International Journal of
Life Sciences *and* Biotechnology

Life Sciences

Plant Biotechnology

Biotechnology

Genetics & Genomics

Agricultural Biotechnology

Bioinformatics

Food Science

Management Office / Yönetim Ofisi

Yildiz Technical University, Faculty of Chemical and Metallurgical Engineering.
ESENLER/İSTANBUL

From The Editor;

Dear Readers and Authors,

As "International Journal of Life Sciences and Biotechnology", we are pleased and honored to present the 20th 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 third issue of 2024 "İnternational Journal of Life Sciences and Biotechnology". Hope to see you in the next issue…

Editörden;

Değerli okurlar ve yazarlar,

"İnternational Journal of Life Sciences and Biotechnology" olarak dergimizin yirminci 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 2024 yılı üçüncü sayısını yayınladığımız "Internatıonal Journal of Lıfe 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…

15.12. 2024
Editör
Prof. Dr. Ali Aslan

Reviewers of the Issue / Sayının Hakemleri[*]

Dr. Azhybekov Baktybek, Ph.D., Kyrgyz - Turkısh Manas University

Dr. Belma Nural Yaman, Ph.D., Eskişehir Osmangazi University

Dr. Dilek Kandemir, Ph.D., Ondokuz Mayıs University

Dr. Erdem Gülümser, Ph.D., Bilecik Şeyh Edebali University

Dr. Fatih Alay, Ph.D., Samsun Black Sea Agricultural Research Institute

Dr. Hossein Zeinalzadeh Tabrizi,Ph.D., Kyrgyz - Turkısh Manas University

Dr. Irgashev Almazbek, Ph.D., Kyrgyz - Turkısh Manas University

Dr. Mahmut Ünver, Ph.D., Kırıkkale University

Dr. Maulanov Amangeldi, Ph.D., Kazakh National Agricultural Research University

Dr. Mehmet Fidan, Ph.D., Siirt University

Dr. Melek Mümine Erol, Ph.D., Istanbul Technıcal University

Dr. Merve Göre, Ph.D., Ege University

Dr. Muhammet Safa Hacıkamiloğlu, Ondokuz Mayıs University

Dr. Muzaffer Mükemre, Ph.D., Hakkarı University

Dr. Onur İnan, Ph.D., Selcuk University

Dr. Sakir Tasdemir, Ph.D., Selcuk University

Dr. Sıtkı Ermiş, Ph.D., Eskişehir Osmangazi University

Dr. Şahane Funda Arslanoğlu, Ph.D., Ondokuz Mayıs University

Dr. Ugur Başaran, Ph.D., Yozgat Bozok University

Dr. Vedat Pirinç, Ph.D., Dicle University

Contents/ İçindekiler

Research Articles/ Araştırma Makaleleri

Erratum / Düzeltme

Antarctic Microalgae Growth in Simulated Wastewater

Deniz Erçetin[1] [iD] **, Benan İnan[2]** [iD] **, Didem Balkanlı[2*]** [iD]

ABSTRACT

In recent years, the number of scientific studies in the Antarctic and Arctic regions has increased considerably. While scientific studies allow us to explore the untouched nature of the region and better understand the global climate, they also raise various ecological concerns such as wastewater, air pollution and habitat destruction. This threatens the flora and fauna of the polar regions, negatively affecting biodiversity. It is crucial that we protect the fragile ecosystems of the polar regions through sustainable research practices and international cooperation to prevent pollution, protect habitats and prevent the introduction of invasive species. The Antarctic Treaty and other protocols prohibit the discharge of wastewater into the sea without treatment. For this reason, many research bases in Antarctica have established facilities to treat wastewater. These facilities ensure that wastewater is treated and returned to the sea without harming the environment. However, the wastewater treatment process generates a significant amount of solid waste. As this waste accumulates in the treatment plants, it has to be transported back to the mainland. Antarctica's remoteness and challenging geographical conditions make solid waste transportation logistically difficult and costly. In order to solve these problems, it was aimed to use the algal blooms occurring in Antarctica in the treatment process by cultivating them in wastewater. In the study, it was found that the Antarctic microalgae can be cultivated in domestic wastewater in Antarctic bases and have a high potential for the proposed activities by having approximately 30% of protein content.

Introduction

Antarctica, the world's fifth largest continent in terms of area, may seem barren and devoid of life at first glance, but it is a real treasure for our planet with its unique ecosystem [1]. It is rich in strategically important resources such as oil, natural gas, minerals and water. The continent is the driest, windiest and coldest region in the world, making life and work very difficult [2]. However, these challenging conditions provide a unique environment for scientific research and an important opportunity to understand the ecological and geological structure of the continent [3].

The Antarctic Treaty and the Madrid Protocol are the principles of peace, science and environmental protection of Antarctica. These treaties aim to protect and sustainably manage Antarctica as a region dedicated to the common good of humanity [4].

The Madrid Protocol includes three important conditions for wastewater management in Antarctica:

- In areas subject to the Antarctic Treaty, there can be no discharge of wastewater that could damage surface ice. This condition aims to protect the continent's fragile ecosystems and ice sheets.
- After certain treatments, wastewater can be discharged to the bottom of deep ice structures. This allows wastewater to be disposed of without harming the environment.
- Wastewater from sparsely populated bases may be discharged from the coast into the sea under specific conditions. However, this discharge must comply with environmental protection standards [5].

Although discharging wastewater into the sea is preferred, especially in low-populated coastal bases, water treatment is necessary in many inland bases. In bases with a permanent population of a few hundred people, water treatment is in many cases inadequate. This is due to technical and infrastructural challenges caused by the isolated nature and extreme conditions of the continent, which render the establishment and operation of

[1] Robert College, Istanbul / Türkiye
[2] Yildiz Technical University, Faculty of Chemical and Metallurgical Engineering, Department of Bioengineering, Istanbul / Türkiye
*Corresponding Author: Didem Balkanlı, e-mail: didem.balkanli@yildiz.edu.tr

wastewater management systems difficult. However, the general trend is towards the development and improvement of wastewater treatment systems, with more effort than the legal limitations demand. Some bases are trying to make wastewater reusable through recycling and treatment, aiming to create a completely "closed system" [6].

Different methods are used for wastewater treatment in Antarctica. Certain physical methods are used to remove physical pollutants that cannot be removed by biological methods, and then biotreatment methods are usually used [7]. The materials required for the functioning of treatment plants usually need to be shipped from the mainland. The transportation of these materials to Antarctica is costly and time consuming. In particular, the transportation and procurement of these materials is challenged by the isolated nature of the continent and the extreme climatic conditions. Moreover, the solid waste remaining after treatment is often sent to the countries of origin and treated as organic matter, as no plant cultivation activities are carried out on many bases. This creates an additional challenge and cost for waste management and disposal.

As for the wastewater in Antarctica, it can be divided as black water and gray water. Black water and gray water are wastewater from human waste and sources such as kitchens and showers and contain trace amounts of organic matter and chemical pollutants, respectively [8]. These two types of wastewaters are usually collected through different water systems. This allows different treatment methods to be applied to black water and gray water. While black water contains more intense pollution due to the human waste it contains and requires more extensive treatment, on the other hand, gray water is less contaminated and has a lower organic matter content, so, it is subjected to physical and chemical treatment processes such as filtration and disinfection [9]. Separate treatment of black water and gray water makes wastewater treatment more effective and efficient, leading to more sustainable water management.

Microalgae are third generation biomass feedstock that have been used in various areas such as biofuel production, pharmaceutical and nutraceutical production and environmental applications [10, 11, 12 ,13, 14]. Today, microalgae stand out as an innovative and sustainable solution for wastewater treatment. Microalgae utilize organic matter and nutrients in wastewater for photosynthesis and produce oxygen [15]. This allows wastewater to be treated and oxygenated. Furthermore, the biomass of microalgae can be collected from treated wastewater and converted into valuable products such as biofuels or fertilizers.

In the literature, the high efficiency of microalgae, especially in black water treatment, has been reported in various sources [16, 17, 18]. However, wastewater treatment studies have not yet been carried out by utilizing Antarctic microalgae which has a significant potential for environmental applications [19]. The target of this study is to explore and evaluate the cultivation of Antarctic microalgae in the wastewater that occurred in scientific bases in Antarctica. Given the unique and extreme environmental conditions of the Antarctic region, the microalgae found there possess distinctive physiological and metabolic adaptations that may render them particularly effective in bioremediation processes. This study aims to determine the biochemical content and physiological responses of Antarctic microalgae when exposed to wastewater. Comparing the performance of Antarctic microalgae with that of microalgae from temperate regions will highlight any superior traits or advantages specific to Antarctic species.

By achieving these targets, the study aims to contribute valuable insights into the potential of Antarctic microalgae as a sustainable and efficient source for wastewater treatment, addressing both environmental pollution and resource management challenges.

Material and Methods

Microalgae culture and artificial wastewater media

Chlorella variabilis YTU.ANTARCTIC.001 (MN372092) (Fig 1) was evaluated in this study. Artificial wastewater media with various chemicals (Yeast Peptone Media, Glucose, NH_4Cl, KCl, $NaHCO_3$, $MgSO_4$-$7H_2O$, $FeSO_4$-$7H_2O$, NaCl) were prepared according to Table 1. The pH of the medium was adjusted to 7.8 and then autoclaved at 121°C for 15 min. All the chemicals used in this study were supplied from Merck.
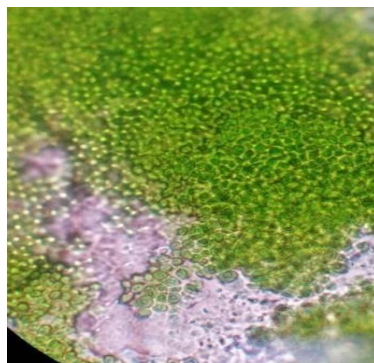
Fig 1 Microscope image of *Chlorella variabilis* YTU.ANTARCTIC.001 cells under (100x)

Antarctic Microalgae Growth in Artificial Wastewater

Microalgae to be grown on solid media and prepared for inoculation were first inoculated into petri dishes from the stock culture. At the end of one week, petri dishes were washed with distilled water and microalgae cells were collected for inoculation and transferred to a modified artificial wastewater medium [20]. A control group was cultivated in BG-11 medium. The experimental study was carried out in 250 mL flasks which were inoculated with 10% inoculum of polar microalgae for 8 days (Fig 2). The cultures were cultivated in a stirred incubator at 24 ± 2 °C and 150 rpm. Continuous illumination was maintained during the growth of the cultures. The pH of the cultures was 7.5- 8.5. The growth of the cultures was monitored by measuring their absorbance using PG 60 Instrument UV-Vis spectrophotometer at 680 nm [21]. The growth rate and doubling time were calculated by Equation 1-2. Cultivation of Antarctic microalgae in artificial wastewater was carried out in two replicates (*C. variabilis* 1 and 2 at growth curves graph). At the end of the cultivation, microalgae culture was centrifuged at 8000 rpm and the media and microalgae culture were separated for characterization studies.

$$\mu = ln\ ln\ \left(\frac{A_t}{A_0}\right)\ /(t - t_0) \tag{1}$$

$$t_d = ln2/\mu \tag{2}$$

where; t: Time (day) μ: Specific growth rate (day-1) A_0: Absorbance at t=0, A_t: Absorbance at time t. t_d is the doubling time.

Table 1 Modified artificial wastewater content

Chemical components	g/L
Yeast Peptone Media	5
Glucose	2
NH_4Cl	0.5
KCl	0.2
$NaHCO_3$	0.5
$MgSO_4\text{-}7H_2O$	0.1
$FeSO_4\text{-}7H_2O$	0.01
NaCl	2.5

Characterization of microalgae and culture media

The clear water separated from the centrifuged microalgae can be proposed to be used for various purposes such as watering the plants at the Antarctic science base. For this purpose, the pH and dissolved oxygen level of the water were analyzed using a Hach-Lange multimeter [22].

Microalgae separated by centrifugation were dried in an oven at 60°C for one day. For the determination of carbohydrate, lipid and protein contents of microalgae, the phenol-sulfuric acid [23], the Bligh-Dyer [24], and the Lowry [25], methods were carried out, respectively. The change in the main compounds that was used by microalgae was evaluated with phenol-sulfuric acid and modified Lowry method [23], [25].

Results and Discussion

Microalgal growth in artificial wastewater

In the experimental study, it was observed that microalgae cultures grew well within 8 days in the prepared wastewater using the components in the wastewater (Fig 2). It was observed that microalgae grew very rapidly in the first 3 days and starts to slow down after the third day due to the pH level of the culture.

Fig 2 Monitoring the growth of Antarctic microalgae

As can be seen in Fig 3, between days 0 and 8, the absorbance increased from 0.1 to 0.48 for C. *variabilis* cultures. It was determined that the microalgae cultures cultivated in artificial wastewater showed similar growth. When the growth results were analyzed, it was found that the growth rate and doubling time of microalgae was 0.15 day^{-1} and 4.6 days, respectively. Although there is no study on Antarctic microalgae cultivation in wastewater, there are many studies on temperate microalgae cultivation in different wastewater types (Table 2). Liu et al. studied growth of *Chlorella vulgaris* and nutrient removal in domestic wastewater, and reported that, the specific growth rate was found as between 0.3-0.5 day^{-1} when the culture was supplied with air [26]. Reyimu and Özçimen investigated the growth of *Nannochloropsis oculata* and *Tetraselmis* suecica in different concentrations of municipal wastewater. It was stated that, both *N. oculata* and *T. suecica* can tolerate and utilize the wastewater and, the specific growth rate of the cultures can up to 0.5430 d^{-1} (75% of wastewater) for *N. oculata* and 0.4778 d^{-1} (25% of wastewater) for *T. suecica* [27]. Similarly, Gani et al. reported that, highest biomass productivity in domestic wastewater carried out with 0.26 day^{-1} of specific growth rate, and a doubling time of 2.63 days. Meanwhile, the lowest biomass productivity was observed with the lowest specific growth rate of 0.1 day^{-1} and the longest doubling time, which reached up to 7.14 day [28]. Rani et al. studied the growth of *Chlorella sorokiniana* in different tertiary wastewater types (synthetic and real). It was reported that, the highest specific growth rate was found as 0.59 day^{-1} when secondary effluent of domestic wastewater was used. As for the growth in synthetic wastewater, the specific growth rate was calculated as 0.11 day^{-1} [29]. Oliviera et al. analyzed the growth of *C. vulgaris* in the effluent of a Biofloc Technology (BFT) system used in the Nile tilapia fingerlings farming. It was found that specific growth rate was 0.77, 0.63 and 0.53 when the microalgae cultivated in 0, 50, and 100% BFT effluent [30]. It was found out that the specific growth rates of the microalgae species given in the Table 2 were similar to our results, but it should be noted that some of the wastewater types had higher nutrient concentration than the prepared artificial wastewater in this study. Moreover, the increased presence of CO_2 led to higher growth rates in these studies [26].

Characterization of microalgae and culture media

After the growth of Antarctic microalgae in artificial wastewater, carbohydrate, protein, lipid, contents of the microalgae were determined (Table 3). It was seen that, cultures grown in artificial wastewater had partially higher protein content than control sample. By considering the usage areas of microalgae culture after grown in wastewater such as plant growing in Antarctic Scientific Base, it was aimed to focus on protein content. In comparison with the literature studies, it was seen that, protein content of the Antarctic microalgae grown in artificial wastewater is similar [33, 34, 35, 36, 37]. Yet, according to the wastewater type and the nitrate content in the wastewater, the protein content of this sample was determined to be lower than some studies. Higher amounts of nitrate compounds in growth media led to higher protein content in microalgae [27]. The comparison of the protein contents of Antarctic microalgae and the literature studies was given in Table 4.
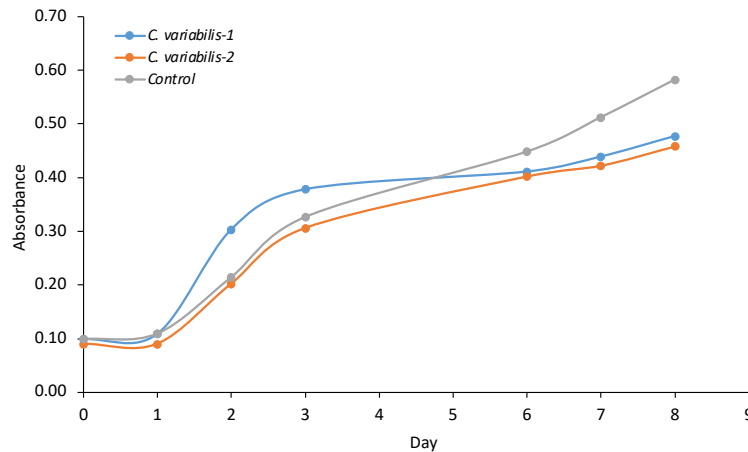
Fig 3 Growth curves of Antarctic microalgae cultivated in artificial wastewater and control samples

After 8 days of cultivation, it was also found that, the wastewater in which the microalgae are cultivated had a pH of 8. The dissolved oxygen level at the end of the day was measured with a multimeter and it was observed that the level increased from 6.2 to 7.6 mg/L. The change in the main compounds that was used by microalgae was evaluated with phenol-sulfuric acid and modified Lowry method. It was seen that, 52% and 48% of the glucose and protein-based compounds in the media were assimilated by microalgae.

Table 2 Specific growth rates of different microalgae species cultivated in various wastewater types

Microalgae	Wastewater type	Specific growth rate (day⁻¹)	References
Botryococcus sp.	Domestic	0.10-0.26	[28]
Chlorella sorokiniana	Domestic	0.59	[29]
Chlorella vulgaris	Domestic	0.3-0.5	[26]
Nannochloropsis oculata	Municipal	0.54	[27]
Chlorella vulgaris	Nile Tilapia Farming	1.18-1.73	[30]
Chlorella vulgaris	Nitrate and Ammonium	0.31	[31]
Chlorella zofingiensis	Swine	0.34	[32]
Chlorella variabilis	Artificial	0.15	This study

Table 3 Biochemical contents of Antarctic microalgae species cultivated in artificial wastewater

Cultures	Carbohydrate (%)	Protein (%)	Lipid (%)
C. variabilis 1	25±1.2	33±1.4	39±1.4
C. variabilis 2	26±1.4	28±1.6	44±1.2
Control sample	28±1.3	21±1.2	49±1.1

Table 4 Protein contents of different microalgae species cultivated in various wastewater types

Microalgae	Wastewater type	Protein content (%)	References
Chlorella pyrenoidosa	Artificial	~50%	[33]
Chlorella sorokiniana AK-1	Swine	42.2%	[34]
Chlorella vulgaris	Artificial (25% diluted)	50.7 %	[33]
Chlorella vulgaris	Hydroponic	50.5%	[35]
Chlorella vulgaris	Municipal	43.2%	[36]
Chlorella vulgaris	Municipal	35%	[37]
Oscillatoria sp	Municipal	32.9%	[37]
Chlorella variabilis	Artificial	33%	This study

Conclusion

Microalgae in Antarctica, have not been discovered to a great extent; their potential in possible agricultural areas such as crop production, food enrichment, soil protection has not been put into practice in many ways.

There are many bioproducts fro microalgae such as biofertilizers, biostimulants, biocontrol agents and soil conditioners that are thought to be developed for agriculture. In this study, it was aimed to investigate the utilization of Antarctic microalgae for the treatment of wastewater occurred in Antarctic Scientific Base and evaluation of its potential as a fertilize for plants by determining the protein content. In here, it was found that, Antarctic microalgae can be proposed for the treatment of domestic wastewater in Antarctic bases and has a high potential for the proposed activities by having approximately 30% of protein content. Using Antarctic microalgae for the wastewater treatment in the Antarctica bases will provide various advantages: no transportation of microalgae cultures to continent, utilization of cultures in compatible with continent's environmental conditions, in situ application. In conclusion, although experiments were conducted on a single Antarctic species, there are dozens of microalgae species isolated only from the Antarctic region and each microalgae species has the potential to remove wastewater as biomass.

Acknowledgements

Availability of data and material

Please contact the corresponding author for any data request.

References

1. Evecen, C., Let's Get to Know Antarctica. Tübitak Marmara Research Center, 2020.
2. Broady, P. A., Life on land: Non-aquatic ecosystems. In Exploring the Last Continent: An Introduction to Antarctica, Springer International Publishing, 2016, p. 201-228.
3. Salihoğlu, B., and B. Öztürk, 2021. Climate Change and Its Effects on the Seas of Turkey (B. Salihoğlu & B. Öztürk, Eds.). Turkish Marine Research Foundation.
4. French, D., Sustainable development and the 1991 Madrid Protocol to the 1959 Antarctic Treaty: The primacy of protection in a particularly sensitive environment. Journal of International Wildlife Law and Policy, 1999, *2*(3), p. 291-317.
5. Tin, T., et al., Impacts of local human activities on the Antarctic environment. Antarctic Science, 2009. *21*(1). p. 3-33.
6. Potter, S. 2003. Approaches to Antarctic solid waste management logistics: past, present, potential (Doctoral dissertation, University of Tasmania).
7. Stark, J. S., et al., Physical, chemical, biological and ecotoxicological properties of wastewater discharged from Davis Station, Antarctica. Cold Regions Science and Technology, 2015, *113*, p. 52-62.
8. Üstün, G. E., and A. Tırpancı, Greywater Treatment and Reuse. Uludağ University Journal of The Faculty of Engineering, 2015, 20(2), 119.
9. Oktor, K., and D. Celik, Treatment of wash basin and bathroom greywater with *Chlorella variabilis* and reusability. Journal of Water Process Engineering, 2019, 31, 100857.
10. Koçer, A. T., et al., Exopolysaccharides from microalgae: production, characterization, optimization and techno-economic assessment. Brazilian Journal of Microbiology, 2021, *52*(4), p. 1779-1790.
11. Sert, B., B. İnan, and D. Özçimen, Effect of chemical pre-treatments on bioethanol production from Chlorella minutissima. Acta Chimica Slovenica, 2018, *65*(1).
12. Vehapi, M., A. Yilmaz, and D. Özçimen, Antifungal activities of *Chlorella vulgaris* and *Chlorella minutissima* microalgae cultivated in Bold Basal medium, wastewater and tree extract water against Aspergillus niger and Fusarium oxysporum. Rom. Biotechnol. Lett, 2018, *1*, p. 1-8.
13. Özçimen, D., Ö. M. Gülyurt, and B. İnan, Optimization of biodiesel production from *Chlorella protothecoides* oil via ultrasound assisted transesterification. Chemical Industry and Chemical Engineering Quarterly, 2017, *23*(3), p. 367-375.
14. İnan, B., and D. Özçimen, Preparation and characterization of microalgal oil loaded alginate/poly (vinyl alcohol) electrosprayed nanoparticles. Food and Bioproducts Processing, 2021, *129*, p.105-114.
15. Alazaiza, M. Y. D., et al., Sewage water treatment using *Chlorella vulgaris* microalgae for simultaneous nutrient separation and biomass production. Separations, 2023, 10(4).
16. Wollmann, F., et al., Microalgae wastewater treatment: Biological and technological approaches. In Engineering in Life Sciences, Wiley-VCH Verlag, 2019, 19, 12, p. 860-871.
17. Slompo, N. D. M., et al., Nutrient and pathogen removal from anaerobically treated black water by microalgae. Journal of Environmental Management, 2020, *268*, 110693.
18. Silva, D. F. S., et al., Separation of microalgae cultivated in anaerobically digested black water using Moringa Oleifera Lam seeds as coagulant. Journal of Water Process Engineering, 2021, *39*, 101738.
19. Kırkıncı, S. F., et al., Antarktika: Yaşam Bilimleri ve Biyoteknoloji Araştırmalarının Gözden Geçirilmesi. International Journal of Life Sciences and Biotechnology, 2021, *4*(1), p.158-177.
20. Shrestha, B., et al., Formulation of a Simulated Wastewater Influent Composition for Use in the Research of Technologies for Managing Wastewaters Generated during Manned Long-Term Space Exploration and Other Similar Situations—Literature-Based Composition Development. BioTech, 2023, 12(1), 8.
21. İnan, B., et al., Interactive effects of cold and temperate conditions on growth and biochemical content of Antarctic microalga *Chlorella variabilis* YTU. ANTARCTIC. 001. Journal of Applied Phycology, 2023, *35*(2), p. 625-637.
22. Tarasenko, S., 2008. Wastewater Treatment in Antarctica. GCAS.
23. Dubois, M., K.A., Gilles, J. K., Hamilton, Colorimetric method for determination of sugars and related substances. Analytical chemistry,1956, 28: p. 350–356.

