blood pressure-lowering, and molluscicidal effects [20].

Pharmacological activities

F. foetida, a traditional medicinal plant, has been historically utilized to address a broad spectrum of health concerns. Renowned for its diverse pharmacological properties, including antibacterial, anti-inflammatory, antioxidant, anticarcinogenic, antitumor, antihelmintic, and hepatoprotective activities, as outlined in Figure

4 below, it harbors numerous bioactive compounds such as umbelliprenin, methyl galbanate, ferutinin, teferin, ferulic acid, and eferidin. Extensively studied through both in vitro and animal model experiments, these compounds have exhibited promising potential in treating neurological disorders, dizziness, asthma, headache, inflammation, bronchitis, rheumatism, and gastrointestinal disorders [28].

Gastrointestinal tract healing: *F. foetida* has traditionally been used or several stomach diseases. Studies have shown that *F. foetida* herb is one of the best remedies available for flatulence and distension of the stomach [20].

Table 1 The chemical constituent accountable for the pharmacological effects [20]

Pharmacological activity	**Responsible chemical constituent**
Anticancer	a-pinene; a-terpineol; diallyl-disulfide; ferulic-acid; isopimpinellin; luteolin; umbelliferone; vanillin
Anti-inflammatory	a-pinene; azulene; B-pinene; ferulic-acid; isopimpinellin; luteolin; umbelliferone
Antileukemic	Luteolin
Antimutagenic	Diallyl-sulfide; ferulic-acid; luteolin; umbelliferone; vanillin
Antineoplastic	Ferulic-acid
Antitumor	Diallyl-disulfide; diallyl-sulfide; ferulic-acid; luteolin; vanillin
Antiviral	a-pinene; diallyl-disulfide; ferulic-acid; luteolin; vanillin
Antibacterial	a-pinene; a-terpineol; azulene; diallyl-disulfide; diallyl-sulfide; ferulic-acid; luteolin; umbelliferone
Antispasmodic	Azulene; ferulic-acid; luteolin; umbelliferone; valeric-acid
Antiseptic	a-terpineol; azulene; B-pinene; diallyl-sulfide; umbelliferone
Lipoxygenase-inhibitor	Luteolin; umbelliferone
Antiulcer	Azulene
Hepatoprotective	Ferulic-acid; luteolin
Anti-HIV	Diallyl-disulfide; luteolin
Antinitrosaminic	Ferulic-acid
Antioxidant	Ferulic-acid; luteolin; vanillin
Antiaggregant	Ferulic-acid
Tranquilizer	a-pinene; valeric-acid
Antiproliferative	Diallyl-disulfide
Apoptotic	Luteolin
Anticarcinogenic	Ferulic-acid; luteolin
B-Glucuronidase-Inhibitor	Luteolin
Immunostimulant	Diallyl-disulfide; ferulic-acid
Antihepatotoxic	Ferulic-acid; glucoronic-acid
Antiprostaglandin	Umbelliferone
Antihyaluronidase	Luteolin
Cytotoxic	Luteolin
Sedative Ornithine-Decarboxylase-Inhibitor	a-pinene; alpha-terpineol; valeric-acid Ferulic-acid
PTK-Inhibitor	Luteolin

Anti-inflammatory and analgesic: *F. foetida* has been reported to have anti-inflammatory and analgesic properties. Studies have shown that root extracts have significant anti-inflammatory, anti-nociceptive, and analgesic effects [29].

Antibacterial: *F. foetida* has potent antibacterial activity against a range of batteries. The dry roots affected *Staphylococcus aureus*, *Bacillus subtili and* Sporosarcina [14].

Antifungal activity: *F. foetida*'s oils have been found to have antifungal activity against several fungal pathogens, including *Aspergillus niger* and *Candida albicans*. The highest antifungal activity of this plant was reported with methanolic extract [31].