24. Bligh E. G., and W. J., Dyer, A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology, 1959, 37: p. 911–917.
25. Lowry, O. H., et al., Protein measurement with the folin. Journal Biological Chemistry, 1951, 193
26. Liu, Xiaoning et. al., Growth of *Chlorella vulgaris* and nutrient removal in the wastewater in response to intermittent carbon dioxide, Chemosphere, 2017, 186, p. 977-985.
27. Reyimu, Z., and D., Özçimen, Batch cultivation of marine microalgae *Nannochloropsis oculata* and *Tetraselmis suecica* in treated municipal wastewater toward bioethanol production. Journal of Cleaner Production, 2017, 150: p. 40–46.
28. Gani, P., et al., Influence of initial cell concentrations on the growth rate and biomass productivity of microalgae in domestic wastewater. Applied Ecology and Environmental Research, 2016, 14(2): p. 399-409.
29. Rani, S., and C.S.P., Ojha, Chlorella sorokiniana for Integrated Wastewater Treatment, Biomass Accumulation and Value-Added Product Estimation under Varying Photoperiod Regimes: A Comparative Study. Journal of Water Process Engineering, 2020, 39, 10188
30. Oliveira, C. Y. B., et. al., Growth of *Chlorella vulgaris* using wastewater from Nile tilapia (*Oreochromis niloticus*) farming in a low-salinity biofloc system. Acta Scientiarum. Technology, 2019, 42, e46232.
31. Babaei, A. et. al., Evaluation of Nutrient Removal and Biomass Production Through Mixotrophic,Heterotrophic, and Photoautotrophic Cultivation of *Chlorella* in Nitrate and Ammonium Wastewater. International Journal of Environmental Research, 2018, 12(2), p.167–178.
32. Zhu, L. et. al., Scale-up potential of cultivating *Chlorella zofingiensis* in piggery wastewater for biodiesel production. Bioresource Technology, 2018, 137, p. 318–325.
33. Miao, M. et. al., Mixotrophic growth and biochemical analysis of *Chlorella vulgaris* cultivated with synthetic domestic wastewater. International Biodeterioration & Biodegradation, 2016, 113, p. 120–125.
34. Chen, C.-Y. et. al., Cultivating Chlorella sorokiniana AK-1 with swine wastewater for simultaneous wastewater treatment and algal biomass production. Bioresource Technology, 2020, 122814.
35. Bertoldi, F. C., E. Sant'Anna, and J. L. Barcelos-Oliveira, *Chlorella vulgaris* cultivated in hydroponic wastewater. Acta Horticulturae, 2009, 843, p. 203–210.
36. Venckus, P., J. Kostkevičienė, and V. Bendikienė, Green algae *Chlorella vulgaris* cultivation in municipal wastewater and biomass composition, Journal of Environmental Engineering and Landscape Management, 2017, 25:1, p. 56-63
37. Madkour, Amany et. al., The Differential Efficiency of *Chlorella vulgaris* and *Oscillatoria* sp. to Treat the Municipal Wastewater, Journal of Biology, Agriculture and Healthcare, 2017, 7: 22.

Estimates of Genetic Variability and Interplay of Germination and Seedling Traits Conferring Salinity Tolerance in Rice (*Oryza sativa* L.)

Abiola Toyin Ajayi [1*] 🆔 ,**Oluwatoyin Sunday Osekita** [1] 🆔 ,**Oloruntoba Emmanuel Oladipo**[1] 🆔 , **Joshua Adewale Akinola** [2] 🆔

ABSTRACT

This study estimates genetic variability and correlations among germination and seedling traits conferring salinity tolerance in rice accessions. Five rice accessions were screened under salinity levels of 0, 50, 100, and 200 mM NaCl in a controlled laboratory setting. Traits such as germination energy, capacity, shoot and root length, and biomass were measured. Data were analyzed for variance and correlations to assess variability and trait relationships. Significant genetic variability was found among accessions for all traits. Germination energy showed the highest coefficient of variation (CV) at 22.29% under control conditions, while fresh shoot weight had the highest CV (34.35%) under 200 mM salinity. Accessions ACC2 and ACC5 consistently demonstrated higher performance in germination energy (23.33 to 53.33% and 10.00 to 41.67%), germination capacity (40.00 to 60.00% and 28.33 to 46.67%), and shoot length (0.67 to 2.97 cm and 0.40 to 3.93 cm) under various salinity stress levels. ACC1, ACC3, and ACC4 showed more variability but maintained some consistency in specific traits, with ACC4 generally showing lower performance across most traits. Genetic parameter estimates indicated high heritability (>60%) for all traits, with the highest in germination capacity (96.88%). High genetic advance (GAM) was observed for all traits (>20%), with germination energy showing the highest (107.00%). Traits with high heritability and genetic advance, such as germination energy, germination capacity, and root length, suggest strong genetic control and potential for improvement through selective breeding. Significant correlations were found between germination energy and capacity (r= 0.89 to 0.96) and between shoot length and leaf length (r= 0.92) under stress conditions. Stress tolerance indices identified accessions ACC2 and ACC5 as the most tolerant, with ACC1 showing consistent performance across traits. This study underscores the importance of identifying resilient traits and accessions to enhance salinity tolerance in rice, contributing to improved productivity in saline-affected regions.

Introduction

Rice (*Oryza sativa* L.) is the preeminent cereal crop globally, playing a vital role in Nigeria's food landscape and presenting a potential catalyst for economic growth in the current challenging circumstances [1]. As the primary sustenance for over half of the world's population, rice is a cornerstone of global nutrition and the most crucial natural energy source worldwide [2]. Rice is an excellent food source rich in vitamins, fiber, and essential micronutrients such as vitamins, minerals, and secondary metabolites [3 – 5]. The dietary minerals in rice include calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, copper, manganese, and selenium [6 – 9]. These minerals are crucial for maintaining human health, and their deficiencies can lead to various pathological conditions [7, 10]. Beyond its culinary significance, rice plays a critical role in multiple industries. It is a vital source of starch, alcoholic beverages such as rice wine, and confectionary flour [7]. Additionally, rice is integral to the pharmaceutical sector for producing phytin and vitamin B, and its oil is extensively utilized in manufacturing soap and candles [11, 12]. Consequently, rice is established as a superior choice for a nutritious and cost-effective energy source [13].

[1]Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria
[2] Department of Plant Biotechnology, Wageningen University, Netherlands
*Corresponding Autor: Abiola Toyin Ajayi, e-mail: toyin.ajayi@aaua.edu.ng

Rice cultivation has a longstanding history in Nigeria, primarily led by smallholder farmers employing rudimentary technology and traditional methods, contributing to over 80% of the national production [14]. The adaptability of rice to diverse ecological conditions across the country, including rain-fed lowlands, irrigated lowlands, and upland areas, further enhances its widespread growth [15]. As of 2009, Nigeria ranked 12th globally in rice consumption, securing the 17th position in global production, the third in Africa, and the foremost in West Africa [16]. Notably, by 2021, Nigeria emerged as Africa's leading rice producer, with a production volume of approximately 8.3 million metric tons, surpassing Egypt and Madagascar, which produced around 4.8 million and 4.4 million metric tons of rice, respectively [17]. Despite the abundant potential of rice in Nigeria, challenges persist in meeting current demand and accommodating a growing population [13]. Various constraints, encompassing climatic, economic, and environmental factors, hinder rice production. Notably, soil salinity stands out as a significant limitation, adversely affecting the survival, biomass production, and yield of rice—an issue posing a substantial threat to global food security [18].

Soil salinity triggers complex morphological and physiological stress in plants, leading to ion imbalance, oxidative stress, and reduced metabolic activity, ultimately decreasing crop productivity [19, 20]. It affects germination, growth, and overall plant vigor [19, 21]. Research shows that over 1000 million hectares of land could become saline, with 25–30% of irrigated areas affected, and about 50% of the global population already living in regions with severe salinity [22 – 24]. Rising salinity due to climate change, sea level rise, and poor irrigation practices threatens to reduce agricultural lands by 50% by 2050 [23, 25].

Global efforts have focused on understanding salinity tolerance and developing salt-tolerant rice cultivars, but the complexity of plant responses to salinity, including osmotic, ionic, and oxidative stress, makes this challenging [26]. Given significant production losses in rice due to saline soils and climate change, cultivating salt-tolerant upland rice in Nigeria is crucial [27]. Breeding programs rely on genetic diversity and germplasm response to salt stress, highlighting the need to evaluate genetic variability in rice [28]. Although several salt-tolerant rice lines exist globally [13, 29–33], many Nigerian varieties remain unexplored. Identifying salinity-tolerant traits is essential for improving rice yields under salt-stress conditions.

The germination phase is one of the most affected by salinity in many crops, with traits like germination percentage, root and shoot length, and dry weight negatively impacted by high salinity levels. A plant's growth and development under salinity stress depend largely on its tolerance at the germination stage [34]. Therefore, assessing variability in salinity tolerance traits during germination is crucial for rice breeding. Screening at this stage is advantageous as it is quicker and ensures uniform salt distribution, offering valuable data for salinity tolerance in crops [21].

The objectives of this study were to rigorously estimate the extent of genetic variability and elucidate the correlations among germination and seedling traits that confer salinity tolerance in selected rice accessions. These accessions were systematically screened under varying levels of salinity stress in a controlled laboratory environment.

Materials and Methods

Plant materials

Five rice accessions were obtained from the National Cereals Research Institute (NCRI) in Badegi, Nigeria, and all these accessions were affiliated with the forest transition/derived savanna Agroecology. The specific details of each genotype are provided in Table 1.

Table 1 List of rice accessions and their origins

S/N	Accession	Origin	Code
1	FARO26	NCRI, Nigeria	ACC1
2	FARO16	NCRI, Nigeria	ACC2
3	FARO21	Philippines	ACC3
4	FARO61	Africa Rice, Nigeria	ACC4
5	FARO15	NCRI, Nigeria	ACC5

Experimental procedure

In February 2023, the study was carried out at the Plant Science and Biotechnology Laboratory, Adekunle Ajasin University, Nigeria, located at a latitude of 7.20°N, longitude of 5.44°E, and an altitude of 423 meters above sea level. The experimental design involved a factorial experiment with a 5 × 4 treatment combination, utilizing a completely randomized design (CRD). Uniform-sized seeds were surface sterilized in a 10% (v/v) sodium hypochlorite (NaClO) solution for 30 minutes, followed by three rinses in distilled water. Seeds of

each accession were exposed to sodium chloride solutions at concentrations of 0 (pH: 7.0), 50 (pH: 7.22), 100 (pH: 7.38), and 200 mM (pH: 7.51) in three replicates. Sterile plastic containers lined with two filter papers each and soaked with 5 ml of NaCl solution were used for seed incubation in the dark for 48 hours, followed by exposure to a photoperiod of 12 hr./12 hr. (day/night) at room temperature for three weeks.

Data Collection

During data collection, germination energy, representing the percentage of germinated seeds on day four after sowing, was determined by dividing the number of germinated seeds by the total planted and multiplying by 100. Similarly, germination capacity, calculated on day ten, followed the same formula. Other measurements included shoot length, assessed from the base of the plant to the first leaf at week three, and the length of the main root per shoot, measured from the base to the tip of the taproot at the same period. Leaf length, number of roots, and leaves per shoot were also recorded in week three. Furthermore, fresh shoot weight was determined by separating seedlings into shoots and roots and recording the shoot weight, while fresh root weight was measured by weighing the separated fresh roots. Dry shoot and root weights were obtained after oven-drying plant shoots and roots for 48 hours at 60°C.

Data analysis

The data underwent analysis of variance (ANOVA) using SPSS version 21, and mean values were separated utilizing Duncan's multiple range test (DMRT) at a significance level of *P ≤ 0.05*. Salinity tolerance indices for all traits were determined using the stress tolerance index (STI) as per Ajayi [35] as follows: $STI = \frac{C*S}{(GM)2}$, where C, S, and GM represent the mean values for an accession under control, mean value under stress, and grand mean over all accessions under control, respectively. Accessions were ranked into different classes of salinity tolerance ranging from 1 (most tolerant) to 5 (least tolerant) for each trait. Genetic parameter estimates were conducted following the methodology outlined by Ajayi *et al*. [36]. Pearson's correlation analysis was conducted using SPSS, and significance levels were compared using Pearson's correlation Table at *P ≤ 0.05* and *P ≤ 0.01*.

Results

ANOVA results indicated significant differences among accessions for all traits across treatments (Table 2), demonstrating ample genetic variability in rice accessions under differential salinity stress. In the control treatment, germination energy showed the highest coefficient of variation (CV) at 22.29%, followed by the number of leaves at 17.79%, with the lowest in dry root weight at 1.05%. In the 50 mM treatment, the number of roots per shoot exhibited the highest CV (33.83%), while root length had the lowest (4.31%) (Table 2). Similarly, the 100 mM treatment showed the highest CV in the number of roots (32.07%) and germination capacity (28.2%), while fresh shoot weight had the lowest (1.09%). However, in the 200 mM treatment, fresh shoot weight had the highest CV (34.35%), while the number of leaves had the lowest (1.922%). Across all factors, accession, treatment, and their interaction, significant effects were observed for all traits, with the highest CV in the number of roots (7.21%) and the lowest in fresh root weight (0.44%).

Table 3 displays mean trait values under control and salinity stress (50, 100, 200 mM). In the control, ACC1 and ACC2 had the highest germination energy (28.33%), while ACC4 had the lowest (6.67%). Across stress levels, ACC2 consistently showed the highest germination capacity (40% to 51.67%), while ACC4 had the lowest (11.67% to 5%). Shoot length's highest mean was 3.73 cm in ACC1 under control and varied from 1.01 cm (ACC1, 200 mM) to 3.93 cm (ACC5, 50 mM) under stress. ACC1 and ACC5 had the highest mean root lengths under control and 50 mM, while ACC4 had the lowest. The number of leaves varied, with ACC1 having the highest under control and ACC4 the lowest across stress levels. Leaf lengths peaked at 8.13 cm (ACC5, control) and dropped to 0.13 cm (ACC5, 200 mM). ACC5 consistently had the highest root lengths, peaking at 10.93 cm (control) and 7.93 cm (50 mM). Fresh root weights ranged from 0.005 g (ACC1, control) to 0.001 g (ACC4, 200 mM). ACC1 consistently had the highest dry root weights, while ACC4 had the lowest across conditions. Dry shoot weights ranged from 0.05 g (ACC5, control) to 0.001 g (ACC4, stress conditions).

Table 4 summarizes the stress tolerance indices (STI) and accession ranks based on germination traits of rice accessions. ACC2 excelled under 50 mM stress, with high values in germination energy (2.49) and germination capacity (2.70), while ACC5 performed well in root length (1.53) and shoot length (1.37). Under 100 mM stress, ACC2 maintained strength, while ACC5 exhibited resilience, particularly in germination energy (2.51) and number of roots (1.46). ACC1 consistently performed well, especially in dry root weight (1.16) and germination capacity (1.31). Under 200 mM stress, ACC2 showed resilience, ACC5 displayed adaptability, and ACC1 exhibited moderate performance. Overall, ACC2 and ACC5 demonstrated strong tolerance across stress levels, while ACC1 showed consistent performance, indicating potential resilience to salinity stress in rice accessions.

Table 2 Mean square values of germination and seedling traits of rice accessions screened under differential salinity stress

Source of variation	DF	GE (%)	GC (%)	SL (cm)	NL	NR	LL (cm)	RL (cm)	FRW (g)	FSW (g)	DRW (g)	DSW (g)
Combine												
Accession (A)	4	1892.82*	2269.792*	2.067*	0.608*	28.975*	9.856*	9.585*	0.00075*	0.00029*	0.000051*	0.000014*
Treatment (T)	3	1293.261*	684.86*	23.267*	3.883*	52.156*	87.456*	100.752*	0.004*	0.003*	0.000098*	0.000061*
A × T	12	206.029*	72.014*	0.525*	0.231*	8.475*	3.155*	5.753*	0.00039*	0.00011*	0.000018*	0.00000034*
Error	40	37.817	36.667	0.082	0.2	2.267	0.385	0.505	0	0	0	0.0000019
CV (%)		6.767	4.98	2.889	2.918	7.213	3.825	4.165	0.663	0.439	1.719	
Control												
Accession	4	237.500*	377.5*	0.924*	0.433*	10.933*	6.152*	14.659*	0.00018*	0.00024*	0.0000921*	0.0000041*
Error	10	23.333	28.333	0.014	0.2	0.6	0.126	0.509	0.000023	0.000029	0.00000071	0.0000006
CV (%)		22.29	9.24	5.68	17.79	11.9	6.13	9.48	12	13.5	1.05	1.54
50 Mm												
Accession	4	526.667*	735.000*	1.158*	0.100*	22.100*	4.599	3.908*	0.00137	0.00017*	0.000009*	0.00000054*
Error	10	41.667	45	0.061	0.133	4.4	0.979	0.081	0.00026	0.00001018	0.000000079	0.000000012
CV (%)		21.28	20.33	7.6	17.2	33.83	18.83	4.31	4.48	10.64	4.68	5.77
100 mM												
Accession	4	1296.767*	800.00*	1.346*	0.267*	16.433	8.154*	3.575*	0.0000296*	0.000124*	0.00000024*	0.000014*
Error	10	66.267	38.333	0.161	0.133	3.467	0.454	1.103	0.0000368	0.0000162	0.00000017	0.00000055
CV (%)		28.2	16.36	14.23	16.36	32.07	1.5	24	20.33	1.09	10.31	12.36
200 mM												
Accession	4	450.00*	573.338	0.214*	0.50*	4.933*	0.414*	4.707*	0.0000102*	0.0000686*	0.00000023*	0.00000027*
Error	10	20	35	0.096	0.33	0.6	0.011	0.327	0.0000205	0.0000118	0.000000027	0.000000027
CV (%)		44.7	26.11	48	43	31.17	1.923	33.73	64.29	34.35	26	26

*: Significant at P ≤ 0.05. DF: Degree of freedom; CV: Coefficient of variation
GE: Germination energy; GC: Germination capacity; NL: Number of leaves; SL: Shoot length; NR: Number of roots; RL: Root length; LL: Leaf length; FSW: Fresh shoot weight; FRW: Fresh root weight; DRW: Dry root weight; DSW: Dry shoot weight.

Table 3 Mean performance of germination and seedling traits of rice accessions screened under differential salinity stress

Accession/ Treatment	GE (%)	GC (%)	SL (cm)	NL	NR	LL (cm)	RL (cm)	FRW (g)	FSW (g)	DRW (g)	DSW (g)
Control											
ACC1	28.33±3.33[b]	30.00±2.89[bc]	3.73±0.06[c]	3.00±0.10[b]	9.00±0.58[d]	5.90±0.23[b]	6.30±0.57[ab]	0.04±0.002[bc]	0.50±0.006[c]	0.02±0.006[c]	0.006±0.006[b]
ACC2	23.33±1.67[b]	40.00±2.89[c]	2.53±0.03[a]	2.33±0.33[ab]	7.33±0.33[c]	4.70±0.20[a]	7.37±0.22[bc]	0.036±0.002[ab]	0.04±0.003[ab]	0.01±0.00[b]	0.006±0.00[b]
ACC3	21.67±4.41[b]	21.67±4.41[b]	3.53±0.06[c]	2.00±0.001[a]	6.67±0.33[bc]	5.67±0.23[b]	7.97±0.62[c]	0.03±0.03[a]	0.04±0.00[b]	0.005±0.001[a]	0.004±0.00[a]

	GE (%)	GC (%)	SL (cm)	NL	NR	LL (cm)	RL (cm)	FRW (g)	FSW (g)	DRW (g)	DSW (g)
ACC4	6.67±1.67[a]	11.67±1.67[a]	2.80±0.10[b]	2.67±0.33[ab]	4.00±0.58[a]	4.47±0.06[a]	5.03±0.29[a]	0.05±0.001[c]	0.03±0.002[a]	0.004±0.001[a]	0.004±0.00[a]
ACC5	28.33±1.67[b]	35.00±2.89[c]	3.70±0.05[c]	2.67±0.33[ab]	5.33±0.33[ab]	8.13±0.24[c]	10.93±0.12[d]	0.05±0.005[c]	0.05±0.001[c]	0.004±0.003[a]	0.05±0.00[ab]
Grand mean	21.67±2.37	27.67±2.92	3.26±0.13	2.53±0.13	6.47±0.49	5.79±0.24	7.52±0.55	0.04±0.02	0.04±0.0025	0.08±0.0015	0.05±0.0003
50 mM											
ACC1	21.67±4.41[a]	20.00±5.00[ab]	3.13±0.13[b]	2.33±0.33[a]	5.33±0.88[a]	5.47±0.74[abc]	5.07±0.07[a]	0.04±0.007[a]	0.03±0.009[ab]	0.01±0.001[b]	0.007±0.001[b]
ACC2	50.00±2.89[c]	51.67±4.41[c]	2.70±0.06[ab]	2.33±0.33[a]	5.33±1.33[a]	4.07±0.39[a]	6.50±0.06[b]	0.02±0.011[a]	0.02±0.004[a]	0.01±0.000[b]	0.007±0.001[b]
ACC3	26.67±3.33[ab]	30.00±2.89[b]	3.87±0.20[c]	2.00±0.00[a]	7.00±1.15[ab]	6.03±0.58[bc]	6.03±0.22[b]	0.04±0.02[a]	0.03±0.001[ab]	0.001±0.001[a]	0.004±0.00[a]
ACC4	16.67±3.33[a]	16.67±3.33[a]	2.63±0.19[a]	2.00±0.00[a]	3.00±0.58[a]	4.27±0.73[ab]	7.47±0.27[c]	0.02±0.02[a]	0.03±0.005[ab]	0.003±0.000[a]	0.004±0.001[b]
ACC5	36.67±4.41[b]	46.67±3.33[c]	3.93±0.07[c]	2.00±0.00[a]	10.33±1.76[b]	7.03±0.24[c]	7.93±0.07[c]	0.07±0.16[b]	0.04±0.006[b]	0.01±0.000[b]	0.005±0.001[a]
Grand mean	30.33±3.47	33.00±4.02	3.25±0.16	2.13±0.91	6.20±0.26	5.37±0.37	6.60±0.28	0.36±0.01	0.03±0.003	0.006±0.003	0.006±0.00

Accession/ Treatment	GE (%)	GC (%)	SL (cm)	NL	NR	LL (cm)	RL (cm)	FRW (g)	FSW (g)	DRW (g)	DSW (g)
100 mM											
ACC1	17.67±4.33[b]	33.33±1.67[b]	2.37±0.26[ab]	2.33±0.33[a]	6.33±1.20[b]	3.07±0.28[a]	2.80±0.58[a]	0.026±0.004[ab]	0.037±0.002[bc]	0.004±0.000[a]	0.008±0.001[ab]
ACC2	53.33±4.41[d]	60.00±2.89[c]	2.97±0.12[bc]	2.00±0.00[a]	8.33±1.20[b]	4.87±0.70[b]	5.10±0.70[b]	0.037±0.002[bc]	0.03±0.03[ab]	0.005±0.001[a]	0.006±0.002[ab]
ACC3	31.67±7.26[bc]	40.00±5.77[b]	3.63±0.29[c]	2.00±0.00[a]	5.67±0.88[b]	6.27±0.37[c]	3.83±0.75[ab]	0.034±0.002[bc]	0.044±0.002[c]	0.004±0.001[a]	0.004±0.002[ab]
ACC4	0.00±0.00[a]	15.00±0.00[a]	1.93±0.27[a]	2.00±0.00[a]	2.00±0.00[a]	2.60±0.26[a]	5.40±0.29[b]	0.015±0.003[a]	0.028±0.001[a]	0.003±0.000[a]	0.0037±0.002[a]
ACC5	41.67±4.41[cd]	43.33±4.41[b]	3.17±0.17[c]	2.67±0.33[a]	6.67±1.45[b]	5.90±0.60[bc]	5.03±0.60[b]	0.039±0.005[c]	0.042±0.003[c]	0.003±0.001[a]	0.0083±0.001[b]
Grand mean	28.87±5.28	38.33±4.13	2.81±0.18	2.20±0.11	5.80±0.69	4.54±0.42	4.43±0.35	0.03±0.03	0.37±0.02	0.004±0.000	0.006±0.001
200 mM											
ACC1	0.00±0.00[a]	15.00±0.00[ab]	1.03±0.37[b]	2.00±0.58[a]	2.33±0.67[ab]	1.13±0.09[c]	3.37±0.24[d]	0.016±0.001[b]	0.017±0.001[c]	0.003±0.001[c]	0.001±0.00[a]
ACC2	30.00±5.77[c]	41.67±6.01[d]	0.67±0.13[ab]	1.33±0.33[a]	4.33±0.33[c]	0.47±0.09[b]	1.43±0.26[bc]	0.007±0.003[a]	0.013±0.003[bc]	0.002±0.000[b]	0.002±0.00[a]
ACC3	10.00±0.00[b]	23.33±1.67[bc]	0.77±0.88[ab]	1.33±0.33[a]	3.00±0.00[bc]	0.37±0.03[b]	1.13±0.18[ab]	0.004±0.003[a]	0.006±0.003[a]	0.0023±0.00[bc]	0.002±0.00[a]
ACC4	0.00±0.00[a]	5.00±0.00[a]	0.40±0.000[a]	1.00±0.00[a]	1.00±0.00[a]	0.50±0.00[b]	0.10±0.00[a]	0.001±0.00[a]	0.011±0.00[ab]	0.001±0.000[a]	0.001±0.00[a]
ACC5	10.00±0.00[b]	28.33±4.41[b]	0.40±0.06[a]	1.00±0.00[a]	1.67±0.67[ab]	0.13±0.09[a]	2.43±0.62[cd]	0.008±0.004[ab]	0.007±0.002[ab]	0.001±0.000[a]	0.001±0.00[a]
Grand mean	10.00±3.09	22.67±3.55	0.65±0.09	1.33±0.16	2.47±0.35	0.52±0.09	1.69±0.32	0.007±0.0017	0.01±0.01	0.002±0.000	0.002±0.000

Mean values followed by the same superscript within a column are not significantly different from one at $P \leq 0.05$ significance level using DMRT.GE: Germination energy; GC: Germination capacity; NL: Number of leaves; SL: Shoot length; NR: Number of roots; RL: Root length; LL: Leaf length; FSW: Fresh shoot weight; FRW: Fresh root weight; DRW: Dry root weight; DSW: Dry shoot weight.

Table 4 Stress tolerance indices (STI) and (accession rank) based on germination and seedling traits of rice accessions screened under differential salinity stress

Accession/Treatment	GE	GC	NL	SL	NR	RL	LL	FSW	FRW	DRW	DSW
50 mM											
	1.31	0.78	1.09	1.10	1.15	0.56	0.96	1.00	0.86	0.81	0.35
ACC1	(3)	(4)	(1)	(3)	(2)	(4)	(3)	(2)	(2)	(1)	(2)
	2.49	2.70	0.85	0.64	0.94	0.85	0.57	0.45	0.42	0.32	0.43
ACC2	(1)	(1)	(2)	(4)	(4)	(2)	(5)	(5)	(5)	(2)	(1)
	1.23	0.85	0.62	1.29	1.12	0.85	1.02	0.73	0.69	0.19	0.32
ACC3	(4)	(3)	(4)	(2)	(3)	(2)	(1)	(3)	(3)	(3)	(3)
	0.24	0.25	0.83	0.69	0.29	0.66	0.58	0.47	0.47	0.06	0.21
ACC4	(5)	(5)	(3)	(5)	(5)	(3)	(4)	(4)	(4)	(4)	(5)
	2.21	2.13	0.83	1.37	1.32	1.53	1.70	1.23	1.93	0.06	0.27
ACC5	(2)	(2)	(3)	(1)	(1)	(1)	(2)	(1)	(1)	(4)	(4)
Grand mean	1.50	1.34	0.84	1.02	0.96	0.89	0.97	0.77	0.87	0.29	0.32
100 mM											
	1.07	1.31	1.09	0.83	1.36	0.31	0.54	1.15	0.64	1.16	2.09
ACC1	(4)	(3)	(2)	(5)	(2)	(5)	(4)	(2)	(3)	(1)	(1)
	2.65	3.14	0.73	0.71	1.46	0.66	0.68	0.79	0.75	0.81	1.65
ACC2	(2)	(1)	(4)	(4)	(1)	(2)	(3)	(4)	(2)	(2)	(3)
	1.46	1.13	0.62	1.21	0.90	0.54	1.06	1.06	0.62	0.30	0.64
ACC3	(3)	(4)	(5)	(1)	(3)	(3)	(2)	(3)	(4)	(3)	(4)
	0	0.23	0.83	0.51	0.19	0.48	0.35	0.50	0.43	0.17	0.58
ACC4	(5)	(5)	(3)	(3)	(5)	(4)	(5)	(5)	(5)	(5)	(5)
	2.51	1.98	1.11	1.10	0.85	0.97	1.43	1.29	1.07	0.20	1.69
ACC5	(1)	(2)	(1)	(2)	(4)	(1)	(1)	(1)	(1)	(4)	(2)
Grand mean	1.54	1.56	0.88	0.87	0.95	0.59	0.81	0.96	0.70	0.53	1.33
200 mM											
	0.0	0.59	0.93	0.36	0.50	0.38	0.20	0.54	0.40	0.81	0.35
ACC1	(4)	(5)	(1)	(1)	(2)	(2)	(1)	(1)	(1)	(1)	(2)
	1.49	2.18	0.48	0.16	0.76	0.19	0.07	0.28	0.15	0.32	0.43
ACC2	(1)	(1)	(2)	(3)	(1)	(3)	(2)	(3)	(3)	(2)	(1)
	0.46	0.66	0.42	0.25	0.48	0.16	0.06	0.13	0.07	0.19	0.32
ACC3	(3)	(3)	(3)	(2)	(3)	(4)	(3)	(5)	(4)	(3)	(3)
	0.0	0.08	0.42	0.11	0.10	0.01	0.07	0.19	0.03	0.06	0.21
ACC4	(4)	(4)	(3)	(5)	(5)	(5)	(2)	(4)	(5)	(4)	(5)
	0.60	1.30	0.42	0.14	0.21	0.47	0.03	0.21	0.23	0.06	0.27
ACC5	(2)	(2)	(3)	(4)	(4)	(1)	(4)	(2)	(2)	(5)	($)
Grand mean	0.51	0.96	0.53	0.20	0.41	0.24	0.09	0.27	0.18	0.29	0.32

GE: Germination energy; GC: Germination capacity; NL: Number of leaves; SL: Shoot length; NR: Number of roots; RL: Root length; LL: Leaf length; FSW: Fresh shoot weight; FRW: Fresh root weight; DRW: Dry root weight; DSW: Dry shoot weight.

Table 5 presents the combined rank sum (RkSum), rank means (RkMean), and standard deviation of ranks (SDRk) among rice accessions under varying salinity stress. ACC1, ACC2, and ACC5 exhibited lower RkSum and RkMean values (< grand mean), indicating high tolerance to stress. Conversely, ACC4 had higher values, suggesting susceptibility to stress.

Table 6 presents combined estimates of genetic parameters for germination traits among rice accessions under varying salinity stress. Across traits, germination capacity exhibited higher GV (186.09), PV (192.09), and heritability (96.88%) suggesting a stronger genetic component influencing the trait. Except for the number of leaves, all traits showed moderate to high variability with GCV and PCV ranging from the lowest (15.54 and 18.50%) in fresh shoot weight to the highest (54.73 and 57.69%) in germination energy indicating considerable phenotypic variability among accessions. High broad-sense heritability was observed in all traits ranging from 61.22% in root length to 96.88% in germination capacity, indicating the proportion of total variation attributable to genetic factors and the degree of genetic control over traits. Genetic advance ranged from 0.002

in dry shoot weight to 27.66 in germination capacity, while GAM ranged from 14.82% in the number of leaves to 107.00% in germination energy, indicating the relative improvement potential.

Table 5 Combined rank sum (RkSum), rank means (RkMean), and standard deviation of ranks (SDRk) among rice accessions screened under deferential salinity stress

Accession	RkSum	RkMean	SDRk
ACC1	79	**3.59**	1.30
ACC2	82	**3.73**	1.30
ACC3	103	4.68	0.93
ACC4	143	6.50	0.85
ACC5	75	**3.41**	1.28
Grand mean	**96.40**	**4.38**	**1.13**

In bold: Highly tolerant (values < grand mean); highly susceptible (values > grand mean)

Table 7 summarizes Pearson's correlation analysis for germination and seedling traits under differential salinity stress. Germination energy showed strong positive correlations with germination capacity under 50 mM (0.89*) and 100 mM (0.96**) salinity stress. The number of leaves correlated highly positively with shoot length (0.88*) under 200 mM salinity stress. Additionally, shoot length was strongly correlated with leaf length (0.92*) under 100 mM salinity stress, while root length showed a strong positive correlation with fresh root weight (0.87*) under 200 mM salinity stress.

Table 6 Combined estimates of genetic parameters of germination and seedling traits among rice accessions screened under deferential salinity stress

Trait	GM	GVT	GV	PV	GCV (%)	PCV (%)	H^{2}B (%)	GA	GAM (%)
GE	22.72	56.07	154.58	171.75	54.73	57.69	90.00	24.30	107.00
GC	30.42	11.78	186.09	192.09	44.85	45.57	96.88	27.66	90.93
NL	2.05	0.01	0.03	0.05	9.00	11.26	63.91	0.30	14.82
SL	2.50	0.15	0.17	0.21	16.30	18.33	79.07	0.74	29.85
NR	5.23	2.07	2.22	2.93	28.51	32.72	75.91	2.68	51.17
RL	5.06	1.75	0.76	1.24	17.19	21.96	61.22	1.40	27.70
LL	4.06	0.90	0.79	1.05	21.90	25.28	75.01	1.59	39.07
FSW	0.03	0.00002	0.00002	0.00003	15.54	18.50	70.53	0.01	26.88
FRW	0.03	0.0001	0.00006	0.00001	25.62	32.39	62.59	0.01	41.76
DRW	0.005	0.000005	0.000004	0.000006	41.94	49.29	72.43	0.004	73.53
DSW	0.004	0.0000005	0.000001	0.00001	22.14	25.16	77.40	0.002	40.12

GM: Grand mean; GVT: Genotype × treatment variance; GV: Genotypic variance; PV: Phenotypic variance; GCV: Genotypic coefficient of variation; PCV: Phenotypic coefficient of variation; H^{2}B: Broad sense heritability; GA: Genetic advance; GAM: Genetic advance as percent over mean; GE: Germination energy; GC; Germination capacity; NL: Number of leaves; SL: Shoot length; NR: Number of roots; RL: Root length; LL: Leaf length; FSW: Fresh shoot weight; FRW: Fresh root weight; DSW: Dry shoot weight, and DRW: Dry root weight.

Discussion

Increasing salt tolerance is crucial for enhancing the growth, development, and yields of crops on saline soils and for reintroducing cropping on salinized lands [36]. Screening and identifying salinity-tolerant traits in rice are essential for developing salt-tolerant cultivars and improving yields under salt-stress conditions [21]. The germination phase, significantly affected by salinity, is critical for plant performance under stress [32]. Traits such as germination percentage, root and shoot length, and dry weight are negatively impacted by high salinity levels. Understanding the variability of these traits among rice genotypes during germination and seedling stage is vital for breeding salinity-tolerant rice. Screening at the germination and seedling stage is efficient and ensures even salt distribution, unlike pot and field techniques, providing valuable data for salinity tolerance [21]. Several studies have shown that using germination and seedling traits for screening can effectively identify genotypes for further field evaluation [36].

The observed significant genetic variability among rice accessions in response to different salt treatments suggests that there is potential for breeding salinity-tolerant rice varieties. Traits such as germination energy, number of roots, and overall root characteristics displayed notable variability, indicating that these traits could be targeted in breeding programs to enhance salinity tolerance. The significant interaction between accession and treatment for all traits indicated that accessions performed differently under varying salinity conditions, similar to findings reported in rice [37]. By critically observing the performance of accessions across treatments, it was found that while lower concentrations of salinity enhanced the performance of tolerant accessions, increased salt concentration generally reduced seed germination, seedling growth performance, and overall seedling biomass similar to findings in sunflowers [36], wheat [38], and rice [39]. The observed trends in the response of various traits to salinity stress conditions provide valuable information for breeding salinity-tolerant rice varieties. Resilient accessions, such as ACC1, ACC2, and ACC5, consistently excel in crucial traits like germination energy and capacity, shoot length, root characteristics, and overall plant health. These accessions demonstrate high performance across multiple traits, underscoring their potential as key contributors to salinity tolerance. This study emphasizes the importance of trait-specific responses, enabling targeted breeding efforts to enhance characteristics crucial for plant resilience under stress, including germination, shoot length, and root development. By quantifying the impact of salinity on each trait and prioritizing resilient accessions, breeding strategies can be fine-tuned to develop rice varieties with improved overall salinity tolerance efficiently.

Table 6 Combined estimates of genetic parameters of germination and seedling traits among rice accessions screened under deferential salinity stress

Trait	GM	GVT	GV	PV	GCV (%)	PCV (%)	H^{2}B (%)	GA	GAM (%)
GE	22.72	56.07	154.58	171.75	54.73	57.69	90.00	24.30	107.00
GC	30.42	11.78	186.09	192.09	44.85	45.57	96.88	27.66	90.93
NL	2.05	0.01	0.03	0.05	9.00	11.26	63.91	0.30	14.82
SL	2.50	0.15	0.17	0.21	16.30	18.33	79.07	0.74	29.85
NR	5.23	2.07	2.22	2.93	28.51	32.72	75.91	2.68	51.17
RL	5.06	1.75	0.76	1.24	17.19	21.96	61.22	1.40	27.70
LL	4.06	0.90	0.79	1.05	21.90	25.28	75.01	1.59	39.07
FSW	0.03	0.00002	0.00002	0.00003	15.54	18.50	70.53	0.01	26.88
FRW	0.03	0.0001	0.00006	0.00001	25.62	32.39	62.59	0.01	41.76
DRW	0.005	0.000005	0.000004	0.000006	41.94	49.29	72.43	0.004	73.53
DSW	0.004	0.0000005	0.000001	0.00001	22.14	25.16	77.40	0.002	40.12

GM: Grand mean; GVT: Genotype × treatment variance; GV: Genotypic variance; PV: Phenotypic variance; GCV: Genotypic coefficient of variation; PCV: Phenotypic coefficient of variation; H^{2}B: Broad sense heritability; GA: Genetic advance; GAM: Genetic advance as percent over mean; GE: Germination energy; GC; Germination capacity; NL: Number of leaves; SL: Shoot length; NR: Number of roots; RL: Root length; LL: Leaf length; FSW: Fresh shoot weight; FRW: Fresh root weight; DSW: Dry shoot weight, and DRW: Dry root weight.

Salinity stress has been reported to inhibit seed germination, decrease plant growth and development, and hinder the survival of seedlings. According to Ali *et al.* [40], the toxic effects of salinity likely caused a significant reduction in germination and seedling trait performance among sensitive accessions, leading to disrupted photosynthesis and increased respiration. This results in a shortage of assimilates essential for the proper growth and development of critical organs, potentially halting growth entirely. According to Zafar *et al.* [41], salinity decreases radicle and plumule growth, worsened at higher levels, by reducing water absorption and osmotic potential, thus hindering cell division and differentiation. It negatively impacts plumule, radicle, biomass, and physiological indices due to ionic toxicity, nutrient uptake issues, and reduced enzyme and hormone biosynthesis. During germination, salinity damages cell membranes, increasing permeability, replacing Ca^{2+} with Na^+, and causing K+ leakage, disturbing osmotic balance. According to Zhang *et al.* [21], salt stress damages cell membranes during seed imbibition, increasing permeability and impairing function. This reduces seed germination, energy, and capacity. Intracellular K^+ and Na^+ exosmosis rise, while amylase activity and soluble sugar content drop, delaying starch hydrolysis and causing starch grains to cluster in rice seed storage tissues. Protease activity, protein conversion rate, and dry matter consumption decrease, increasing residual protein in the aleurone cytoplasm.

Table 7 Pearson's correlations among germination traits of rice accessions screened under defferential salinity stress

Trait	Treatment	GE	GC	NL	SL	NR	RL	LL	FSW	FRW	DRW	DSW
GE	**Control**	1	0.79	0.02	0.52	0.57	0.53	0.52	0.82	0.23	0.41	0.46
	50	1	0.89*	0.22	0.04	0.26	0.15	-0.01	-0.29	0.04	0.39	0.42
	100	1	0.96**	0.11	0.57	0.78	0.26	0.65	0.38	0.81	0.39	0.28
	200	1	0.85	-0.19	-0.09	0.71	-0.06	-0.30	-0.15	-0.05	0.00	0.23
GC	**Control**		1	-0.07	0.02	0.42	0.46	0.29	0.49	-0.27	0.32	0.66
	50		1	0.05	0.21	0.45	0.32	0.22	0.01	0.38	0.31	0.2
	100		1	0.00	0.49	0.77	0.09	0.55	0.29	0.80	0.44	0.27
	200		1	-0.02	0.02	0.68	0.28	-0.35	-0.09	0.25	0.05	0.39
NL	**Control**			1	-0.07	0.19	0.09	-0.17	0.09	0.15	0.55	0.23	0.18
	50			1	0.05	-0.10	-0.22	-0.29	-0.34	-0.61	-0.41	0.31	0.66
	100			1	0.00	-0.01	0.43	-0.08	0.05	0.19	0.22	0.20	0.17
	200			1	-0.02	0.88*	0.31	0.33	0.55	0.68	0.37	0.46	0.29
SL	**Control**				1	0.23	0.44	0.74	0.69	0.08	0.11	-0.09
	50				1	0.71	0.08	0.82	0.37	0.64	0.09	-0.09
	100				1	0.39	0.004	0.92*	0.79	0.55	0.19	0.04
	200				1	0.43	0.28	0.53	0.56	0.39	0.63	0.26
NR	**Control**					1	-0.01	-0.02	0.52	-0.39	0.83	0.58
	50					1	0.26	0.69	0.42	0.70	0.29	-0.07
	100					1	-0.06	0.34	0.29	0.57	0.57	0.33
	200					1	0.09	0.04	0.19	0.05	0.39	0.44
RL	**Control**						1	0.83	0.58	-0.09	-0.23	0.01
	50						1	0.14	0.2	0.27	-0.25	-0.43
	100						1	0.09	-0.23	0.03	0.01	-0.27
	200						1	0.42	0.37	0.87*	0.38	-0.04
LL	**Control**							1	0.64	0.22	-0.16	-0.05
	50							1	0.56	0.67	0.12	-0.08
	100							1	0.79	0.62	0.01	0.12
	200							1	0.71	0.52	0.58	-0.1
FSW	**Control**								1	-0.07	0.51	0.35
	50								1	0.78	0.19	-0.3
	100								1	0.51	0.09	0.32
	200								1	0.55	0.49	-0.09
FRW	**Control**									1	-0.19	-0.12
	50									1	0.26	-0.22
	100									1	0.31	0.19
	200									1	0.47	-0.09
DRW	**Control**										1	0.55
	50										1	0.77
	100										1	-0.27
	200										1	0.01
DSW	**Control**											1
	50											1
	100											1
	200											1

*: Significant at P ≤ 0.05; **: Significant at P ≤ 0.01

GE: Germination energy; GC: Germination capacity; NL: Number of leaves; SL: Shoot length; NR: Number of roots; RL: Root length; LL: Leaf length; FSW: Fresh shoot weight; FRW: Fresh root weight; DRW: Dry root weight; DSW: Dry shoot weight.
LL: Leaf length; FSW: Fresh shoot weight; FRW: Fresh root weight; DRW: Dry root weight; DSW: Dry shoot weight.

Consequently, rice seedlings show stunted shoot and root length, number of roots, fresh plant weight, and seedling vigor index. Therefore, identifying specific genotypes with favorable traits under different salt concentrations provides valuable information for developing rice varieties that can thrive in varying salinity conditions, contributing to the resilience and productivity of rice crops in salt-affected regions.

Understanding the genetic variability and heritability of germination and seedling traits allows for focused efforts on traits that offer the greatest potential for improvement. The results from estimates of genetic parameters of germination and seedling traits under differential salinity stress have several implications in breeding for salinity tolerance in rice such as identifying high-performing traits, targeted breeding efforts, utilization of genetic resources, and accelerated breeding programs. In the present study, all traits consistently exhibited a high level of genetic variability under varied salinity stress except the number of leaves, shoot length, root length, and fresh shoot weight with low to moderate variability. Traits with high heritability and high genetic advance as a percent of the mean (GAM) such as germination energy, germination capacity, shoot length, number of roots, leaf length, fresh shoot and root weights, and dry shoot and root weight indicate additive gene effects and their suitability for targeted breeding. Mohammadi *et al*. [42] reported high broad-sense heritability estimates for seven seedling traits in rice, with high values for salinity score and shoot height, moderate values for shoot and root dry weight, and low values for Na^+, K^+, and K^+/Na^+ ratio, indicating varying levels of genetic and environmental influence. Hence, breeders can prioritize these traits for selection to develop rice varieties with improved salinity tolerance. In a similar study on the rice recombinant inbred line (RIL) population [25] evaluated under salinity stress based on seed germination traits, it was reported that these traits are controlled by two or three major genes plus polygenes, with major genes exhibiting high heritability values. By selecting germination traits with high heritability and GAM, breeders can expedite the development of salinity-tolerant rice varieties. Accessions such as ACC1, ACC2, and ACC5 exhibiting favorable trait performance under varied salinity stress will serve as valuable genetic resources for the breeding program. They can be used as parents in breeding crosses to introduce salinity tolerance into elite rice varieties. Employing germination trait STIs and ranking genotypes also have multifaceted implications for breeding salinity-tolerant rice varieties. These include the identification of high-performing accessions exhibiting higher STIs and superior rankings which can serve as valuable genetic resources, targeted selection of parental breeding lines, accelerated breeding programs, enhanced breeding strategies, and improved crop resistance thereby contributing to sustainable agriculture and food security in salinity-affected regions. One of the biggest strengths of STI is its capacity to pinpoint high-performing and consistent genotypes under both normal and stressed conditions an attribute that has been proven successful in several crops such as rice for salinity tolerance [36], and cowpea for aluminum stress [38]. Also, the effectiveness of various salt tolerance indices has been reported in several crop species [40, 42, 43]. Based on the rankings of accessions in the results, where 1 indicates a high ranking and above-average performance of trait across salinity stress conditions and 5 indicates high susceptibility, ACC2, ACC1, and ACC5 were pinpointed as highly tolerant which may be utilized in a breeding program. At the same time, ACC3 and ACC4 were highly susceptible. The tolerant accessions may be used as tolerant breeding stock for further crop improvement programs.