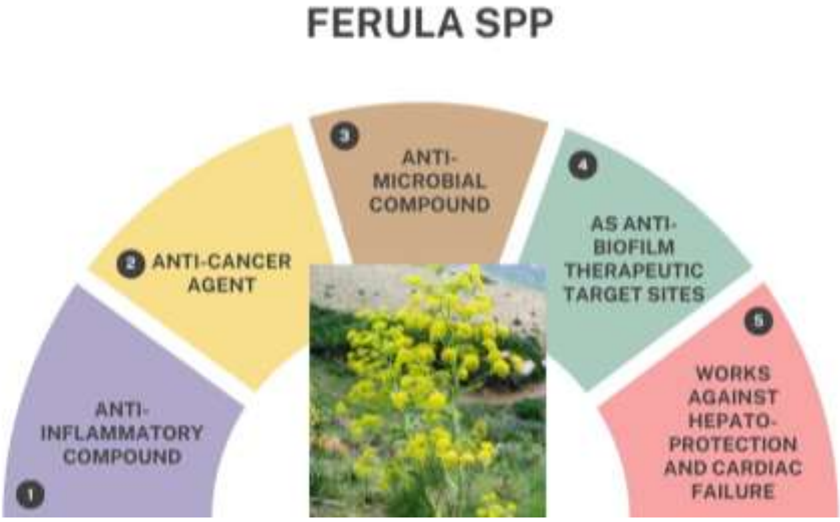

Fig 3 Multi-faced action of Ferula species

Antioxidant activity: Studies have shown that *F. foetida*'s essential oil extracts obtained from gum resins have significant antioxidant activity that may be used as safe and effective natural antioxidants in the food industry to improve the oxidative stability of fatty foods during storage [32].
Immunomodulatory effects: Some compounds, such as terpenes and several other natural agents present in essential oils of Ferula species have shown immunomodulatory properties [33].
Anticancer activity: Research has indicated that *F. foetida* demonstrates anticancer activity owing to its antiproliferative characteristics, notably by compounds like serotonin. These substances have demonstrated efficacy in restraining the proliferation of breast cancer cells [34]. Umbelliprenin compounds induced apoptosis and showed cytotoxic and cytostatic effects against human solid cancer cells (melanoma) [35].
Hepatoprotective: *F. foetida* has been documented to possess hepatoprotective attributes, shielding the liver from harm induced by diverse toxins and pharmaceutical agents. The extract was effective against oxidative damage induced by CCl_4 [36].
Neuroprotective: *F. foetida* has been found to exhibit neuroprotective and nerve-stimulating effects in glutamate-induced neurotoxicity. The extract demonstrated antiapoptotic effects in cerebellar granule neurons, shedding light on the advantageous impacts of *F. foetida* extract in treating neurological ailments [37].
Antiulcer properties: *F. foetida* is reported to have gastric antiulcer properties. The gum resin has shown that this effect may be or mainly related to antioxidant, anticholinergic, and antihistaminergic effects [30].
Antihelmintic activity: *F. foetida* is used in traditional medicine to treat diabetes. Aqueous and dry root latex powder extracts from Ferula have exhibited significant antihelmintic activity against *Pheretima posthuma* and liver fluke *Fasciola gigantic* [38].
Antispasmodic activity: *F. foetida* has been reported to have antihelmintic properties. Studies have shown that *F. foetida* aqueous gum extract has significant antispasmodic and hypotensive activity in reducing blood pressure and decreased contractions induced by acetylcholine, histamine, and KCl in the isolated guinea-pig ileum [39].
Antitumor activity: *F. foetida* has been found to have potential anticancer and antitumor activity. Studies have shown that *F. foetida*'s flowers and leaves have a cytotoxic effect on cancer cell lines, including liver (HEPG2) carcinoma cells, cervical (HELA), and breast (MCF7) [40]. Toxic effect: *F. foetida* has been linked to potentially increasing the effectiveness of warfarin [41]. Chromosomal damage has been linked to the coumarin compounds found in *F. foetida* [20].