The considerable connections between germination and seedling characteristics at various degrees of salt stress have many major implications for developing saline-tolerant rice varieties. Strong positive associations between germination energy and germination capacity, especially under low to moderate salt stress, suggest that selecting for high germination energy can enhance overall germination success in saline conditions. This finding is consistent with reports by Zhang *et al*. [23] and Ding *et al*. [43]. Breeders should prioritize rice lines with high germination energy due to its strong correlation with germination capacity, crucial for seedling establishment in salty environments. Additionally, the significant link between the number of leaves and shoot length under high salt stress indicates that plants continuing vegetative growth (more leaves and longer shoots) are better adapted to high-salinity conditions. Selecting rice cultivars that produce more leaves and longer shoots under severe salt stress may improve plant resistance and overall development in saline soils. Moreover, the strong association between shoot length and leaf length under salt stress implies that longer shoots correspond to longer leaves, potentially enhancing light uptake and photosynthetic efficiency. Breeding for longer shoots may indirectly select for longer leaves, boosting the plant's ability to thrive under mild salt stress through increased photosynthetic capacity and growth. Lastly, the substantial correlation between root length and fresh root weight under severe salt stress suggests that longer roots accumulate more biomass, improving water and nutrient uptake in saline environments. Focusing on root traits such as root length and fresh root

weight can enhance salt tolerance, as plants with robust root systems are more efficient at absorbing water and nutrients, providing a significant advantage in saline soils. These results align with findings by Zhang *et al.* [23] in rice and Wu *et al.* [34] in oilseed rape. Therefore, breeding programs should take a holistic approach, selecting for high germination energy, vigorous vegetative development (more leaves and longer shoots), and strong root systems. Understanding trait correlations enables breeders to pick numerous desirable qualities simultaneously, increasing breeding program efficiency. The differences in responses under differing salt levels highlight the necessity for focused breeding techniques adapted to specific salinity circumstances (for example, moderate vs. high salinity). By focusing on these essential qualities, breeders can create rice varieties better suited to establishing, growing, and producing yields under salty circumstances, thereby contributing to food security in salinized areas.

Conclusion

This study successfully estimated genetic variability and correlations among germination and seedling traits conferring salinity tolerance in rice. Screening five rice accessions under various salinity levels in a controlled laboratory setting revealed significant genetic variability across all traits. Notably, germination energy and fresh shoot weight exhibited the highest coefficients of variation under control and 200 mM salinity conditions, respectively.

Accessions ACC2 and ACC5 consistently outperformed others, showing higher values for germination energy, germination capacity, and shoot length under different salinity levels. In contrast, ACC4 generally showed lower performance, indicating higher susceptibility to salinity stress. Estimates of genetic parameters indicated high heritability and genetic advance for all traits, with germination capacity and germination energy standing out, suggesting strong genetic control and potential for improvement through selective breeding. Significant correlations between germination energy and capacity, as well as between shoot length and leaf length under stress conditions, were observed. Stress tolerance indices further identified ACC2 and ACC5 as the most tolerant accessions, highlighting their potential for breeding programs aimed at enhancing salinity tolerance in rice.

This study underscores the importance of identifying resilient traits and accessions to improve salinity tolerance in rice, ultimately contributing to increased productivity in saline-affected regions.

Competing Interest
None declared by the authors.

References

1. Mohammed, U.A., S. Ibrahim, M. Hayatu, and F.A. Mohammed, Rice (*Oryza sativa* L.) production in Nigeria: Challenges and prospects. Dutse Journal of Pure and Applied Sciences, 2019. 5 (2b): p. 67–75.
2. Long, S.P. and D.R. Ort, More than taking the heat: crops and global change. Current Opinion in Plant Biology, 2010. 13: p. 241–248.
3. World Health Organization, and Food and Agriculture Organization of the United Nations. Vitamin and mineral requirements in human nutrition (2nd Ed.), 2004. https://www.who.int/publications/i/item/9241546123
4. Fernando, B., Rice as a source of fibre. Rice Research: Open Access, 2013. 1 (2): p. 6–10.
5. Smanalieva, J., K. Salieva, B. Borkoev, E.J. Windhab, and P. Fischer, Investigation of changes in chemical composition and rheological properties of Kyrgyz rice cultivars depending on long-term stack-storage after harvesting. LWT-Food Science and Technology, 2015. 63 (1): p. 626–632.
6. Vunain, E., F. Chirambo, S. Sajidu, and T.T. Mguntha, Proximate composition, mineral composition, and phytic acid in three common Malawian white rice grains. Malawi Journal of Science and Technology, 2020. 12 (1): p. 87–108.
7. Gnanamanickam, S.S., Biological Control of Rice Diseases. Progress in Biological Control, 2009. 8: p. 1 – 11.
8. Kaya, Y. and S. Karakütük, Effects of different growth regulators on regeneration of Turkish upland rice. Anadolu Journal of Agricultural Sciences, 2018. 33: p. 226–231.
9. Taşeva, G. et al., An overview of rice production in Kyrgyzstan. Frontiers in Life Sciences and Related Technologies, 2022. 3 (2): p. 86–94.
10. Chang, T., The Impact of Rice on Human Civilization and Population Expansion. Interdisciplinary Science Reviews, 1987. 12 (1): p. 63–69.
11. Arvas, Y.E., I. Kocacaliskan, S. Erisen, and E. Ordu, Antioxidant and molecular response of mutant and native rice (Oryza sativa L.) varieties grown under salt stress. Biologia, 2023. 78 (4): p. 1199–1210.
12. Fraga, C.G., Relevance, essentiality, and toxicity of trace elements in human health. Molecular Aspects of Medicine, 2005. 26: p. 235–244.

13. Iwo, G. and E.E. Obok, Agronomic performance and estimate of genetic variability of upland rice genotypes on Cross River State acid soil. Global Journal of Agricultural Sciences, 2010. 9 (1): p. 1–7.
14. Udo, I.A. and M.A. Ittah, Performance of five swamp rice (*Oryza saliva* L.) varieties at different sodium chloride levels. Global Journal of Agricultural Sciences, 2006. 5 (2): p. 117–121.
15. Afiukwa, C.A. et al., Screening of some rice varieties and landraces cultivated in Nigeria for drought tolerance based on phenotypic traits and their association with SSR polymorphism. African Journal of Agricultural Research, 2016. 11: p. 2599–2615.
16. Omoare, A.M. and W.O. Oyediran, Factors affecting rice farming practices among farmers in Ogun and Niger States, Nigeria. Journal of Agricultural Extension, 2020. 24 (4): p. 92–103.
17. Omoare, A.M., Production of Ofada rice (*Oryza* spp.) for sustenance in Obafemi Owode Local Government Area of Ogun State, Nigeria. Multidisciplinary Journal of Research and Methods in Tertiary Education, 2016. 2 (2): p. 301–314.
18. FAO, FAOSTAT, 2011. Retrieved from http://faostat.fao.org/
19. Statista, Rice production in Africa in 2021, by country, 2023. Retrieved from https://www.statista.com/statistics/1322372/rice-production-in-africa-by-country/
20. Butcher, K. et al., Soil salinity: A threat to global food security. Agronomy Journal, 2015. 108 (6): p. 2189–2200.
21. Munns, R. and M. Tester, Mechanisms of salinity tolerance. Annual Review of Plant Biology, 2008. 59: p. 651–681.
22. Umego, C. et al., Screening of rice accessions for tolerance to drought and salt stress using morphological and physiological parameters. American Journal of Plant Sciences, 2020. 11: p. 2080–2102.
23. Zhang, R. et al., Comprehensive evaluation of salt tolerance in rice (*Oryza sativa* L.) germplasm at the germination stage. Agronomy, 2021. 11 (8): p. 1569.
24. Shahid, S.A., M. Zaman, and L. Heng, Soil salinity: Historical perspectives and a world overview of the problem, M. Zaman, S.A. Shahid, and L. Heng, Editors. 2018, Springer International Publishing. p. 43–53.
25. Chen, T. et al., Molecular mechanisms of salinity tolerance in rice. The Crop Journal, 2021. 9: p. 506–520.
26. Liu, M. et al., Crop halophytism: An environmentally sustainable solution for global food security. Trends in Plant Science, 2020. 25: p. 630–634.
27. Wang, W., B. Vinocur, and A. Altman, Plant responses to drought, salinity, and extreme temperatures: Towards genetic engineering for stress tolerance. Planta, 2003. 218: p. 1–14.
28. Pareek, A., O.P. Dhankher, and C.H. Foyer, Mitigating the impact of climate change on plant productivity and ecosystem sustainability. Journal of Experimental Botany, 2020. 71: p. 451–456.
29. Sakina, A. et al., Genetic variation for salinity tolerance in Pakistani rice (*Oryza sativa* L.) germplasm. Journal of Agronomy and Crop Science, 2016. 202: p. 25–36.
30. De Leon, T.B. et al., Genetic variation in Southern rice genotypes for seedling salinity tolerance. Frontiers in Plant Science, 2015. 6: p. 374.
31. Shereen, A. et al., Salt tolerance potential of upland and lowland rice in physiological perspectives. Pakistan Journal of Botany, 2015. 47: p. 2055–2061.
32. Uyoh, E.A. et al., Morphological and molecular screening of rice accessions for salt tolerance. African Journal of Biotechnology, 2019. 18: p. 612–621.
33. Ahmad, M.S. et al., Field screening of rice germplasm (*Oryza sativa* L. ssp. japonica) based on days to flowering for drought escape. Plants, 2020. 9: p. 609.
34. Wu, H. et al., An effective screening method and a reliable screening trait for salt tolerance of *Brassica napus* at the germination stage. Frontiers in Plant Science, 2019. 10: p. 1–12.
35. Ajayi, A., Genotypic differences in aluminum tolerance of cowpea accessions utilizing germination parameters. International Journal of Life Sciences and Biotechnology, 2021. 4 (2): p. 254–273.
36. Ajayi, A.T. et al., Genetics of aluminum tolerance in cowpea accessions screened in pots under field conditions. Proceedings 45th Annual Conference of Genetics Society of Nigeria, 2023. p. 23-28.
37. Thoday-Kennedy, E. et al., Digital phenotyping to delineate salinity response in safflower genotypes. Frontiers in Plant Science, 2021. 12: p. 662498.
38. Farid, M. et al., Interaction of rice salinity screening in germination and seedling phase through selection index based on principal components. Chilean Journal of Agricultural Research, 2021. 81 (3): p. 368–377.
39. Zafar, S. et al., Evaluation of wheat genotypes for salinity tolerance using physiological indices as a screening tool. Pakistan Journal of Botany, 2015. 47 (2): p. 397–405.
40. Negm, M.E., W. El-Kallawy, and A.G. Hefeina, Comparative study on rice germination and seedling growth under salinity and drought stresses. Environment, Biodiversity and Soil Security, 2019. 3: p. 109–120.
41. Ali, M.N. et al., Screening of rice landraces for salinity tolerance at the seedling stage through morphological and molecular markers. Physiology and Molecular Biology of Plants, 2014. 20 (4): p. 411–423.
42. Mohammadi, R. et al., Genetic analysis of salt tolerance at seedling and reproductive stages in rice (*Oryza sativa* L.). Plant Breeding, 2014. 133 (5): p. 548–559.
43. Chaurasia, S., Identification of salt-tolerant wheat genotypes (*Triticum aestivum* L.) through physiological and ionic studies under salinity stress. Journal of Crop Science and Biotechnology, 2023. 27 (1): p. 273–285.
44. Ding, T. et al., Evaluation of salt-tolerant germplasm and screening of the salt-tolerance traits of sweet sorghum in the germination stage. Functional Plant Biology, 2018. 45 (10): p. 1073–1081.

Trends and Insights in *Lavandula angustifolia* Research: A Critical Literature Review (CLR) and Bibliometric Analysis of Scopus and PubMed Databases

Mir Abdullatif Yahya [1]*

ABSTRACT

English Lavender (*Lavandula angustifolia*) is considered one of the major sources of aromatic and pharmaceutical plants, as well as a source of several volatile organic compounds (VOCs). This aromatic plant has been widely studied for its diverse range of applications in the healthcare, cosmetic, and food industries. A critical literature review (CLR) and bibliometric analysis of the Scopus and PubMed databases were conducted, focusing on extracting and analyzing metadata related to publication trends, influential authors and institutions, and the thematic focus of the existing literature. This study reveals three major findings: spikes in research publications in 2006, 2014, and 2020, suggesting heightened interest on *L. angustifolia* research during these periods; significant contributions from the United States, Iran, and China, with prominent institutions like CNRS and Tehran University of Medical Sciences reflecting its global and multidisciplinary appeal; and a wide range of scientific applications, particularly in agricultural sciences, medicine, and biochemistry, with a strong emphasis on peer-reviewed papers and reviews. The study concludes that utilizing VOS-viewer software for bibliometric analyses plays a pivotal role in identifying novel works and collaboration networks, thereby guiding future research directions. This comprehensive analysis underscores *L. angustifolia* 's significant and evolving impact across various scientific fields.

Introduction

Medicinal plants have historically provided useful natural chemicals with diverse medicinal qualities. Researchers are using *in vitro* approaches to address the increasing demand for herbal medicines and the decreasing availability of these plants. The growing demand for herbal medicines and the dwindling supply of these plants have driven researchers to explore alternative methods of propagation and cultivation, including *in vitro* techniques [4, 22]. *Lavandula angustifolia*, sometimes referred to as English lavender, is a plant that has immense significance in terms of its medical and commercial value. Given its wide geographical range and its valuable attributes in terms of decoration, pharmaceutical, and fragrance, this species contributes significantly to the economies of many countries. *In vitro* production of *L. angustifolia* has been documented as a successful technique to produce many genetically identical plantlets, which may be used to produce valuable bio chemicals [28].

L. angustifolia, sometimes referred to as English lavender, is a very adaptable plant that has attracted considerable interest across several sectors, including horticulture, medicine, and cosmetics. It has a total of 32 species, along with other infraspecific taxa and hybrids [23]. *L. angustifolia* originates from the Mediterranean area, has a significant historical background of customary use, and has subsequently emerged as an essential component in contemporary applications, highlighting its versatile array of advantages. Moreover, *L. angustifolia* has been highly valued in the field of horticulture for its visual attractiveness and pleasant scent. The plant's vivid purple blossoms and subtle scent make it a favored option for landscaping, garden beds, and floral displays.

In vitro propagation, or micropropagation, is a groundbreaking approach in plant production that provides a very efficient and dependable way for safe, sustainable, and mass production of plant components. Enhancements to the micropropagation of economic plants have pronounced economic benefits through reduced losses and waste [27]. Moreover, advancements in biotechnology for propagating ornamental plants

---

[1] Konya Food and Agriculture University, Konya, Türkiye
*Corresponding Author: Mir Abdullatif Yahya, e-mail: latif.yahya1@gmail.com

may enhance characteristics like color, aroma, disease resistance, and vase life. This can result in the development of novel and superior plant types that possess both high aesthetic appeal and commercial value [19]. Numerous studies indicate that biotechnology breakthroughs including tissue culture techniques enhance plant propagation by improving features like color, disease resistance, and yield. They also enable precise genetic alterations and accelerate breeding procedures [14, 6, 7, 28].

A SLR is a rigorous procedure for performing comprehensive evaluations of existing literature. The process includes evaluating different techniques and examining strategies to improve the effectiveness and comprehensiveness of education and research [25]. However, bibliometric studies are considered essential for assessing scientific content. Through the examination of trends in publications, citations, and co-authorship, we may gain useful insights into various academic settings. The use and combination of these two methodologies show that they can positively contribute to literature by offering a full comprehension of the subject matter [26].

The aims of this study are:

• To perform a critical literature review (CLR) to gather insights into the current methodologies, challenges, and breakthroughs on *L. angustifolia.*
• To employ bibliometric analysis to identify research trends and patterns in the literature on *L. angustifolia.*

Material and Methods

Bibliometric Analysis and SLR

Bibliometric analysis is a method used to examine bibliographic data, including articles, books, reviews, and conference papers, particularly in the fields of science, library science, and information science. This analysis often relies on comprehensive databases such as Scopus or Web of Science [24]. Efficient tools for conducting bibliometric and scientometric studies include Bibliometrix, VOS-viewer, and SciMAT. These tools offer unique features and capabilities that can be tailored to meet specific user requirements [17]. The analysis of metrological literature frequently depends on extensive databases like Scopus or Web of Science. Scopus provides extensive coverage across diverse fields and offers access to a wide variety of document types and citation data. This enables detailed analysis of scholarly impact and precise literature searches with high accuracy and retrieval rates [29, 30, 31]. Bibliometric databases like Scopus and PubMed are useful for keyword searches and citation analysis and provide reliable and consistent coverage for making comparisons across different disciplines [8]. Moreover, Dimensions covers 96.42% of Scopus-indexed journals, making it very suitable for doing such analyses [32].

In this research, the Scopus and PubMed databases served as the primary source for both bibliometric analysis and the critical literature review (CLR). Additionally, VOS-viewer version 1.6.20 was utilized to conduct the analysis, leveraging its advanced capabilities for visualizing, and interpreting bibliometric data. For the SCR, the top fifteen articles resulting from the bibliometric analysis were selected from both databases for a more in-depth review. Part of the data collection and analysis was conducted using Microsoft 365, which facilitated the creation of statistical analysis charts. The literature search was executed in the Scopus and PubMed databases in July 2024. To comprehensively retrieve relevant publications, the search results were categorized into four types: (*i*) articles, (*ii*) conference papers, (*iii*) reviews, and (*iv*) conference reviews. The search formula applied was TITLE-ABS-KEY (title-abstract-keyword), ensuring that any term appearing in the title, abstract, or keywords of a publication would be included in the search results. The specific advanced search strings used in the Scopus and PubMed databases for this study are as follows:

Scopus: TITLE-ABS-KEY (*Lavandula* AND *angustifolia*) OR TITLE-ABS (lavender) AND (LIMIT-TO (DOCTYPE , "ar") OR LIMIT-TO (DOCTYPE , "re") OR LIMIT-TO (DOCTYPE , "cp") OR LIMIT-TO (DOCTYPE , "cr"))

PubMed: (*Lavandula angustifolia* [Title/Abstract]) OR (lavender [Title/Abstract]).

Keywords and search terms included in the search formula were: *Lavandula*, *angustifolia*, and lavender. The search results acquired using the described method were saved as a CSV and PubMed file. The relevant files included details such as the author(s), document title, year, source title, citation count, source and document type, affiliations, publisher, correspondence address, author keywords, and indexed keywords. The obtained files were then loaded into VOS-viewer version 1.6.20 software.

Based on the extracted data, the study performed CLR as well as analyses of the following aspects:

- • Analysis of documents per year by source
- • Analysis of documents per year
- • Analysis of documents by country or territory

- Analysis of documents by affiliation
- Analysis of documents by subject area
- Analysis of documents by type
- Citation analysis of documents and authors
- Co-authorship analysis of authors
- Co-occurrence analysis of all keywords.

These analyses enabled the creation of network visualizations, overlay visualizations, and density visualizations, completing the analysis process. The layout and clustering settings were fine-tuned to improve the visual representation, considering the content and data. Larger nodes represent higher connectivity and greater significance. Additionally, some statistical charts were generated using Microsoft 365.

Result and Discussion

Bibliometric Analysis of Scopus Database
Trends in publication output over time
Fig 1 illustrates the publication trends of *L. angustifolia* research across four key journals from 1990 to 2024. Notable peaks in publication numbers are observed in 2006, suggesting a significant research interest or breakthrough during that period, particularly in "Acta Horticulturae," which led to the surge. Subsequent years show fluctuating publication trends with additional peaks around 2014 and in recent years, indicating ongoing but variable interest in the topic. Overall, "Industrial Crops and Products" and "Acta Horticulturae" have been consistent contributors, while "Evidence Based Complementary and Alternative Medicine" and the "Journal of Essential Oil Research" show more sporadic publication activity. This pattern reveals both sustained and episodic research efforts, reflecting the multidisciplinary nature of *L. angustifolia* studies.
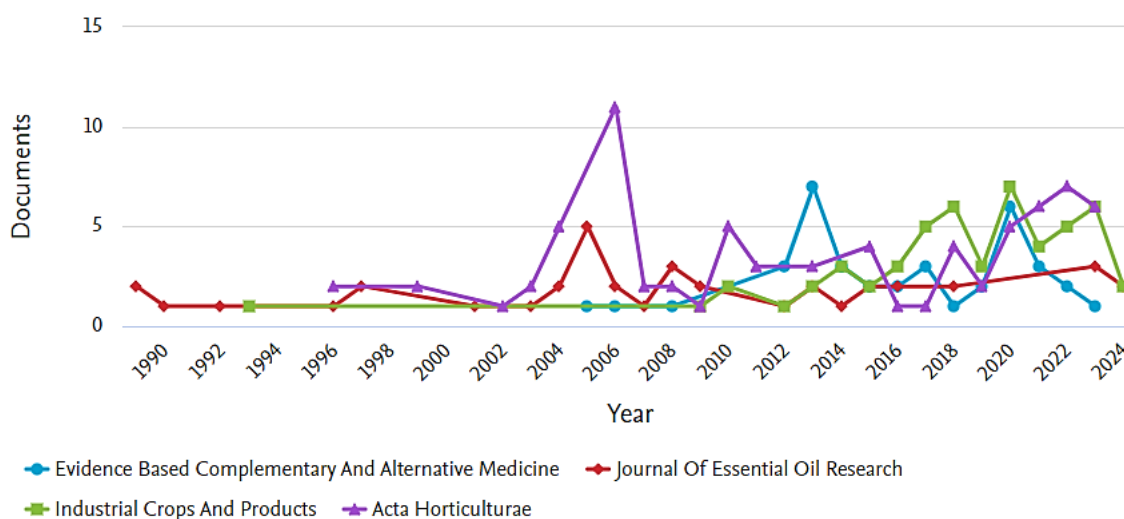

Fig 1 Annual Publication Trends by Journal for *L. angustifolia* Research (1990-2024)

During the early period from 1901 to around 1990, the research output was minimal, maintaining a very low and steady rate of publications. This suggests that *L. angustifolia* was not a major focus of scientific inquiry during these years, possibly due to limited recognition of its potential benefits or a lack of advanced research methodologies. Starting in the early 1990s, there is a noticeable uptick in the number of publications, indicating a growing interest in this plant. This growth phase continues steadily until around 2010, reflecting an increasing awareness of the significance of *L. angustifolia* in various fields such as complementary medicine, essential oils, and horticulture (Fig 2). The consistent rise in publications during this period suggests an expanding body of research and a broadening scope of studies.
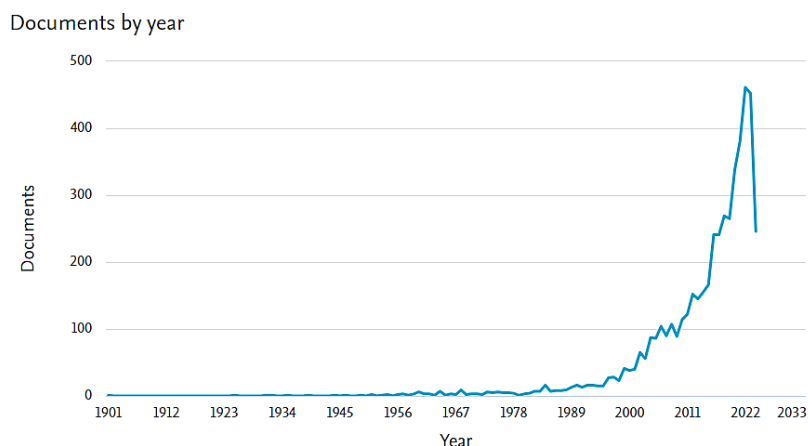
Fig 2 General Publication Trends for *L. angustifolia* Research (1901-2023)

Post-2010, the results show a rapid increase in the number of documents regarding *L. angustifolia* published per year, peaking significantly around 2020. This surge implies a heightened research interest and possibly major breakthroughs or innovations in the study of *L. angustifolia*. The peak around 2020 could also be attributed to an intensified focus on natural and alternative remedies in response to global health trends. However, there is a sharp decline in the number of publications after the peak, which could indicate a saturation point in research or shifts in research funding and priorities
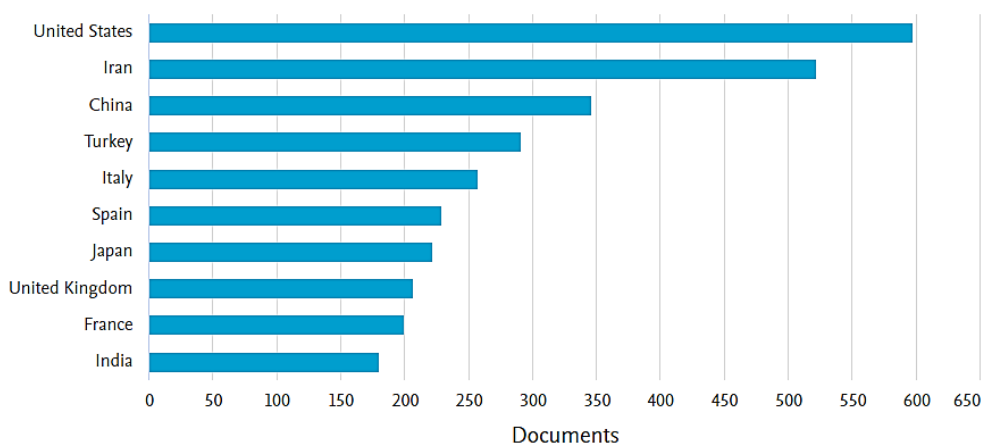

Fig 3 Global Distribution of *L. angustifolia* Research Publications by Country in the Scopus Database (1990-2024)

The United States stands out with the highest number of documents, surpassing 600 publications, indicating a significant research focus and academic interest in *L. angustifolia* within the country. Iran follows closely with nearly 550 documents, reflecting a strong research output in this area as well. China also shows substantial engagement with approximately 450 publications. Türkiye, Italy, and Spain form the next tier of contributors, each with document counts ranging between 250 and 350. This suggests a moderate but significant level of research activity in these countries. Japan, the United Kingdom, and France exhibit similar levels of research output, with each producing between 200 and 250 documents. India, while having the lowest count among the top contributors, still shows considerable research activity with around 200 documents. This indicates that *L. angustifolia* research has a broad international scope, encompassing contributions from various regions around the world. Fig 3 underscores the global nature of research on *L. angustifolia*, with a diverse array of countries actively contributing to the body of knowledge in this field. The high number of publications from multiple countries highlights the widespread scientific interest and the importance of this plant in various research domains.
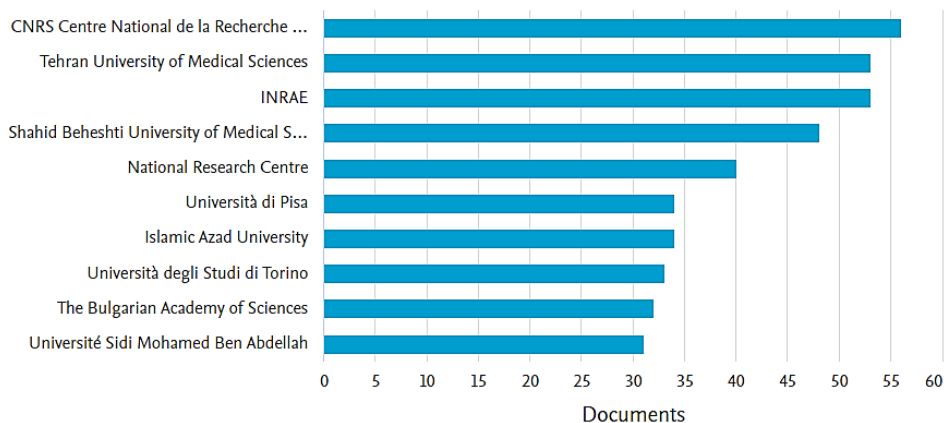
Fig 4 Top Institutional Contributors to *L. angustifolia* Research Publications in the Scopus Database

Fig 4 provides a comparison of the top 10 affiliations contributing to this field. The CNRS Centre National de la Recherche Scientifique (France) is the leading institution with close to 60 documents, highlighting its significant contribution to *L. angustifolia* research. The Tehran University of Medical Sciences (Iran) and INRAE (France) follow closely, each contributing over 50 documents, demonstrating their active involvement in this research area. Other notable institutions include the Shahid Beheshti University of Medical Sciences (Iran) and the National Research Centre (Egypt), both producing around 45 documents. The Università di Pisa (Italy), Islamic Azad University (Iran), and Università degli Studi di Torino (Italy) each have contributed approximately 35 documents, indicating a strong research presence in these universities. The Bulgarian Academy of Sciences (Bulgaria) and the Université Sidi Mohamed Ben Abdellah (Morocco) round out the list with 30 documents each, showing their active participation in *L. angustifolia* research. This distribution underscores the collaborative and international nature of research on *L. angustifolia*, with significant contributions from institutions across Europe, the Middle East, and North Africa.

Fig 5 presents the distribution of research publications related to *L. angustifolia* across various academic disciplines, as recorded in the Scopus database.
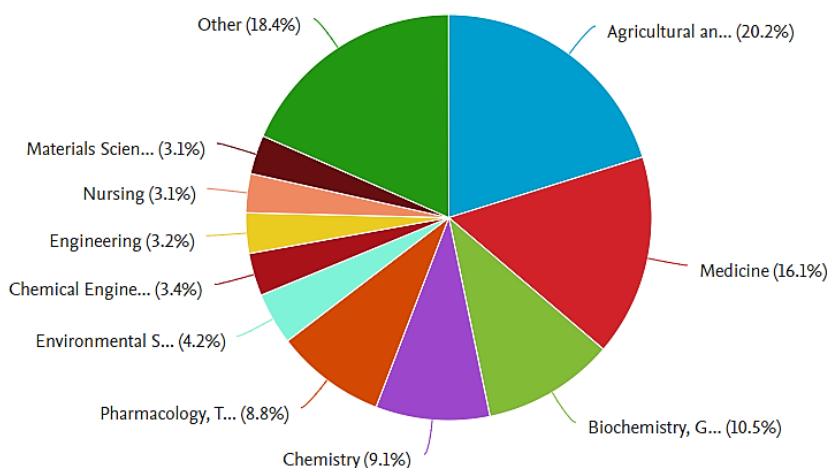
Fig 5 Distribution of *L. angustifolia* Research Publications by Subject Area in the Scopus Database

The largest segment is "Agricultural and Biological Sciences," which accounts for 20.2% of the publications. This reflects the significant interest in the agricultural aspects and biological studies of *L. angustifolia*. "Medicine" follows with 16.1%, indicating a strong focus on the medicinal properties and health-related research of this plant. "Biochemistry, Genetics, and Molecular Biology" represents 10.5% of the documents,

165

highlighting the molecular and biochemical research conducted on *L. angustifolia*. "Chemistry" constitutes 9.1% of the publications, underscoring the chemical analysis and studies related to this species. "Pharmacology, Toxicology, and Pharmaceutics" also have a notable share with 8.8%, indicating research into the pharmacological effects and potential toxicology of *L. angustifolia*. Other subject areas with significant contributions include "Environmental Science" (4.2%), "Chemical Engineering" (3.4%), "Engineering" (3.2%), "Nursing" (3.1%), and "Materials Science" (3.1%). These fields represent various interdisciplinary applications and studies involving *L. angustifolia*. The "Other" category accounts for 18.4% of the publications, which likely includes various other disciplines contributing to the research on *L. angustifolia*. Nevertheless, Fig 5 demonstrates the diverse range of academic fields engaged in research on *L. angustifolia*, highlighting its multifaceted importance across different scientific domains.

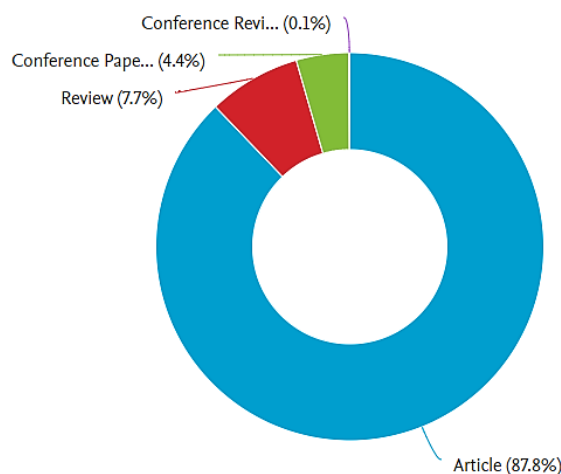**Fig 6** Distribution of *L. angustifolia* Research Publications by Document Type in the Scopus Database

Fig 6 provides a breakdown of the different types of research publications related to *L. angustifolia* in the Scopus database. The data is represented in a doughnut chart, highlighting the proportion of each document type. Most of the publications are "Articles," which make up 87.8% of the total documents. This indicates that the primary mode of disseminating research findings on *L. angustifolia* is through peer-reviewed journal articles. "Reviews" account for 7.7% of the documents, reflecting a substantial number of publications that synthesize existing research and provide comprehensive overviews on various aspects of *L. angustifolia*. "Conference Papers" constitute 4.4% of the publications, showing the contributions from academic conferences where researchers present their latest findings. "Conference Reviews" are the least represented, making up only 0.1% of the documents. This suggests that review papers specifically presented at conferences are relatively rare in this research area. This result underscores that most *L. angustifolia* research is published in the form of articles and reviews, with a smaller proportion presented at conferences. This distribution highlights the emphasis on rigorous peer-reviewed dissemination in the academic community studying this plant (Fig 6).

Citation Analysis of Documents

Fig 7 visualizes the interconnections between various research papers through citation links, highlighting influential works and clustering related studies based on citation patterns. The network is divided into multiple-colored clusters, each representing a thematic area within *L. angustifolia* research. These clusters indicate groups of documents with high citation interconnectivity, suggesting shared research interests and focus areas. Central nodes within each cluster represent highly cited papers, signifying their influence and foundational role in the research domain.

Notable papers include [16] which is central in its cluster, indicating significant influence, and [3] and [9] which are also highly cited and prominently positioned within their respective clusters. Researchers associated with these highly cited papers, such as [16], [3], and [9], are highlighted as key contributors to the field. Their work forms the backbone of current *L. angustifolia* research and has guided subsequent studies. The centrality and connectivity of their papers in the network emphasize their role in advancing the research landscape. Inter-cluster connections illustrate interdisciplinary research connections, indicating how findings in one thematic area are utilized or referenced in another. This highlights the integrative nature of the research field, where

studies on the chemical properties of lavender, for instance, may link to agricultural practices or therapeutic applications. These connections suggest potential areas for interdisciplinary collaboration, fostering innovation and comprehensive studies. Emerging research areas are also evident in the network, represented by smaller or newer clusters. These areas, while not yet extensively cited, hold potential for future growth and innovation. Researchers can identify these nodes to explore novel topics and contribute to the expanding body of knowledge in *L. angustifolia* research. Ultimately, the citation network provides a comprehensive overview of the most cited papers and influential researchers in *L. angustifolia* research. By examining the clusters, seminal works, and interconnections, researchers can gain valuable insights into the current state of the field, identify critical studies, and explore opportunities for future research and collaboration. This analysis serves as a vital tool for navigating the extensive literature and advancing the knowledge frontier in *L. angustifolia* research.

Analysis of Documents by Author

Fig 8 presents a document network analysis of authors, highlighting their influence based on document counts. Each node in this network represents an author, with the size of the node indicating the volume of the published document. Larger nodes signify more frequently published documents by authors, suggesting their substantial impact within the research domain. The lines connecting the nodes represent citation relationships between authors, with thicker lines indicating stronger document publishing connections. Key observations from the network include:
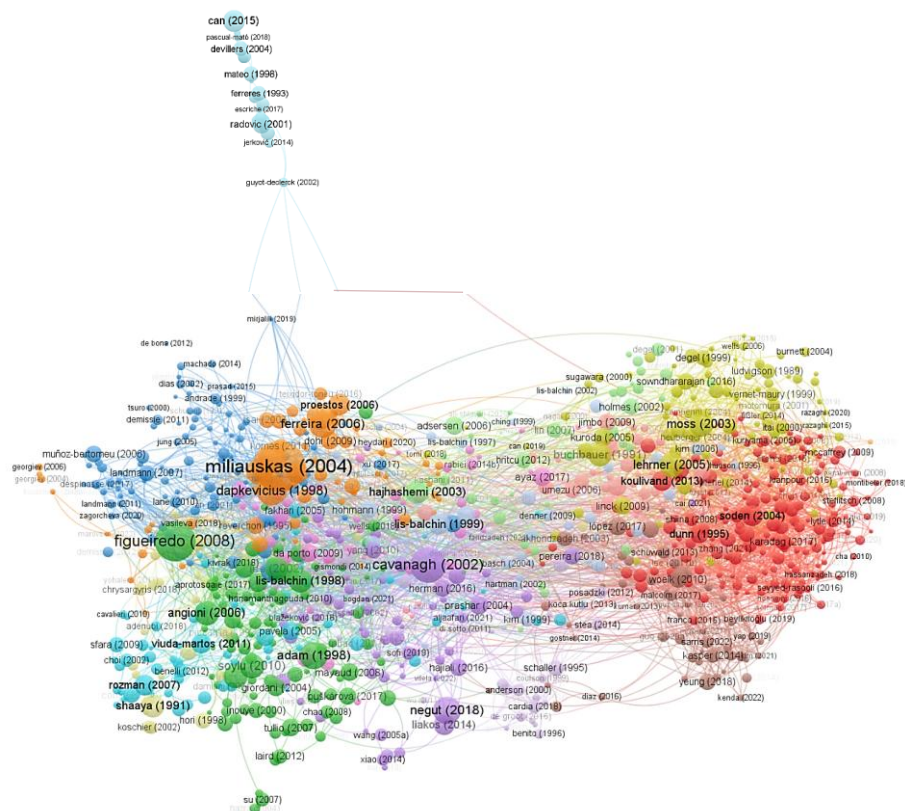

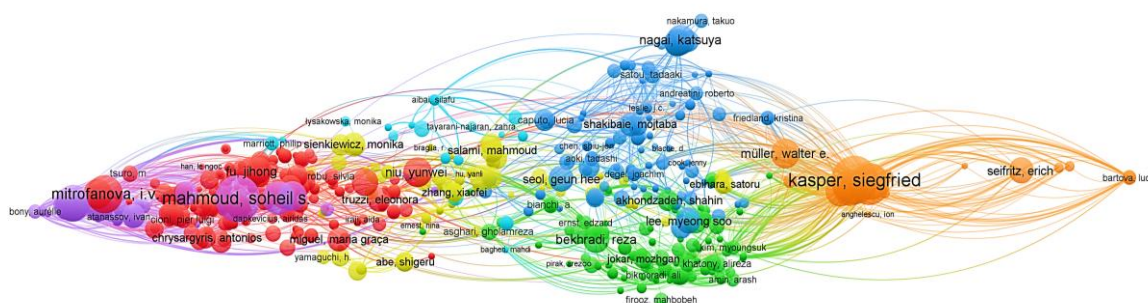**Fig 7** Citation Network Analysis of *L. angustifolia* Research Papers

Fig 8 Analysis of documents by Authors

Kasper, Siegfried stands out as a prominent node with extensive connections, indicating a significant citation impact and influence on the research community. Other notable authors include Mahmoud, Soheil S., Volz, H.P, and Mitrofanova, I.V., who are also central Figures in the network with numerous document counts. The network is divided into several colored clusters, each representing groups of authors with strong document interconnections. The clusters highlight the key research areas and the most influential contributors within each area.
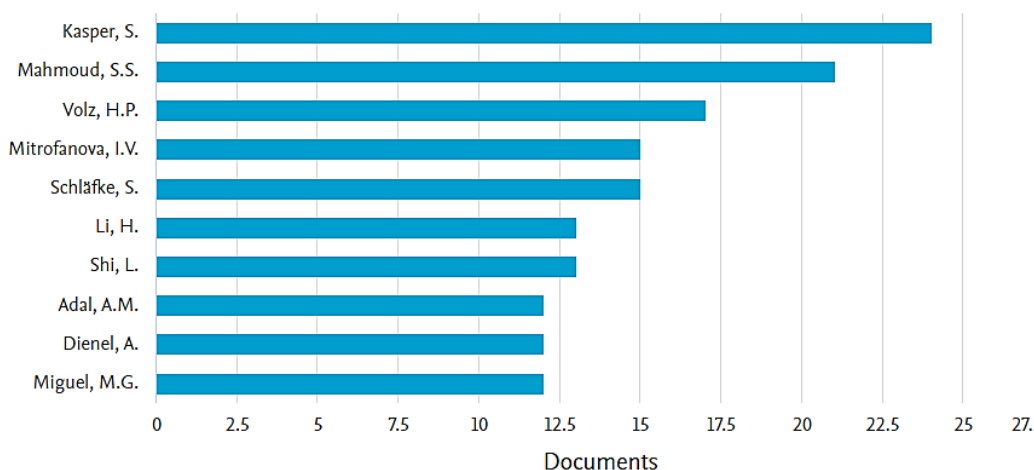

Fig 9 Document Count by Author in *L. angustifolia* Research

These findings underscore the importance of both citation influence and research output in identifying key contributors and understanding collaborative dynamics within the field. This comprehensive analysis highlights the significant roles played by authors like Kasper, Siegfried, Mahmoud, Soheil S., and Mitrofanova, I.V., emphasizing their substantial contributions (Fig 9).

Co-authorship Analysis of Authors

Fig 9 highlights the collaborative relationships among researchers, showing how different authors are connected through co-authored publications. Each node in the network represents an author, with the size of the node indicating the number of co-authored publications. Larger nodes represent authors with a higher number of co-authored works, suggesting they are key collaborators within the field. The lines connecting the nodes represent co-authorship relationships, with thicker lines indicating stronger or more frequent collaborations between authors. The network is divided into multiple-colored clusters, each representing a group of authors who frequently collaborate with one another. Notable clusters include those led by authors such as Zou, Junbo, Yang, Ming, and Wang, [25], who are central Figs within their respective clusters. Zou, Junbo stands out with a large node and numerous connections, indicating a significant role in fostering collaborative research efforts.
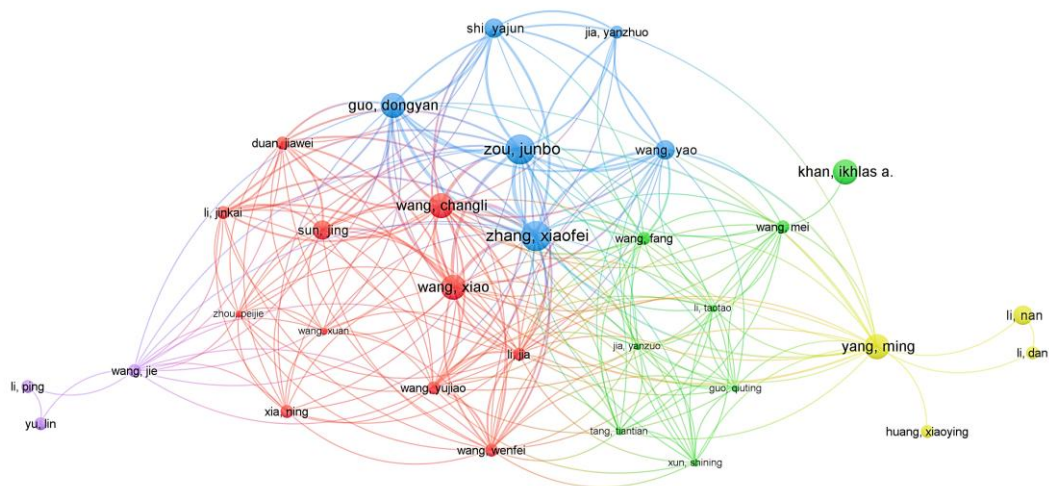
Fig 10 Co-Authorship Network Analysis of Authors in *L. angustifolia* Research

Other prominent authors include Sun, Jing, Li, Jinkai, and Wang, Changli. These researchers have substantial co-authorship networks, suggesting their work is highly collaborative and they play pivotal roles in their research communities. Their central positions within their clusters indicate that they are key facilitators of research collaboration in *L. angustifolia*. Inter-cluster connections illustrate interdisciplinary collaborations and the integration of diverse research perspectives. Authors such as Khan, Ikhlas A. and Yang, Ming have connections spanning multiple clusters, indicating their collaborative efforts extend across different research groups and thematic areas within *L. angustifolia* research. By examining the centrality and connectivity of the nodes, it is clear which researchers are key collaborators and how collaborative efforts are structured within the field. The clusters highlight major collaborative groups and the interconnected nature of the research community, emphasizing the importance of teamwork and interdisciplinary collaboration in advancing the research on *L. angustifolia.*

Co-Occurrence Analysis of All Keywords

The co-occurrence network analysis was conducted with a minimum number of occurrences of a keyword set to 5. Out of the total 31.239 keywords identified, 3.785 keywords met this threshold. Fig 10 shows how different keywords are related based on their co-occurrence in research documents, highlighting key topics and themes within the field.