Fig 4 Different bioactivities of Ferula species

Future considerations

Extensive research and awareness efforts are necessary in Batken, Jalal-Abad regions and neighboring areas of Kyrgyzstan. While *F. foetida*'s leaves, flowers, and root extracts remain integral to ethnomedicinal therapies, comprehensive knowledge of their formulations and applications is primarily preserved in literature and among a select cadre of regional physicians. As root harvesting threatens plant sustainability, proactive measures must be taken to protect these invaluable botanical resources from overexploitation. Though some facets of ethnomedicinal science have been explored, there remains a compelling need for further investigation across diverse fronts, including cancer treatment, antioxidant properties, geographical mapping, metabolomics, bioinformatics, genomics, proteomics, and data-driven analyses. Examination of secondary metabolites like ferulic compounds should leverage in vitro methodologies to circumvent animal-related ethical concerns. While much research has centered on the pharmacological and therapeutic potentials of *F. foetida*, its biotechnological dimensions, such as cell culture techniques, warrant greater scrutiny. The natural habitats of *F. foetida* in Batken, particularly in Leilek and Margun villages, necessitate commercial viability and ecological preservation attention. Collaborative efforts among forestry agencies, research entities, and non-governmental organizations are imperative to safeguard *F. foetida* and its wild endemic species, employing a blend of scientific innovations and traditional conservation practices.

Future considerations for the study of *F. foetida* should include the preservation and sustainable use of this critical species and the advancement of biotechnological methods, such as tissue culture, to promote conservation and commercial viability. Tissue culture techniques can play a crucial role in propagating *F. foetida*, especially given the plant's slow natural reproduction rates and the threats posed by overharvesting. By establishing in vitro propagation protocols, it is possible to produce large quantities of the plant without further depleting wild populations. This method also allows for the genetic enhancement of plant, potentially introducing traits that could increase resistance to diseases and environmental stresses, thereby supporting the development of varieties suited for different climatic and soil conditions.

Integrating these biotechnological strategies with traditional cultivation practices will help conserve this valuable species and ensure its sustainable commercial production. This dual approach promises to secure the future of *F. foetida* both as a cultural heritage and an economic resource in Kyrgyzstan and beyond, aligning with global efforts in conservation biology, biodiversity and sustainable agriculture.

Conclusion

In summation, this comprehensive literature review on the phytochemistry and pharmacological activities of *F. foetida* underscores its profound significance within Kyrgyzstan and throughout Central Asia. Scientific inquiry has illuminated the traditional applications of this botanical treasure across a spectrum of health concerns, validating its efficacy by identifying a plethora of bioactive compounds with considerable therapeutic potential. Indeed, the role of *F. foetida* across Central Asian nations is immeasurable, its historical reputation as a panacea for neurological, gastrointestinal, and cytotoxic maladies enriched by the discovery of phytochemical gems like umbelliprenin, ferulic acids, azulene, luteolin, and vanillin derivatives. These bioactive constituents exhibit various pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, and antihyperglycemic effects, harmonizing with the plant's traditional applications. However, beyond its medicinal virtues, *F. foetida* assumes a pivotal economic role in Central Asian countries. However, rampant over-harvesting and habitat degradation underscore pressing sustainability concerns. *F. foetida* transcends mere botanical taxonomy; it embodies a cultural and medicinal legacy deeply ingrained in diverse traditions worldwide.

Biotechnological approaches, including genetic engineering and molecular breeding, could be harnessed to enhance the production of specific bioactive compounds found in *F. foetida*. These compounds, particularly ferulic acids and other secondary metabolites, are of significant pharmaceutical and industrial interest due to their medicinal properties. Advanced biotechnological tools can help identify and amplify the critical genes involved in the biosynthesis pathways of these compounds, leading to increased yields and more efficient use of the plant in pharmaceuticals and nutraceuticals. Furthermore, community involvement and robust legislative protections are essential to ensure sustainable management and preservation of *F. foetida*, combining traditional knowledge with advanced biotechnological strategies to safeguard this valuable botanical legacy for future generations.