In the co-occurrence network, each node represents a keyword, with the size of the node indicating the frequency of its appearance across research documents. Larger nodes signify more frequently used keywords, suggesting their importance and centrality within the research domain. The lines connecting the nodes represent the co-occurrence relationships between keywords, with thicker lines indicating stronger or more frequent co-occurrence (Fig 10).
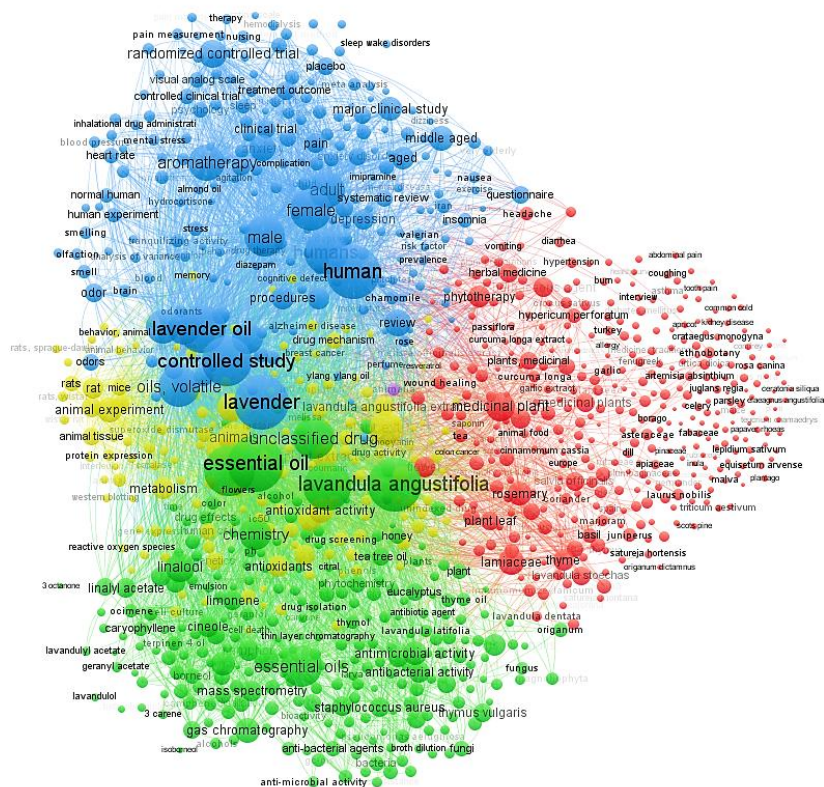
Fig 11 Co-Occurrence Network Analysis of All Keywords

The network is divided into multiple-colored clusters, each representing a group of related keywords that frequently appear together in the literature. Notable clusters include those centered around terms such as "human", "lavender oil", and "essential oil", which are key focal points in *L. angustifolia* research.

- **Human Studies (Blue Cluster):** Keywords such as "clinical trial", "randomized controlled trial", "aromatherapy", and "depression" are prominent in this cluster. This indicates a significant focus on the human health applications and therapeutic benefits of *L. angustifolia*, particularly in clinical and controlled study settings.
- **Chemical and Biological Properties (Green Cluster):** This cluster includes keywords like "essential oil", "antioxidant activity", "chemistry", and "mass spectrometry". The emphasis here is on the chemical composition and biological activities of lavender, including its antioxidant and antimicrobial properties.
- **Phytotherapy and Herbal Medicine (Red Cluster):** Terms such as "phytotherapy", "medicinal plant", and "herbal medicine" dominate this cluster. It highlights the use of *L. angustifolia* in traditional and herbal medicine practices, and its integration with other medicinal plants.
- **Experimental Studies (Yellow Cluster):** Keywords in this cluster, like "animal experiment", "rats", "metabolism", and "volatile oils", suggest a focus on experimental studies involving animal models and the examination of lavender's physiological effects and metabolic pathways.

The central positioning of keywords such as "*Lavandula angustifolia*", "essential oil", and "lavender oil" indicates their broad relevance and connectivity across different research themes. Inter-cluster connections illustrate the interdisciplinary nature of *L. angustifolia* research, where findings in one area, such as chemical properties, may inform and enhance studies in human health applications or experimental models. Through the analysis of pivotal and interconnected keywords, researchers may ascertain the primary areas of concentration, comprehend the interdisciplinary connections, and investigate prospective novel avenues for their investigations. This study is an effective instrument for mapping the research environment and providing guidance for future studies around *L. angustifolia.*

Bibliometric Analysis of PubMed Database

Co-authorship analysis of authors

Co-authorship analysis of authors was conducted with a minimum number of documents of an author set to 1. A total of 8751 authors were identified. Prominent nodes such as Zair, Touriya and Bourhia, Mohammed stand out due to their large sizes, indicating their central roles and numerous co-authorships in PubMed database.

These authors are influential within the research community, having collaborated extensively with others. Their centrality in the network suggests they have contributed significantly to advancing the research on *L. angustifolia* through their numerous collaborative works (Fig 12).
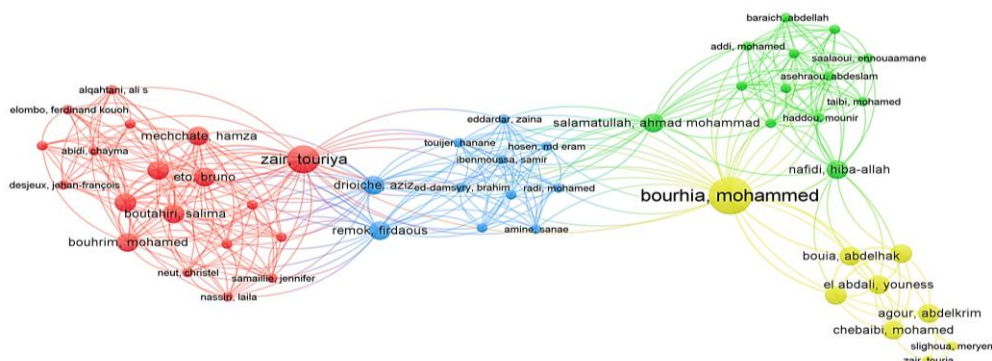

Fig 12 Co-Authorship Network Analysis of Authors in PubMed Database

The network is partitioned into many distinct clusters, each denoting a cohort of writers who engage in regular collaborations. The Red Cluster, led by Zair, Touriya, includes authors like Mechchate, Hamza, Eto, Bruno, and Boutahir, Salima. This cluster is tightly connected, indicating strong collaborative relationships within the group. The Green Cluster, dominated by Bourhia, Mohammed, includes collaborators such as Salamatullah, Ahmad Mohammad, Nafidi, Hiba-Allah, and Bouia, Abdelhak, reflecting a similarly high level of interconnectivity and extensive collaboration. Additionally, the Blue Cluster contains significant authors like Driouiche, Aziz and Remok, Firdaous, highlighting another important collaborative group. Inter-cluster connections are evident, illustrating interdisciplinary collaboration and the integration of diverse research perspectives. For example, Zair, Touriya has connections extending to other clusters, suggesting cross-group collaborations. These inter-cluster connections highlight the interdisciplinary nature of research in *L. angustifolia*, where findings and expertise are shared across different research groups. The dense interconnections within clusters and between key nodes indicate a robust collaborative network. Authors with central positions and numerous connections, such as Zair, Touriya and Bourhia, Mohammed, play pivotal roles in fostering collaboration and driving research forward. This co-authorship network analysis provides valuable insights into the collaborative landscape of *L. angustifolia* research, emphasizing the importance of teamwork and interdisciplinary collaboration in advancing knowledge in this field.

Co-occurrence analysis of all keywords

Co- occurrence analysis of all keywords was conducted with a minimum occurrence of a keyword set to 5. Out of the total 6521 keywords identified, 625 keywords met this threshold. Within the co-occurrence network, every node symbolizes a term, and the node's size corresponds to the frequency of its occurrence in research materials. Nodes of greater size indicate terms that are used more often, indicating their significance and vital role within the study field. The lines that link the nodes in the diagram depict the relationships of co-occurrence between terms. Thicker lines indicate a stronger or more frequent co-occurrence (*Fig* 12).
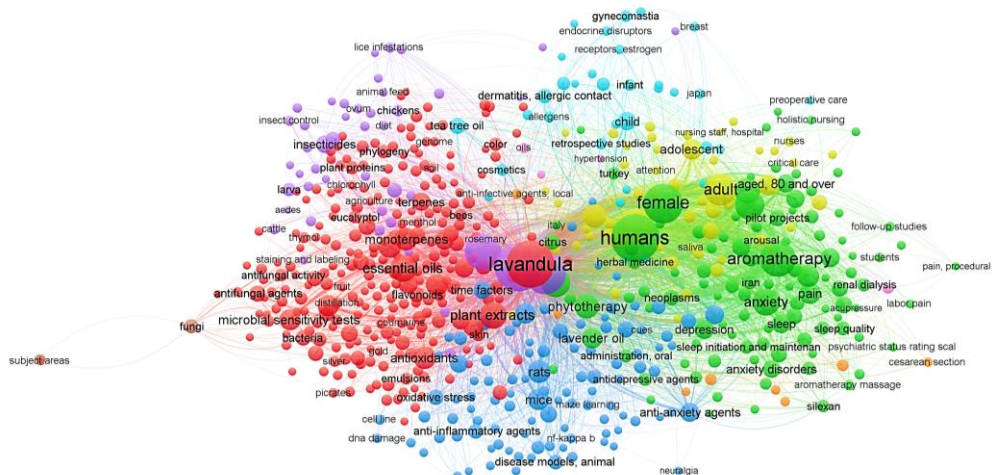

Fig 13 Co-Occurrence Network Analysis of Keywords in PubMed Database

The network is divided into multiple-colored clusters, each representing a group of related keywords that frequently appear together in the literature. Notable clusters include those centered around terms such as **"*Lavandula*", "humans"**, and **"aromatherapy"**, which are key focal points in *L. angustifolia* research.

- *Lavandula* **and Plant Extracts (Red Cluster)**: This cluster includes keywords such as **"*Lavandula*", "plant extracts", "essential oils"**, and **"monoterpenes"**. This suggests a significant focus on the chemical properties, extraction methods, and components of lavender and its essential oils.
- **Human Studies and Health Applications (Green Cluster)**: Keywords like **"humans", "female", "anxiety"**, and **"aromatherapy"** are prominent in this cluster. It indicates a strong emphasis on the health applications of lavender, particularly in human studies related to anxiety, depression, and aromatherapy practices.
- **Experimental and Disease Models (Blue Cluster)**: This cluster includes terms such as **"rats", "oxidative stress", "antioxidants",** and **"disease models, animal"**. The focus here is on experimental studies involving animal models and the examination of lavender's physiological effects and potential therapeutic benefits.
- **Dermatological and Cosmetic Applications (Purple Cluster)**: Keywords such as **"dermatitis", "allergic contact", "insecticides"**, and **"cosmetics"** are found in this cluster, suggesting research interests in the dermatological and cosmetic applications of lavender, including its use in treating skin conditions and as a natural insect repellent.

The central positioning of keywords like **"*Lavandula*", "essential oils"**, and **"aromatherapy"** indicates their broad relevance and connectivity across different research themes. Inter-cluster connections illustrate the interdisciplinary nature of *L. angustifolia* research, where findings in one area, such as chemical properties, may inform and enhance studies in human health applications or experimental models. Through the analysis of pivotal and associated terms, researchers may ascertain the primary areas of concentration, comprehend the interdisciplinary connections, and investigate prospective novel avenues for their study. This analysis could be an important tool for mapping the research environment and providing guidance for future studies in *L. angustifolia*.

Critical Literature Review (CLR)

For the CLR, a total of 15 articles (10 from Scopus and 5 from PubMed) were selected based on their citation count and connectivity in the network visualization (Fig 7) during the analysis process in VOS-viewer.

1.   Miliauskas, G., 2004 [16]:

This study investigates the antioxidant properties of extracts from 12 medicinal and aromatic plants through radical scavenging assays using DPPH and ABTS. The study aims to identify potential sources of natural antioxidants for various applications like food and cosmetics. The plants studied include various species of *Salvia, Lavandula, Calendula, Matricaria, Echinacea, Rhaponticum, Juglans, Melilotus, Geranium,* and *Potentilla*.

Methodologically, dried plant materials were extracted using acetone, ethyl acetate, and methanol. Radical scavenging activity was evaluated using spectrophotometric assays, correlating these activities with the content of total phenolic compounds, flavonoids, and flavonols in the extracts. Key findings from this study include:

1. **Radical Scavenging Activity**: Methanol extracts generally exhibited higher radical scavenging activity compared to acetone and ethyl acetate extracts. Particularly, extracts from *Geranium macrorrhizum* and *Potentilla fruticosa* showed very high activity in both DPPH and ABTS assays, surpassing the well-known antioxidant Salvia officinalis.
2. **Correlation with Phenolic Compounds**: There was a positive correlation between the radical scavenging capacities of the extracts and their total phenolic compound content. This suggests that phenolic compounds are likely major contributors to the observed antioxidant activity.
3. **Differences Between Assays**: While there was generally good correlation between DPPH and ABTS assays, some plants showed different levels of activity in each assay. Echinacea purpurea, for instance, exhibited higher activity in the ABTS assay compared to the DPPH assay.

2.   Cavanagh, H. M. A., & Wilkinson, J. M., 2002 [3]:

This research explores the various biological activities of lavender essential oil, examining its therapeutic and cosmetic applications, and highlights the challenges in evaluating its effectiveness due to methodological issues.

Lavender essential oil, derived from species such as *L. angustifolia*, *L. latifolia*, *L. stoechas*, and *Lavandula x intermedia*, has been traditionally used for its purported antibacterial, antifungal, carminative, sedative,

antidepressive, and insect repellent properties. The review investigates the scientific evidence supporting these claims and identifies areas needing further research. Lavender oil demonstrates activity against various bacteria and fungi, including antibiotic-resistant strains like MRSA and VRE. However, the efficacy of lavender oil varies significantly with different types and origins of lavender, as well as the methods of extraction and testing . Some studies suggest lavender oil is effective as a topical agent but not for deep-seated infections.

The study mentioned that aromatherapy using lavender oil is linked to reduced anxiety, improved mood, and enhanced sleep quality in various clinical settings. The oil's components, such as linalool and linalyl acetate, are thought to have sedative effects through their actions on the central nervous system. Additionally, lavender oil has been traditionally used for wound healing and treating skin conditions such as eczema and psoriasis. It may also help reduce discomfort post-labor when added to bath water. Nonetheless, there are concerns about allergic reactions and skin irritation, though some studies suggest lavender is less likely to cause dermatitis compared to other essential oils. Lavender oil has also shown potential as a pesticide and in promoting hair growth in conditions like alopecia. Its use in midwifery and palliative care for pain relief and anxiety reduction has been explored, although more rigorous scientific studies are needed to substantiate these claims. The review highlights several methodologies used in studying lavender oil's effects. Methods like disc/well diffusion and agar/broth dilution are commonly used in antimicrobial testing, though they are not always suitable for essential oils due to their hydrophobic nature. Clinical trials, such as those assessing hair growth and anxiety reduction, are mentioned. However, inconsistencies in methodologies and small sample sizes often limit these studies' reliability. Techniques like gas chromatography with mass spectrometry (GC/MS) are used to determine the composition of lavender oils, which is crucial for understanding their varying biological activities.

The main challenges in evaluating the therapeutic potential of lavender oil include variability in oil composition. Differences in species, extraction methods, and geographical origins lead to significant variability in oil composition, complicating the assessment of its biological activities. Methodological inconsistencies, such as the lack of standardized methodologies and detailed reporting in studies, make it difficult to compare results and draw definitive conclusions. There is a call for standardized, reproducible methods to assess essential oils' biological activities accurately.

The article concludes that while lavender essential oil shows promise in various therapeutic applications, more rigorous and standardized scientific research is needed to fully understand and validate its biological activities and clinical potential.

3. Figueiredo, A. C., et al., 2008 [9]:

This study examines the various factors that influence the presence, yield, and composition of secondary metabolites in plants, particularly focusing on volatile components and essential oils. The study identifies several key variables that can affect these secondary metabolites from their formation in the plant to their eventual isolation. These factors are critically analyzed to optimize cultivation conditions and harvesting times for commercially important crops, aiming to produce higher yields of high-quality essential oils that meet market standards.

The researchers found that understanding the chemical variability and yield determinants is essential not only for commercial purposes but also for using essential oils and volatiles as chemotaxonomic tools. The factors influencing the chemical variability and yield include physiological variations, environmental conditions, geographic variations, genetic factors and evolution, political and social conditions, and the amount of plant material, space, and manual labor required.

The methodology employed in the study involved a comprehensive review of the factors affecting secondary metabolite production in plants. This included analyzing physiological and environmental conditions, geographic and genetic variations, and socio-political influences. The research underscores the importance of each factor in determining the quality and yield of essential oils, highlighting the complexity of optimizing cultivation practices to meet both commercial and scientific needs.

4. Dapkevicius, A., et al., 1998 [5]:

This article investigates the potential of several aromatic herbs grown in Lithuania as sources of natural antioxidants in response to the growing demand for natural additives over synthetic ones. The herbs examined include marjoram (*Majorana hortensis Moench*), catnip (*Nepeta cataria L.*), oregano (*Origanum vulgare L.*), lavender (*Lavandula angustifolia Mill*), thyme (*Thymus vulgaris L.*), hyssop (*Hyssopus officinalis L.*), anise hyssop (*Lophanthus anisatus* Benth), and sage (*Salvia officinalis L.*).

To evaluate their antioxidant potential, dried herb samples underwent extraction using supercritical CO_2, acetone, methanol/water, and hydro-distillation. Additionally, deodorized herb samples (after the removal of essential oils) were extracted with acetone. The antioxidant activities of these extracts, essential oils, and dried

deodorized aqueous extracts were assessed using the β-carotene bleaching test through diffusion and spectrophotometric methods.

The findings indicated that the highest yields of extracts were obtained using polar solvents. Specifically, thyme and sage acetone oleoresins exhibited significant antioxidant activity in the tests conducted, suggesting that these two herbs are the most promising sources of natural antioxidants among the samples tested. The methodology included the use of various extraction techniques to isolate potential antioxidant compounds from the herbs, followed by rigorous testing of these extracts' antioxidant activities. The study highlights the effectiveness of polar solvents in yielding extracts with high antioxidant potential, particularly emphasizing the promise of thyme and sage as valuable natural sources of antioxidants.

5. Adam, K., et al., 1998 [2]:

The study explores the antifungal properties of essential oils from four plant species: *Origanum vulgare* subsp. hirtum, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa*, against human pathogens Malassezia furfur, Trichophyton rubrum, and Trichosporon beigelii. Among these, *O. vulgare* subsp. hirtum oil demonstrated the highest fungicidal activity, achieving a 95% reduction in the number of metabolically active cells at a dilution of 1/50000 within 6 hours of exposure. The main components of these essential oils, carvacrol and thymol, were identified as having the highest levels of antifungal activity. The therapeutic efficacy of *O. vulgare* subsp. hirtum essential oil was further validated through experiments on rats infected with T. rubrum, showing promising results. Additionally, the essential oils were assessed for mutagenic activity using the Ames test and were found to be non-mutagenic.

The methodology involved:

- Testing the antifungal properties of essential oils against specific human pathogens.
- Identifying and analyzing the main components of the oils for their antifungal activity.
- Conducting in vivo experiments to assess therapeutic efficacy.
- Using the Ames test to evaluate potential mutagenic effects.

The findings underscore the significant antifungal potential of *O. vulgare* subsp. hirtum essential oil, particularly due to its components carvacrol and thymol, and confirm its safety in terms of non-mutagenicity.

6. Lehrner, J., et al., 2005 [13]:

The study aimed to examine the effects of essential oils of orange and lavender on anxiety, mood, alertness, and calmness in dental patients. The research involved 200 patients aged 18 to 77 years, equally divided between men and women. Participants were randomly assigned to one of four groups: ambient odor of orange, ambient odor of lavender, music, or a control condition (no odor, no music).

While waiting for their dental procedures, patients' anxiety, mood, alertness, and calmness were measured. Statistical analyses indicated that both ambient odors of orange and lavender significantly reduced anxiety and improved mood compared to the control condition. The methodology included:

- Randomly assigning patients to different sensory conditions (orange odor, lavender odor, music, control).
- Assessing emotional states (anxiety, mood, alertness, calmness) while patients waited for dental treatment.
- Conducting statistical analyses to compare the effects of the different conditions.

The findings support the notion that ambient odors can influence emotional states, suggesting that the use of essential oils, particularly orange and lavender, can be beneficial in reducing anxiety and improving mood in dental patients.

7. K. Benachour, K., 2017 [10]:

The study conducted an inventory of insects visiting *Lavandula officinalis* flowers over three years (2009, 2010, and 2013) in the Constantine region of northeast Algeria. Researchers recorded insects from three orders: Hymenoptera, Lepidoptera, and Diptera. Most taxa identified (81%) exhibited very low average abundances (≤1 individual/day), with 73% being bees, including the honeybee (Apis mellifera), the bumblebee (Bombus terrestris), *Ceratina cucurbitina*, and two M*egachilid*ae species, *Anthidium florentinum* and A. *manicatum*.

The study found that bees had higher visitation rates than Lepidopterans, which included Iphiclides podalirius, Pieris rapae, and Polyommatus dorylas. Quantitative analysis, based on abundance and visitation rates, identified *B. terrestris* and *A. mellifera* as the most dominant pollinators of *L. officinalis*. Qualitative analysis, which involved counting pollen grains on the bodies of the five major bee species, revealed that *B. terrestris* was not only the most dominant but also the most efficient pollinator.

The methodologies used in the study included:

- Long-term observation and recording of insect visits to *L. officinalis* flowers over multiple years.
- Quantitative analysis of insect abundance and visitation rates.

- Qualitative analysis of pollen grains transported by major pollinators to assess pollination efficiency.

The findings underscore the significant role of bees, particularly *B. terrestris* and *A. mellifera*, in the pollination of *L. officinalis*, with *B. terrestris* being the most efficient pollinator due to its high dominance and pollen transport capacity.

8.   Abuhamdah, S., & Chazot, P. L., 2008 [1]:

The article explores the potential of natural therapeutics, specifically essential oils, as a strategy for treating emotional and neurodegenerative disorders. It highlights the historical and ongoing relevance of deriving novel treatments from natural sources, particularly in addressing the limitations of current medications for anxiety, depression, and psychotic disorders, which often suffer from inefficacy and multiple side effects. The review focuses on essential oils from Melissa and Lavender plants, noting their beneficial properties in alleviating emotional disorders. Melissa oil is particularly noted for its dual benefits: reducing agitation and maintaining attention in patients with dementia. Lavender oil is also recognized for its anti-agitation properties.

The methodology of the review involves examining existing evidence on the efficacy of these essential oils in treating emotional and neurodegenerative disorders. The analysis indicates that both Melissa and Lavender essential oils offer promising alternative treatments, particularly in neurodegenerative disease contexts where current medications fall short. In summary, the article emphasizes the therapeutic potential of Melissa and Lavender essential oils in managing emotional disorders and highlights their specific benefits for patients with neurodegenerative diseases. Their findings suggest a valuable role for natural therapeutics in addressing the shortcomings of conventional medications.

9.   Lane, A., et al., 2010 [12]:

This study aims to develop *L. angustifolia* as a model system to investigate the molecular regulation of essential oil production, which comprises mono- and sesquiterpenes. As an initial step towards establishing the necessary genomic tools for this species, researchers constructed two cDNA libraries from lavender leaves and flowers and sequenced 14,213 high-quality expressed sequence tags (ESTs). The EST collection includes orthologs for genes involved in the 1-deoxy-D-xylulose-5-phosphate (DXP) and mevalonic acid (MVA) pathways of terpenoid biosynthesis, as well as known terpene synthases and prenyl transferases.

To understand the regulation of terpene metabolism in lavender flowers, the study evaluated the transcriptional activity of genes encoding 1-deoxy-D-xylulose-5-phosphate synthase (DXS) and HMG-CoA reductase (HMGR) in glandular trichomes (oil glands) using real-time PCR. The results showed that HMGR transcripts were barely detectable, while DXS was highly expressed in glandular trichomes, suggesting that essential oil constituents are predominantly produced through the DXP pathway in these tissues. Additionally, the gene responsible for linalool production (linalool synthase, LinS) was strongly expressed in glandular trichomes.