References

1. Caretta, M.A., A. Mukherji, M. Arfanuzzaman, R.A. Betts, A. Gelfan, Y. Hirabayashi, T.K. Lissner, J. Liu, E. Lopez Gunn, R. Morgan, S. Mwanga, and S. Supratid, 2022: Water. In: Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp. 551–712, doi:10.1017/9781009325844.006
2. Usupbaev A.K., Milko D.A.: «Zelenoe zoloto Kyrgyzstana», ili k oprosu o strategicheskom potenciale poleznyh dikorastushih rastenii // Vestnik mezhdunarodnogo medicinskogo universiteta, Bishkek, 2022, № 4 C. 27-36. (Усупбаев А.К., Милько Д. А. «Зелёное золото Кыргызстана», или к опросу о стратегическом потенциале полезных дикорастущих растений // Вестник международного медицинского университета, Бишкек, 2022, № 4 С. 27-36)
3. Baylea, A., & Kaya, Y. (2023): Chemical Characteristics and Biotechnological Potentials of Mumio. Manas Journal of Agriculture Veterinary and Life Sciences, 13(2), 187-195. https://doi.org/10.53518/mjavl.1327332
4. Taşeva, G., Sabırkulova, T., Kıdıralıyeva, B., Omurzakova, N., et al. (2022): Kırgızistan çeltik üretimine genel bir bakış. Frontiers in Life Sciences and Related Technologies, 3(2), 86-94. https://doi.org/10.51753/flsrt.1122101
5. FAO (2022): Comprehensive analysis of the disaster risk reduction system for the agricultural sector in Kyrgyzstan. Budapest, https://doi.org/10.4060/cb8418en
6. Narbayep, Marat and Pavlova, Vera. The Aral Sea, Central Asian Countries and Climate Change in the 21st Century. United Nations ESCAP, IDD, April 2022. Bangkok.
7. Tojibaev KS, Karimov FI, Hoshimov HR, Gulomov R, Lazkov GA, Jang C-G, Gil H-Y, Jang J-E, Batoshov AR, Iskandarov A, Choi HJ (2023): Important plant areas (IPAs) in the Fergana Valley (Central Asia): The Bozbu-Too-Ungortepa massif. Nature Conservation 51: 13-70. https://doi.org/10.3897/natureconservation.51.94477
8. Камелин Р. В. Ботанико-географические районы Киргизии / Р. В. Камелин // Зонтичные Киргизии. – Москва : KMK Scientific Press Ltd, 2002. – С. 1-18.
(Kamelin R. V. Botaniko-geograficheskiye rayony Kirgizii / R. V. Kamelin // Zontichnyye Kirgizii. – Moskva : KMK Scientific Press Ltd, 2002. — C. 1–18.)
9. Shalpykov K. T., (2015): Lekarstvennyye i aromaticheskiye rasteniya v Kyrgyzstane. Ohrana i ustoychivoye ispol'zovaniye resursov lekarstvennykh rasteniy // Sbornik materialov ostayetsya mezhdunar. nauch. prakt. konf. — Bishkek, 2015. — S. 122–127. (Шалпыков К. Т., (2015): Лекарственные и ароматические растения в Кыргызстане. Охрана и устойчивое использование ресурсов лекарственных растений // Сборник материалов третьей междунар. науч. практ. конф. — Бишкек, 2015. — С. 122–127)
10. Sagyndykova, M.S. & Imanbayeva, Akjunis & Belozerov, I.F.. (2018): Polymorphism of morphological characteristics of *Ferula foetida* (Bunge) regel in the natural populations of the Mangyshlak peninsula. Journal of Pharmaceutical Sciences and Research. 10. 2084-2091.
11. Kareparamban, J.A. & Nikam, P.H. & Jadhav, A.P. & Kadam, V.J.. (2012): *Ferula foetida*" hing": A review. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 3. 775-786.
12. Salehi M., Naghavi M.R., Bahmankar M.: A review of Ferula species: Biochemical characteristics, pharmaceutical and industrial applications, and suggestions for biotechnologists. Ind. Crops Prod. 2019;139:111511. doi: 10.1016/j.indcrop.2019.111511.
13. Znati M., Hichem B.J., Cazaux S., Bouajila J.: Chemical composition, biological and cytotoxic activities of plant extracts and compounds isolated from Ferula lutea. Molecules. 2014;19:2733–2747. doi: 10.3390/molecules19032733.
14. Bahetjan, Y.; Muhaxi, M.; Pang, K.; Kizaibek, M.; Tang, H.; Sefidkon, F.; Yang, X.: Chemistry, Bioactivity, and Prediction of the Quality Marker (Q-Marker) of Ferula
15. Lin, J.R.; Jin, M.; Wu, C.M.: Advances in studies on chemical constituents and pharmacological effects of Ferula. Strait Pharm. J. 2014, 26, 1–3.
16. Liu, Q.X.; Hui, H.: The chemical constituents of volatile oil from *Ferula* L. in China and its taxonomical significance. J. Plant Resour. Environ. 1997, 6, 27–32.
17. Ying, F.H.; Qian, X.Q.; Liu, B.R.: Progress in the study of the anti-tumor mechanism of action of ferulic acid. Mod. J. Integr. Tradit. Chin. West. Med. 2010, 19, 4238–4240.

18. Suzuki, K.; Okasaka, M.; Kashiwada, Y.; Takaishi, Y.; Honda, G.; Ito, M.; Takeda, Y.; Kodzhimatov, O.K.; Ashurmetov, O.; Sekiya, M.; et al.: Sesquiterpene Lactones from the Roots of Ferula Waria and Their Cytotoxic Activity. J. Nat. Prod. 2007, 70, 1915–1918.
19. Wang, S.; Sheng, P.; Yao, L.; Du, B.J.: GC-MS fingerprint of in vitro anti-gastric cancer active parts from roots of Uygur medicine *Ferula ferulaeoides*. Chin. Tradit. Herb. Drugs 2015, 46, 2874–2879.
20. Mahendra P, Bisht S.: Ferula *asafoetida*: Traditional uses and pharmacological activity. Pharmacogn Rev. 2012 Jul;6(12):141-6. doi: 10.4103/0973-7847.99948. PMID: 23055640; PMCID: PMC3459456.
21. Divya, K.; Ramalakshmi, K.; Murthy, P.S.; Jagan Mohan Rao, L.: Volatile oils from *Ferula asafoetida* varieties and their antimicrobial activity. LWT Food Sci. Technol. 2014, 59, 774–779.
22. Javanshir, S.; Soukhtanloo, M.; Jalili-Nik, M.; Yazdi, A.J.; Amiri, M.S.; Ghorbani, A.: Evaluation Potential Antidiabetic Effects of *Ferula latisecta* in Streptozotocin-Induced Diabetic Rats. J. Pharmacopunct. 2020, 23, 158–164.
23. Lee, C.L.; Chiang, L.C.; Cheng, L.H.; Chuang, L.C.; Mohamed, H.A.E.-R.; Chang, F.R.; Wu, Y.C.: Influenza A (H1N1) Antiviral and Cytotoxic Agents from *Ferula assa-foetida*. J. Nat. Prod. 2009, 72, 1568–1572.
24. Duke JA, Ayensu ES: Medicinal plants of China. Vol. 1. Algonac, Michigan: Reference Publications Inc; 1985. pp. 52–361.
25. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Eslami B, Dehpour AA.: Antioxidant and antihaemolytic activities of *Ferula foetida* regel (Umbelliferae). Eur Rev Med Pharmacol Sci. 2011 Feb;15(2):157-64. PMID: 21434482.
26. Ghasemi Z, Rezaee R, Aslani MR, Boskabady MH.: Anti-inflammatory, anti-oxidant, and immunomodulatory activities of the genus Ferula and their constituents: A review.
27. Kamboj, V.P. (1988): A review of Indian medicinal plants with interceptive activity. Indian J. Med. Res., 3: 336-355
28. Nazari ZE, Iranshahi M.: Biologically active sesquiterpene coumarins from Ferula species. Phytother Res. 2011 Mar;25(3):315-23. doi: 10.1002/ptr.3311. Epub 2010 Oct 28. PMID: 21031633.
29. Bagheri SM, Hedesh ST, Mirjalili A, Dashti-R MH.: Evaluation of anti-inflammatory and some possible mechanisms of antinociceptive effect of *Ferula assafoetida* oleo gum resin. Evid Based Complement Alternat Med. 2016; 21:271–276.
30. Bagheri SM, Yadegari M, Zare-Mohazabiye F, Momeni-Asl H, Mirjalili A, Anvari M, et al.: Effect of *Ferula assa foetida* oleogum-resin on gastric ulcer in indomethacin-ulcerated rats. J Curr Res Sci Med 2018; 4:42-6
31. Vilas A. Kamble & Sahadeo D. Patil (2008): Spice-Derived Essential Oils: Effective Antifungal and Possible Therapeutic Agents, Journal of Herbs, Spices & Medicinal Plants, 14:3-4, 129-143, DOI: 10.1080/10496470802598677
32. G. Kavoosi, V. Rowshan.: Chemical composition, antioxidant and antimicrobial activities of essential oil obtained from *Ferula asafoetida* oleo-gum-resin: effect of collection time. Food Chem, 138 (2013), pp. 2180-2187
33. Oüzek G, Schepetkin IA, Utegenova GA, Kirpotina LN, Andrei SR, Oüzek T, et al.: Chemical composition and phagocyte immunomodulatory activity of *Ferula iliensis* essential oils. J Leukoc Biol. 2017;101:1361–1371.
34. Safi R, Rodriguez F, Hilal G, Diab-Assaf M, Diab Y, El-Sabban M, et al.: Hemisynthesis, antitumoral effect, and molecular docking studies of ferutinin and its analogues. Chem Bio & Drug Design. 2016;87:382–397.
35. Barthomeuf C, Lim S, Iranshahi M, Chollet P.: Umbelliprenin from *Ferula szowitsiana* inhibits the growth of human M4Beu metastatic pigmented malignant melanoma cells through cell-cycle arrest in G1 and induction of caspase-dependent apoptosis. Phytomedicine. 2008;15:103–111.
36. Deniz G, Laloglu E, Koc K, Geyikoglu F.: Hepatoprotective potential of *Ferula communis* extract for carbon tetrachloride induced hepatotoxicity and oxidative damage in rats. Biotech Histochem. 2019;94:334–340.
37. G.S. Tayeboon, F. Tavakoli, S. Hassani, M. Khanavi, O. Sabzevari, S.N. Ostad.: Effects of Cymbopogon citratus and *Ferula asafoetida* extracts on glutamate-induced neurotoxicity. In vitro Cell Dev Biol-Anim, 49 (2013), pp. 706-715
38. R. Gundamaraju.: Evaluation of anti-helmintic activity of *Ferula foetida* “Hing- A natural Indian spice” aqueous extract. Asian Pac J Trop Dis, 3 (2013), pp. 189-191
39. M. Fatehi, F. Farifteh, Z. Fatehi-Hassanabad.: Antispasmodic and hypotensive effects of *Ferula asafoetida* gum extract. J Ethnopharmacol, 91 (2004), pp. 321-324
40. Elghwaji W., El-Sayed A. M., El-Deeb K. S., ElSayed A. M. (2017): Chemical composition, antimicrobial and antitumor potentiality of essential oil of *Ferula tingitana* L. Apiaceae grow in Libya. Phcog. Mag. 13 (3), 446–451. 10.4103/pm.pm_323_15
41. Heck AM, DeWitt BA, Lukes AL.: Potential interactions between alternative therapies and warfarin. Am J Health Syst Pharm. 2000;57:1221–7.
42. Aldayarov, N., Tulobaev, A., Salykov, R., Jumabekova, J., Kydyralieva, B., Omurzakova, N., Kurmanbekova, G., Imanberdieva, N., Usubaliev, B., Borkoev, B., Salieva, K., Salieva, Z., Omurzakov, T., & Chekirov, K. (2022). An ethnoveterinary study of wild medicinal plants used by the Kyrgyz farmers. Journal of Ethnopharmacology, 285, 114842. https://doi.org/10.1016/j.jep.2021.114842