Interestingly, the most abundant transcript in floral glandular trichomes corresponded to a sesquiterpene synthase (cadinene synthase, CadS), even though sesquiterpenes are minor constituents of lavender essential oils. This finding, along with the weak activity of the MVA pathway (the primary route for sesquiterpene production) in trichomes, suggests that precursor supply might be a limiting factor in sesquiterpene biosynthesis in lavender flowers.

Methodologies:

- Construction of cDNA Libraries: Libraries were constructed from lavender leaves and flowers.
- Sequencing and EST Collection: Sequencing yielded 14,213 high-quality ESTs.
- Homology Analysis: ESTs were analyzed for homology to sequences in GenBank to identify genes involved in terpenoid biosynthesis.
- Transcriptional Activity Evaluation: Real-time PCR was used to evaluate the transcriptional activity of DXS and HMGR in glandular trichomes.
- Gene Expression Analysis: Expression levels of LinS and CadS were assessed in floral glandular trichomes.

Findings:

- Essential oil production in lavender glandular trichomes predominantly occurs through the DXP pathway, indicated by high DXS expression.
- LinS, responsible for linalool production, is strongly expressed in glandular trichomes.
- Despite being the most abundant transcript, CadS expression suggests a bottleneck in sesquiterpene biosynthesis due to limited precursor supply from the MVA pathway.

This research provides a foundation for further genomic studies on lavender, aiming to enhance the understanding of essential oil biosynthesis and regulation in plants.

10.   Yahya, M.A., et al., 2024a [28]:

The article details the development of an *in vitro* propagation protocol *for L. angustifolia* Miller. Researchers used Murashige and Skoog (MS) media supplemented with various concentrations of Plant Growth Regulators (PGRs) to study the growth parameters of nodal segments and assess callus formation and shoot proliferation. Nodal explants supplemented with 2 mg/L of 6-Benzylaminopurine (BAP) successfully formed callus. The optimal hormonal concentration for shoot proliferation from callus cultures (39.33%) was determined to be 5 mg/L of 2-Isopentenyl adenine (2iP). Additionally, the best media for callus growth were found to be those containing 1 and 2 mg/L of BAP, achieving a 98% formation rate.

The researchers performed qualitative and quantitative analyses on the callus cultures and *in vitro* propagated seedlings, examining phenolic profiles, flavonoids, and phenolic acids using High-Performance Liquid Chromatography (HPLC), as well as analyzing volatile compounds through headspace-SPME. Seedlings grown in media with 1 mg/L of 6-Furfurylaminopurine (KIN) had the highest yield of phenolic acids (11.95 ± 0.01 mg GAE/g). Conversely, the lowest concentration of phenolic acids (2.17 ± 0.04 mg GAE/g) was found in media containing 0.5 mg/L of BAP and 0.5 mg/L of Naphthaleneacetic acid (NAA).

The study also revealed that plantlets grown in media with 0.5 mg/L of BAP and 0.5 mg/L of NAA exhibited the highest flavonoid yield (31.67 ± 0.06 μg/g QE/g), whereas callus samples in media supplemented with 0.5 mg/L of BAP had the lowest flavonoid yield (11.59 ± 0.02 μg/g QE/g). HPLC analysis showed variability in phenolic acid content among callus cultures and plantlets, identifying gallic acid, 4-OH benzoic acid, chlorogenic acid, vanillic acid, caffeic acid, cinnamic acid, and rosmarinic acid as the main constituents. Headspace-SPME analysis identified twenty-two chemicals, with eucalyptol, nonanal, borneol, carvone, and β-caryophyllene being the most abundant. The study concludes that micropropagation of *L. angustifolia* is an effective method for producing large quantities of genetically identical plantlets to produce high-value bio compounds.

11. Nakajima, D., et al., 2024 [18]:

The study aimed to explore the relationship between oxytocin secretion and the effects of aromatherapy with lavender oil on anxiety levels in healthy individuals. The hypothesis was that if aromatherapy could promote oxytocin secretion, it might improve mood and alleviate anxiety. The investigation was carried out through a randomized open crossover trial involving 15 men and 10 women. Each participant experienced both a placebo intervention (control group) and an aromatherapy session (aromatherapy group). The aromatherapy intervention included a 30-minute session in a room with diffused lavender oil followed by a 10-minute hand massage using a carrier oil containing lavender oil.

Anxiety levels were measured using the State-Trait Anxiety Inventory (STAI) at three time points: before the intervention, 30 minutes after the start of the intervention, and after the hand massage. Saliva samples were collected at the same time points to measure oxytocin levels.

The findings revealed that in women, both aromatherapy and hand massage independently led to a reduction in anxiety levels and an increase in salivary oxytocin levels after aromatherapy. In men, anxiety levels decreased following both aromatherapy and hand massage, regardless of the presence of lavender oil, but there were no significant changes in salivary oxytocin levels between the control and aromatherapy groups during the intervention period. Furthermore, a positive correlation was found between anxiety levels and salivary oxytocin levels before the intervention, which shifted to a negative correlation after the hand massage with lavender oil. In conclusion, the study suggests that aromatherapy with lavender oil effectively reduces anxiety and increases oxytocin levels in women, whereas in men, aromatherapy's impact on anxiety and oxytocin levels is less clear, though it does alter the correlation between anxiety and oxytocin. These findings indicate that the effects of aromatherapy can vary by sex, highlighting the need for further research to understand the underlying mechanisms and potential sex-specific responses.

12. Mazraeh, A., Tavallali, H., & Tavallali, V., 2024 [15]:

The article investigates the synthesis of copper nano complexes (Cu-NCs) using aqueous extracts of jujube and neem leaves and examines their effects on Lavandula sublepidota Rech. f., an Iranian native medicinal herb. Copper (Cu) is recognized as an essential micronutrient in plant physiology and biochemistry. The study aimed to determine the impact of foliar application of Cu-jujube and Cu-neem Cu-NCs at various concentrations (0, 10, 25, and 50 mg L^-1) on the bioactive compounds, antioxidant capacity, and essential oil content of the lavender plant.

The methodology involved synthesizing Cu-NCs from jujube and neem leaf extracts and applying these complexes as foliar sprays to the lavender plants. The concentrations used were 0, 10, 25, and 50 mg L^-1. The researchers then measured several parameters, including levels of flavonoids and polyphenols, essential oil yield, secondary metabolite content, antioxidant activity, and antibacterial activity against three pathogen strains.

The findings revealed that the highest levels of flavonoids and polyphenols were observed in plants treated with Cu-NCs at a concentration of 25 mg L^-1, with no significant difference between the effects of Cu-jujube and Cu-neem complexes. Additionally, at the same concentration, the essential oil yield increased by 48% with Cu-jujube and 52% with Cu-neem compared to the control. This suggests an optimal concentration threshold, beyond which toxicity effects may occur. The study also reported that the amount of commercially significant secondary metabolites was highest at the 25 mg L^-1 concentration compared to 10 and 50 mg L^-1. Moreover, the maximum antioxidant activity was found in lavender extracts treated with 25 mg L^-1 Cu-NCs. The exogenous application of Cu-NCs significantly enhanced the antibacterial activity of the extracts against the three pathogen strains, demonstrating greater effectiveness at the optimal concentration of 25 mg L^-1.

In conclusion, the study suggests that Cu-NCs, particularly at a concentration of 25 mg L^-1, are effective in enhancing the production of essential oil and bioactive compounds in Lavandula sublepidota Rech. f. This research highlights the importance of synthesizing nano complexes using plant extracts and emphasizes the potential for using plant-based Cu-NCs to increase the medicinal and commercial value of lavender through improved yields of essential oils and bioactive compounds. The results underscore the necessity of understanding the phytochemical changes in lavender plants resulting from such treatments.

13. Kim, Y. J., et al., 2024 [11]:

The article addresses the ongoing health issue of smoking in patients with type 2 diabetes mellitus by investigating the impact of high glucose (HG) and nicotine on intracellular calcium ion concentrations ([Ca2+]i) in microglia, neurons, and astrocytes. The study's primary objective was to compare [Ca2+]i levels in these cells under HG and nicotine conditions and to evaluate the potential modulatory effects of *L. angustifolia* Mill. essential oil (LEO).

The researchers employed Fura-2 acetoxymethyl ester fluorescence to measure [Ca2+]i concentrations. Their results showed that treatment with HG and nicotine significantly increased [Ca2+]i in microglia and neurons through Ca2+ influx from extracellular sources. Notably, LEO was found to significantly reduce this increased Ca2+ influx in microglia, an effect that was partially inhibited by the Na+/Ca2+ exchanger (NCX) inhibitor Ni2+. Similarly, in neuron-like cells pretreated with HG and nicotine, LEO significantly decreased Ca2+ influx, with this effect partially blocked by the L-type Ca2+ channel blocker nifedipine and the T-type Ca2+ channel blocker mibefradil. Additionally, the study found that either LEO treatment or a two-fold increase in the number of astrocytes attenuated the Ca2+ influx caused by high glucose and nicotine in mixed cultures of microglia, neuron-like cells, and astrocytes. These findings suggest that LEO can regulate HG and nicotine-induced Ca2+ influx into microglia and neurons through distinct mechanisms, highlighting its potential therapeutic role in mitigating the cellular effects of smoking in diabetic patients.

14. Shan, J., et al., 2024 [20]:

In this study researchers utilized a replicative lifespan (RLS) experiment involving K6001 yeast to identify anti-aging compounds in lavender extract (*L. angustifolia* Mill.), leading to the discovery of β-cyclocitral (CYC) as a promising anti-aging agent. Concurrent validation was achieved through chronological lifespan (CLS) experiments with YOM36 yeast and mammalian cells, confirming the anti-aging properties of CYC. This compound was shown to extend the yeast lifespan and inhibit cell senescence induced by etoposide (ETO).

The study delved into the mechanisms underlying CYC's anti-aging effects, focusing on telomere biology, oxidative stress, and autophagy. Administration of CYC resulted in significant increases in telomerase content, telomere length, and expression levels of telomeric shelterin proteins, including telomeric-repeat binding factor 2 (TRF2) and repressor activator protein 1 (RAP1). Furthermore, CYC reversed H2O2-induced telomere damage, highlighting its potent antioxidant capacity. It also improved the survival rate of BY4741 yeast under oxidative stress induced by 6.2 mM H2O2 by enhancing antioxidant enzyme activity and reducing levels of reactive oxygen species (ROS), reactive nitrogen species (RNS), and malondialdehyde (MDA). Moreover, CYC was found to enhance autophagic flux and the expression of free green fluorescent protein (GFP) in the YOM38-GFP-ATG8 yeast strain, suggesting an upregulation of autophagy. However, CYC did not extend the RLS of K6001 yeast mutants deficient in antioxidant enzymes (Δsod1, Δsod2, Δcat, Δgpx) or autophagy-related genes (Δatg2, Δatg32), indicating that its anti-aging effects are dependent on these pathways.

These findings collectively reveal that CYC functions as an anti-aging agent by modulating telomeres, oxidative stress, and autophagy. The promising results warrant further investigation of CYC as a potential therapeutic compound for aging-related interventions.

15. Soden, K., et al., 2004 [21]:

The research explores the use of aromatherapy and massage in patients with advanced cancer, focusing on their physical and psychological benefits. Given the growing popularity of these therapies in palliative care,

the study aimed to compare the effects of four-week courses of aromatherapy massage and massage alone on various symptoms in this patient group. Forty-two patients were randomly assigned to three groups: one receiving weekly massages with lavender essential oil and an inert carrier oil (aromatherapy group), one receiving massages with an inert carrier oil only (massage group), and one receiving no intervention.

The outcome measures included a Visual Analogue Scale (VAS) for pain intensity, the Verran and Snyder-Halpern (VSH) sleep scale, the Hospital Anxiety and Depression (HAD) scale, and the Rotterdam Symptom Checklist (RSCL). The study found no significant long-term benefits of either aromatherapy or massage in terms of pain control, anxiety, or overall quality of life. However, there were notable improvements in sleep scores in both the massage and combined massage (aromatherapy and massage) groups. Additionally, the massage group showed statistically significant reductions in depression scores.

The results indicate that the addition of lavender essential oil did not enhance the beneficial effects of massage. Nevertheless, the findings suggest that patients experiencing high levels of psychological distress may derive the most benefit from these therapies. Overall, while aromatherapy did not show added advantages, massage alone was effective in improving sleep and reducing depression in patients with advanced cancer.

Conclusion

This study demonstrates notable patterns and key elements across time, showcasing both enduring and fluctuating interest in the research around *L. angustifolia*. The growing interest in alternative and complementary medicine in response to lifestyle diseases and antibiotic resistance has driven increased research in the medicinal uses of lavender. Additionally, the shift in research funding toward biotechnology and sustainable agricultural practices has contributed to advances in lavender cultivation techniques. The publication patterns show distinct spikes in research effort, particularly in 2006, 2014, and 2020. These peaks suggest periods of increased interest, possibly driven by significant advancements or global health phenomena. The 2006 spike corresponds to major advances in the extraction and commercialization of lavender essential oils, particularly for medical and cosmetic purposes. The 2014 increase can be attributed to a surge in research on natural antioxidants, driven by the global push for more sustainable health solutions. The sharp rise in 2020 is likely linked to the COVID-19 pandemic, where there was a global shift toward exploring alternative remedies and herbal treatments. This aspect provides a more comprehensive understanding toward social, economic, and scientific factors that potentially influence research activity.Publications such as "Acta Horticulturae" and "Industrial Crops and Products" have consistently made contributions, while others have shown more irregular activity, highlighting the wide appeal of the research within multidisciplinary studies. An examination of geographic distribution (Scopus and PubMed databases) reveals that the United States, Iran, and China are the primary publishers, indicating a significant worldwide interest. Institutional analysis highlights prominent actors such as the CNRS in France and Tehran University of Medical Sciences. The examination of subject areas highlights *L. angustifolia* 's wide range of scientific uses, with notable contributions from fields like agricultural sciences, medicine, and biochemistry. The distribution of document types shows that peer-reviewed papers and reviews are the most prevalent, highlighting the strong emphasis on academic rigor in studying Lavender. This study suggests that using citation and co-authorship analysis in Vos-viewer software, researchers may identify pivotal works and authors, as well as reveal crucial collaboration networks as well as researchers can then use this data to inform and steer future research endeavors. In conclusion, this study offers a thorough and inclusive examination of the research environment using Vos-viewer software, highlighting the *L. angustifolia* 's substantial and ever-changing influence on various scientific fields.

Abbreviations
2,2-diphenyl-1-picrylhydrazyl (DPPH); Critical Literature Review (CLR); Centre National de la Recherche Scientifique (CNR); 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS); High-quality expressed sequence tags (ESTs); Mevalonic acid (MVA); 1-deoxy-D-xylulose-5-phosphate synthase (DXS); HMG-CoA reductase (HMGR); High-performance liquid chromatography (HPLC); State-Trait Anxiety Inventory (STAI); Copper nano complexes (Cu-NCs); High glucose (HG); β-cyclocitral (CYC); Replicative lifespan (RLS); Chronological lifespan (CLS); Visual Analogue Scale (VAS); Snyder-Halpern (VSH); Hospital Anxiety and Depression (HAD); Rotterdam Symptom Checklist (RSCL).

Availability of data and material
Please contact the corresponding author for any data request.

References

1. Abuhamdah, S., and P. L. Chazot, Lemon balm and lavender herbal essential oils: old and new ways to treat emotional disorders?. *Current Anaesthesia & Critical Care*, 2008. *19*(4), 221-226.
2. Adam, K., et al., Antifungal activities of Origanum vulgare subsp. hirtum, Mentha spicata, *Lavandula angustifolia*, and Salvia fruticosa essential oils against human pathogenic fungi. *Journal of agricultural and food chemistry*, 1998. *46*(5), 1739-1745.
3. Cavanagh, H. M. A., and J. M. Wilkinson, Biological activities of lavender essential oil. Phytotherapy research, 2002. 16(4), 301-308.
4. Chen, C. C., et al., *In vitro* propagation and analysis of secondary metabolites in Glossogyne tenuifolia (Hsiang-Ju)-a medicinal plant native to Taiwan. *Botanical Studies*, 2004. *55*, 1-9.
5. Dapkevicius, A., et al., Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. Journal of the Science of Food and Agriculture, 1998. 77(1), 140-146.
6. Davison, B., et al., The impact of biotechnological advances on the future of US bioenergy. Biofuels, 2015. 9. https://doi.org/10.1002/bbb.1549.
7. Enfissi, E., et al., New plant breeding techniques and their regulatory implications: An opportunity to advance metabolomics approaches.. Journal of plant physiology, 2021. 258-259, 153378 . https://doi.org/10.1016/j.jplph.2021.153378.
8. Falagas, M., et al., Comparison of PubMed, Scopus, Web of Science, and Google Scholar: strengths and weaknesses. The FASEB Journal, 2007. 22, 338 - 342. https://doi.org/10.1096/fj.07-9492LSF.
9. Figueiredo, A. C., et al., Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour and Fragrance journal, 2008. 23(4), 213-226.
10. Benachour, K., Insect visitors of lavender (Lavandula officinalis L.): Comparison of quantitative and qualitative interactions of the plant with its main pollinators. African Entomology, 2007. 25(2), 435-444.
11. Kim, Y. J., et al., *Lavandula angustifolia* Mill. inhibits high glucose and nicotine-induced Ca2+ influx in microglia and neuron-like cells via two distinct mechanisms. *Biomedicine & Pharmacotherapy*, 2024. *177*, 117062.
12. Lane, A., et al., A genomics resource for investigating regulation of essential oil production in *Lavandula angustifolia*. *Planta*, 2010. *231*, 835-845.
13. Lehrner, J., et al., Ambient odors of orange and lavender reduce anxiety and improve mood in a dental office. Physiology & Behavior, 2005. 86(1-2), 92-95.
14. Limera, C., et al., New Biotechnological Tools for the Genetic Improvement of Major Woody Fruit Species. Frontiers in Plant Science, 2017. 8. https://doi.org/10.3389/fpls.2017.01418.
15. Mazraeh, A., H. Tavallali and V. Tavallali, Variations in the biochemical characteristics of Lavandula sublepidota Rech. f. in response to the foliar enrichment of green-synthesized copper nano complexes from extract of Neem and Jujube. *Plant Physiology and Biochemistry*, 2024. 108885.
16. Miliauskas, G., et al., Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food chemistry, 2024. 85(2), 231-237.
17. Moral-Muñoz, J., et al., Software tools for conducting bibliometric analysis in science: An up-to-date review. El Profesional de la Información. 2020. https://doi.org/10.3145/epi.2020.ene.03.
18. Nakajima, D., et al., Sex differences in the effects of aromatherapy on anxiety and salivary oxytocin levels. *Frontiers in Endocrinology*, 2024. *15*, 1380779.
19. Noman, A., et al., Biotechnological Advancements for Improving Floral Attributes in Ornamental Plants. Frontiers in Plant Science, 2017. 8. https://doi.org/10.3389/fpls.2017.00530.
20. Shan, J., et al., β-Cyclocitral from *Lavandula angustifolia* Mill. Exerts Anti-Aging Effects on Yeasts and Mammalian Cells via Telomere Protection, Antioxidative Stress, and Autophagy Activation. *Antioxidants*, 2024. *13*(6), 715.
21. Soden, K., et al., A randomized controlled trial of aromatherapy massage in a hospice setting. *Palliative medicine*, 2004. *18*(2), 87-92.
22. Tasheva, K., and G. Kosturkova, Role of biotechnology for protection of endangered medicinal plants. *Environmental biotechnology-New approaches and prospective applications*, 2013. 235-238.
23. Upson, T., The taxonomy of the genus Lavandula L. In Lavender 2002. pp. 16-48. CRC Press.
24. Van Eck, N., and L. Waltman, Software survey: VOSviewer, a computer program for bibliometric mapping. Scientometrics, 2009. 84, 523 - 538. https://doi:10.1007/s11192-009-0146-3
25. Xiao, Y., and M. Watson, Guidance on conducting a systematic literature review. Journal of Planning Education and Research, 2017. 39, 112 - 93. https://doi:10.1177/0739456X17723971
26. Yahya, M. A., and H. Mammadzada, Targeting generation Z: A systematic literature review (SLR) and bibliometric analysis for effective marketing. Journal of Politics Economy and Management, 2024c. 7(1), 21-44.
27. Yahya, M. A., et al., *In vitro* production of *Phalaenopsis* orchids. Turkish Journal of Biodiversity, 2024b. 7(1), 41-53. https://doi.org/10.38059/biodiversity.1452374
28. Yahya, M.A., et al., Phenolic profile and volatiles of *in vitro* propagated *Lavandula angustifolia* mill. seedlings. Phyton-International Journal of Experimental Botany, 2024a. 93(3), 427-444. https://doi.org/10.32604/phyton.2024.046271
29. Vieira, E., and J. Gomes, A comparison of Scopus and Web of Science for a typical university. Scientometrics, 2009. 81, 587-600. https://doi:10.1007/s11192-009-2178-0
30. Burnham, J., Scopus database: a review. Biomedical Digital Libraries, 2006. 3, 1. https://doi:10.1186/1742-5581-3-1
31. Zhu, J., and W. Liu, A tale of two databases: the use of Web of Science and Scopus in academic papers. Scientometrics, 2020. 123, 321-335. https://doi:10.1007/s11192-020-03387-8
32. Singh, V., et al. The journal coverage of Web of Science, Scopus and Dimensions: A comparative analysis. Scientometrics, 2020. 126, 5113-5142. https://doi:10.1007/s11192-021-03948-5

The Effect of Kuchala (*Arum korolkowii* Regel, 1873) Tuber Tincture To Increase Of The Serum Testosterone In The Adult Male Guinea Pigs (*Cavia porcellus* Linnaeus, 1758)

Nurbek Aldayarov[1*] iD , Askarbek Tulobaev[2] iD

ABSTRACT

Kuchala (*Arum korolkowii* Regel) is a medicinal plant commonly used in folk medicine among the Kyrgyz people. The tuber tincture of kuchala is traditionally used in small doses to enhance human sexual potency. However, there is no scientific evidence supporting these medicinal effects. Therefore, we decided to study the effect of kuchala tuber tincture on the sexual potency of adult male guinea pigs. We investigated the effect of kuchala on 12 male guinea pigs, each approximately 48 months old. A 10% tuber tincture of kuchala in 70% ethanol was prepared and administered orally at a daily dose of 150 µl for 30 days. The study employed ethological, hematological, serum biochemistry, gross anatomical, histological, and statistical methods to collect and analyze the data. The hematological and serum biochemistry parameters showed significant differences between the control and experimental groups. In the experimental group, the percentage of neutrophils was significantly lower ($^d p < 0.001$) than in the control group, while lymphocyte counts were significantly higher ($^d p < 0.001$). Additionally, RBC counts, hemoglobin (Hgb), hematocrit (Hct), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were all significantly higher in the experimental group ($^d p < 0.001$; $^d p < 0.001$; $^c p < 0.01$; $^d p < 0.001$; $^d p < 0.001$, respectively) compared to the control group. Conversely, the color indicator and mean platelet volume were higher ($^b p < 0.05$) and significantly higher ($^d p < 0.001$) in the control group than in the experimental group. The levels of alanine transaminase (ALT) and aspartate transaminase (AST) were lower in the experimental group than in the control group (both $^d p < 0.001$). Notably, the serum testosterone concentration was much higher ($^d p < 0.001$) in the experimental group. Microscopic examination revealed minor structural damage in the liver tissue of the experimental group, indicating a metabolic disorder. However, the testes in the experimental group showed an improvement in spermatogenesis compared to the control group, suggesting a positive effect on reproductive health. The 10% kuchala tuber tincture in 70% ethanol has a positive effect on improving the sexual potency of older guinea pigs by increasing testosterone production and enhancing spermatogenesis.