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Kocalishkan, I., Allelopathy. 2006, Ankara, Turkey: Our Office Press-In Turkish. ISBN:xxxxxxxxxxxxxx

Example for Book Chapter;

Kaya, Y., F.Z. Huyop, and M.F. Edbeib, Genetic Diversity in Plants, Advances in Biosciences, F.Z. Huyop and S. Mohammed, Editors. 2019, Penerbit UTM Press. Malaysia: Malaysia. p. 04-24. Doi:xxxxxxxxxxxxxxxxxxxxxxxxx or ISBN:xxxxxxxxxxx

Thesis
Kaya, Y., Regeneratıon of Dalapon Tolerant Nicotıana Tabacum By Expressıng Dehalogenase E Gene Through Agrobacterıum Tumefaciens – Medıated Transformatıon. 2013, Universiti Teknologi Malaysia: Malaysia.

Example for congress/conferences paper;

Arvas, Y.E., et al., Possible Effects of Genetically Modified Plants on Biodiversity, in I. International Organic Agriculture and Biodiversity Symposium. 2017: Berlin- Germany. p. 65.

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DECLARATION:

This work is part of Gülfidan KUYUMCU's MA thesis. Articles on 27-29 September 2017

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Genetic Analysis Related To Organized Genetic Changes in Potato And Processed Potatoes

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