Background

Since ancient times, people have successfully used folk medicine, which contains an inexhaustible wealth of information about medicinal plants as important therapeutic agents in both human and veterinary medicine [1, 2, 3, 4, 5]. Many treatment methods have been passed down through generations and have been adapted for use in modern medical practice. Kyrgyz folk medicine, in particular, has occupied an important place in the nomadic civilization of the Kyrgyz people. The Kyrgyz Republic is a mountainous country in Central Asia. Due to its extreme environment and climate, there is a diverse range of plant species, including more than 200 species of medicinal plants. Many of these medicinal plants used in Kyrgyz folk medicine have not been studied using modern scientific techniques [6]. *Arum korolkowii* Regel is one of the medicinal plants often used in the folk medicine of Central Asia, and it remains relevant today. The vernacular name of this medicinal plant is kuchala. A. korolkowii, described in 1873, belongs to the genus Arum L. of the family Araceae Juss.

---

[1] Kırgızistan-Türkiye Manas Üniversitesi, Faculty of Science, Department of Biology
[2] Kyrgyz - Turkish Manas University, Faculty of Veterinary Medicine
*Corresponding Author: Nurbek Aldayarov, e-mail: nurbek.aldayarov@manas.edu.kg

It grows in soil pockets of rocky hillsides, beneath low scrub. Its native range includes Central Asia, North-Western China, Northern Iran, and Afghanistan. *A. korolkowii* is a perennial tuberous herb that sprouts in early spring from a discoid, vertically oriented tuber. It has well-described biological characteristics [7, 8]. However, there is another plant also called kuchla or Chinese kuchla (*Strychnos nux-vomica*). *S. nux-vomica* is an evergreen tree that can reach up to 25 meters in height. Its dried seeds (Nux vomica) are used in both modern and traditional medicine [9, 10].

Arum korolkowii Regel is a very poisonous herb. In folk medicine, a tincture of its tubers is used in small doses as a medicinal raw material to increase human potency and immunity, treat infertility and stomach ulcers, and address diseases of the nasopharynx and respiratory tract. It is also believed to eliminate fatigue and increase strength. The powdered tuber is used to treat poisonous snake and scorpion bites, fungal skin diseases, and hemorrhoids [7]. The medicinal properties of kuchala are mentioned in the works of Avicenna and in the Kyrgyz folk epics "Manas" 11] and "Semetey" [12] .

According to these sources, milky and sour-milky (kumys) tinctures of the tubers are often used among the elderly (over 70 years) to increase male sexual potency. However, there are no modern scientific data proving the medicinal properties of kuchala, particularly its effect on human potency. Additionally, the chemical composition of *A. korolkowii* has not been studied yet. In this regard, we decided to experiment with kuchala tuber tinctures on laboratory animals, with a particular focus on adult male guinea pigs. Thus, the purpose of this study was to investigate the effect of tincture of kuchala tubers (*Arum korolkowii* Regel) on hematological and biochemical parameters of blood serum, as well as on the structure of the testes and liver of adult male guinea pigs (*Cavia porcellus* Linnaeus, 1758).

Materials and Methods

Kuchala tubers and tincture

Dried kuchala tubers of 5 pieces (49.62 g) were procured from local markets in Bishkek, Kyrgyz Republic. Each tuber piece underwent cleaning with warm water followed by 70% ethanol, and subsequently dried in ambient air at room temperature. The tubers, including their peels, were then shredded using a manual grinder. The ground tuber material was weighed using an electronic Precisa scale (Switzerland) and used to prepare a 10% tincture in 70% ethanol. The resulting tincture was poured into a dark glass bottle with a tight seal and stored in a dark place at room temperature. Twice daily, the tincture was agitated, and this process continued for 14 days until the tincture reached readiness. On the 15th day, the tincture was filtered through dense gauze and subsequently filtered through filter paper. The prepared 10% tincture in 70% ethanol was then stored in a refrigerator (+4 °C) and utilized for the experimental study.

Experimental animals and husbandry

We acquired 22 male Abyssinian breed guinea pigs, all approximately 48 months old and with an average weight of 682 g (ranging from 489 to 792 g), from a private guinea pig producer. The animals were clinically healthy at the time of purchase. They were housed in two isolator cages: one measuring 98.7 cm x 347.89 cm x 54.3 cm for 10 control animals and the other measuring 110.3 cm x 398.73 cm x 54.6 cm for 12 experimental animals. The cages were handmade and contained sun-dried clean straw bedding, as well as cardboard huts for enrichment. Bedding was changed every two days or more frequently if necessary. The animals were kept in a room with a temperature ranging from 22 to 26 °C and humidity between 40% and 75%. Guinea pigs had ad libitum access to rodent chow and water. The conditions of animal maintenance and nutrition were in accordance with previously described guidelines [13,14].

The animals were allowed to acclimate in the vivarium for 10 days after delivery before they were used for the study. The research protocol was approved by the Ethics Committee for Animal Experimentation of Kyrgyz-Turkish Manas University, Kyrgyz Republic (№7, 20/12/2019).

Treatments and handling

During the 10-day adaptation period, we closely observed the behavior of the guinea pigs, monitoring their physical activity and body weight. Based on these observations, the experimental animals were divided into two groups. Both the control and experimental groups were formed according to the aforementioned principle, with the control group consisting of 10 male animals and the experimental group consisting of 12 male animals. Both groups were maintained under the same conditions.

Each day, from 8:00 to 9:00 a.m., the experimental animals received 150 μl of kuchala tubers tincture orally, while the control group received 150 μl of normal water orally, for a duration of 30 days. Subsequently, we observed the behavior of the animals through a window in the adjacent room and documented any changes in their behavior.

Blood collection

Blood samples were collected from each guinea pig twice: once five days before the start and once after the completion of administering the tuber tincture, while the animals were under isoflurane anesthesia (3% to 5%). Blood was drawn from the cranial vena cava using a method previously well described in the literature [15]. Throughout the phlebotomy procedure, strict adherence to aseptic and antiseptic protocols was maintained. A 25-gauge, 5/8-inch needle attached to a 3 ml syringe (Zhejiang Huafu Medical Equipment Co. LTD, China) was used to obtain blood from the cranial vena cava. The collected blood was immediately transferred into Gel/Clot Activator (GD060SGC) tubes for serum collection and EDTA.K3 (GD060EK3) tubes for general blood analysis. Additionally, blood smears were prepared for cytological examination.

Blood analysis

The collected blood samples were stored for up to 2-3 hours before processing in a refrigerator at 4°C. Subsequently, the blood samples were submitted to a human clinical-diagnostic laboratory for processing and analysis. Hematological analysis was performed using an APUIA 560 Hematology System (Siemens, Germany) for 1–2 minutes, while serum biochemistry analysis was conducted using a Beckman Coulter AU 480 analyzer (USA-Japan) for 15-20 minutes. Immune chemiluminescence tests were analyzed using an ImmuLite 2000 XPi Immunoassay system (Siemens, Germany) for 1 hour and 6 minutes. These machines undergo routine calibration every 6 months by service professionals using commercial calibration standards. An Erythrocyte Sedimentation Rate (ESR) was determined manually. Blood smears were prepared and stained with MGG Quick Stain (04-090805, Bio Optica Milano s.p.a.) using a flooded slide preparation method for microscopic examination.

Necropsy

The anesthetized animals were euthanized by exsanguination and then underwent necropsy following standard procedures [16]. The heart, liver, and testes were extracted, and their gross anatomy data, including color, consistency, and blood filling, were studied. Morphometric parameters of the liver and testes, such as length, width, and thickness, were recorded. The weight of the organs was measured using an electronic weight scale (Precisa, Switzerland).

Histology

Tissue samples from the testes and liver for microscopic study were fixed in neutral buffered 4% formaldehyde (pH 7.4) overnight at room temperature. Following standardized histological processing to paraffin, sections 4 μm thick were cut using an automated Leica RM2255 rotary microtome. These sections were then stained with hematoxylin and eosin. Observation and photography were conducted using a Nikon ECLIPSE 50i microscope equipped with a Nikon Digital Sight DS-Fi1 camera.

Statictical analysis

The hematological and serum biochemical data obtained were subjected to statistical processing using software (Microsoft Excel). Mean, standard deviations (SDs), median, Student's t-test, minimum, and maximum values were calculated. A p-value of less than 0.05 was considered statistically significant.

Results

Physical characteristics of tubers and tincture

The dried kuchala tubers are primarily discoid, ranging from 2 to 6 cm across and 2 to 2.3 cm thick. The peel is light brown and hard, flaking off into small, hard scales during cleaning. Beneath the hard peel, there is a soft, thin, easily removable shell of yellowish-white color. Upon transverse sectioning, the tuber exhibits a yellow-white color, is easily cut, and has a soft consistency akin to plasticine. The tubers can be easily pressed, forming a mushy oily mass. They do not emit a pungent smell, but upon tasting, a strong bitter taste reminiscent of spicy pepper develops after a few moments, lingering for an extended duration. The prepared 10% tincture of tubers in 70% ethanol is transparent, viscous, and yellowish-reddish in color. It possesses a distinctive bitter smell, distinguishable from the odor of alcohol.

Animal behavior

The behavior of animals in both the control and experimental groups was observed from an adjacent room through a window, and the observations were recorded. Both groups exhibited an active lifestyle, frequently engaging in running, playing, and occasional fighting among themselves. They also displayed healthy appetites and consumed food well. However, over time, a noticeable increase in appetite and activity was observed in the experimental animals compared to the control group. Experimental animals displayed heightened aggression, often engaging in fights among themselves and occasionally climbing on the sidewalls of the isolator cage.

Hematology

We observed no adverse effects in terms of clinical signs of anemia or other disorders after guinea pigs were phlebotomized under isoflurane anesthesia. Common hematology parameters including WBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils, RBC, Hgb, Hct, MCV, MCH, MCHC, color indicator, erythrocyte sedimentation rate, platelets, and mean platelet volume were evaluated (Table 1). The results of the study revealed several significant differences in blood parameters between the control and experimental groups (Fig. 1).

Neutrophil percentage in experimental animals was significantly lower ([d]$p<0.001$) than in control animals, while lymphocyte counts were significantly higher in experimental animals ([d]$p<0.001$). RBC counts, Hgb, Hct, MCH, and MCHC were significantly higher in experimental animals ([d]$p<0.001$; [d]$p<0.001$; [c]$p<0.01$; [d]$p<0.001$; [d]$p<0.001$, respectively) compared to control guinea pigs. However, the color indicator and mean platelet volume were significantly higher in control animals ([b]$p<0.05$; [d]$p<0.001$, respectively) compared to experimental animals.

Other hematological parameters such as WBC, monocytes, eosinophils, basophils, MCV, erythrocyte sedimentation rate, and platelets between the animal groups were not statistically significant.

Note: The hematological and serum biochemistry parameters of the animals obtained before the experiment showed almost no difference compared to the animals in the control group. Therefore, only the parameters of the control group animals were presented.

Table 1 Hematological parameters for control and experimental guinea pigs

Blood parameters	Control group (n = 10)					Experimental group (n = 12)				
	Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max
WBC (x10⁹/L)	7.24 ± 0.343	1.084	7.1	5.5	9.1	7.94 ± 1.00	3.48	7	4	15.2
Neutrophils (%)[d]	53.6 ± 1.258 [d]	3.978	53	48	61	27.08 ± 2.62	9.07	26.5	14	46
Lymphocytes (%)[d]	39.4 ± 0.872	2.757	40	35	43	56.08 ± 3,08[d]	10.66	56.5	40	71
Monocytes (%)	4.8 ± 0.291	0.919	5	4	7	4.58 ± 0,89	3.09	3.5	2	11
Eosinophils (%)	2.4 ± 0.499	1.578	3	0	4	5.42 ± 1,65	5.71	4	0	20
Basophils (%)	0.4 ± 0.163	0.516	0	0	1	0.08 ± 0,08	0.29	0	0	1
RBC (x10¹²/L)[d]	4.53 ± 0.110	0.347	4.6	3.9	4.9	5.38 ± 0,03[d]	0.10	5.35	5.3	5.6
Hgb (g/dL)[d]	144.5 ± 0.934	2.953	145.5	139	148	154.58 ± 1,14[d]	3.96	154.5	147	160[d]
Hct (%)[c]	43.6 ± 0.340	1.075	44	42	45	45.33 ± 0.37 [c]	1.27	45.1	44	48[c]
MCV (fL)	76.6 ± 1.222	3.864	77	70	81	74.61 ± 0.95	3.30	73.8	70.4	84.2
MCH (pg) [d]	24.27 ± 0.495	1.566	24.2	21.9	26.3	53.89 ± 5.94[d]	20.57	51.5	25.7	95.5
MCHC (g/dL) [d]	32.36 ± 0.264	0.834	32.45	30.9	33.3	752.83 ± 132.66[d]	459.54	685	352	1910
Color indicator[b]	0.88 ± 0.007[b]	0.022	0.88	0.83	0.9	0.85 ± 0,00	0.01	0.855	0.83	0.87
Erythrocyte sedimentation rate (mm/hour)	2.6 ± 0.163	0.516	3	2	3	2.25 ± 0.13	0.45	2	2	3
Platelets (x10⁹/L)	301.9 ± 2.364	7.475	303	289	310	343.50 ± 22.05	76.38	335.5	235	494
Mean platelet volume (fL)[d]	7.43 ± 0.037[d]	0.116	7.4	7.3	7.7	4.58 ± 0.30	1.04	4.8	3.1	6

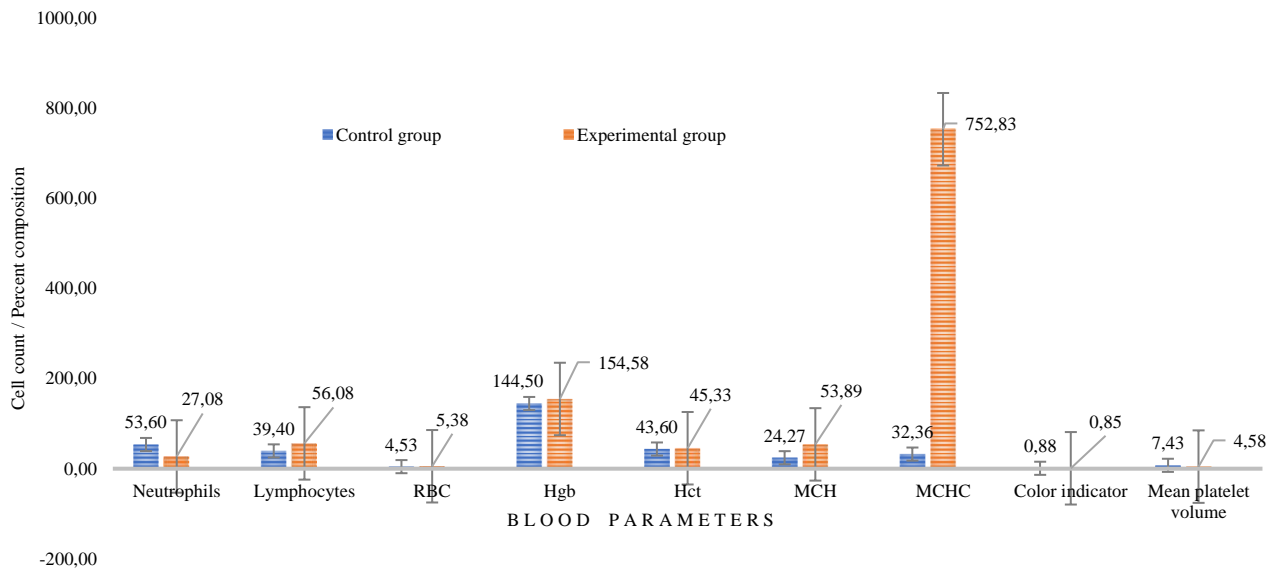**Note:** [b]$P < 0.05$; [c]$P < 0.01$; [d]$P < 0.001$.

Fig 1 Hematological parameters (mean±s.e.m.) that were significantly different between the control and experimental groups of guinea pigs. Notable differences were observed in the percentage of neutrophils ([d]p<0.001), lymphocytes ([d]p<0.001), RBC ([d]p<0.001), Hgb ([d]p<0.001), Hct ([c]p<0.01), MCH ([d]p<0.001), MCHC ([d]p<0.001), color indicator ([b]p<0.05), and mean platelet volume ([d]p<0.001).

Serum biochemistry

In this study, several serum biochemical parameters including ALT (alanine aminotransferase), AST (aspartate aminotransferase), glucose, and testosterone were evaluated (Table 2). As a result, three of these parameters (ALT, AST, and testosterone) showed significant differences between the control and experimental groups of animals (Fig. 2). ALT and AST percentages in experimental animals were significantly lower than those in control animals (both [d]p<0.001). Additionally, the testosterone concentration was considerably higher ([d]p<0.001) in experimental guinea pigs. However, the glucose percentage in serum was not statistically significant between the studied animal groups.

Table 2 Serum biochemistry parameters for control and experimental guinea pigs

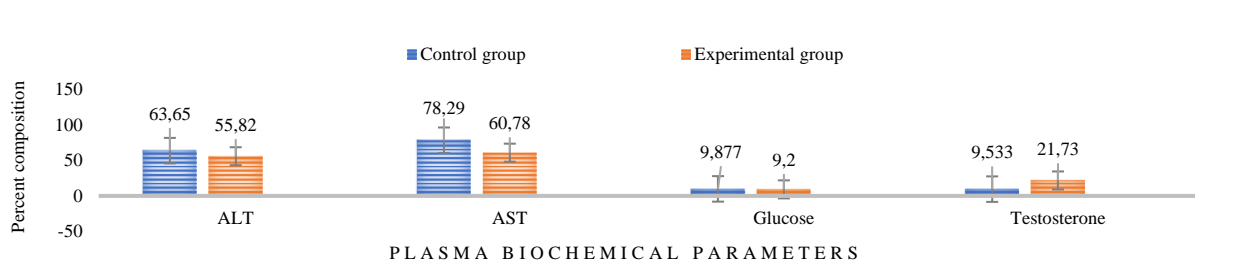| Serum parameters	Control group (n = 10)					Experimental group (n = 12)				
	Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max
ALT (U/L)[d]										
	63.65 ± 0.521[d]	1.647	64.25	60.7	65.4	55.82 ± 1.33	4.59	55.6	48.6	63.93
AST (U/L)[d]										
	78.29 ± 3.096[d]	9.790	80.05	58.2	90.9	60.78 ± 2.00	6.94	62.85	47.67	69.9
Glucose (mmol/L)										
	9.877 ± 0.621	1.963	9.92	6.91	13.5	9.20 ± 0.39	1.35	9.5	6.99	11.08
Testosterone (nmol/L)[d]										
	9.533 ± 0.184	0.583	9.615	8.63	10.4	21.73 ± 2.11[d]	7.32	20.95	11.4	31.5

Note: [d]P < 0.001.

Fig 4 Serum biochemistry parameters (mean ± s.e.m.) of guinea pigs in control and experimental groups are presented. Apart from the percentage of glucose in serum, notable differences were observed in the percentages of ALT ([d]p<0.001), AST ([d]p<0.001), and testosterone ([d]p<0.001).

Gross anatomy and histology

A comparative analysis was conducted on all internal organs, with special attention given to the heart, liver, kidneys, and testes in both control and experimental groups. Results from the visual examination, including color, consistency, and degree of blood filling of the aforementioned organs in necropsied guinea pigs from both groups, did not reveal any significant differences. Similarly, comparative morphometric studies, encompassing organ weight, width, length, and thickness, for the liver and testes showed no statistically significant disparities between the groups. As a result, comparative morphometric data for the studied organs were not provided.

Microscopic examination of blood smears also demonstrated no noticeable differences or changes between the control and experimental groups of animals. The only distinction observed was in the white blood cell (WBC) count of guinea pigs in the experimental group, as depicted in Figure 3, without a detailed description of the structural features of each cell.
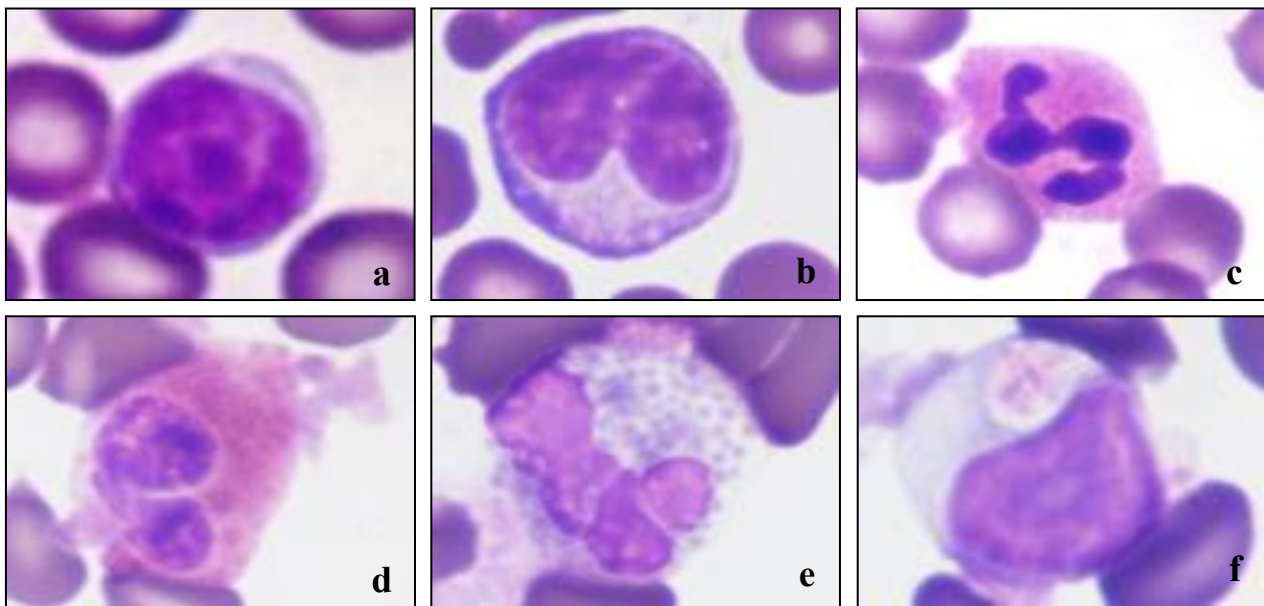

Fig 3 White blood cells (WBCs) commonly observed in guinea pig blood smears. (a) Lymphocyte with the largest nucleus and a small cytoplasmic rim, (b) bean-shaped nuclear monocyte, (c) segmented neutrophil, (d) eosinophil with purple-colored granules in the cytoplasm, (e) basophil with characteristic blue-purple granules, and (f) Foa-Kurloff cell with a pink intracytoplasmic inclusion body. Staining: MGG Quick Stain, magnification x100 (oil immersion).

Microscopic examination of the control guinea pig liver revealed normal hexagonal hepatic lobules of various sizes, containing hepatocytes, a central vein with blood cells, sinusoids with some empty spaces, and Kupffer cells lining the sinusoids in different locations (Fig. 4a). In comparison, liver sections from experimental animals appeared paler than those from the control group. The liver cords, sinusoids, intensely stained Kupffer cells, several apoptotic figures, and apoptosomes were clearly visible (Fig. 4a*).

Histological examination of the control guinea pig testes section showed tubular glands and intertubular connective tissue, characterized by Leydig cells. The round-oval seminiferous tubules varied in size and were surrounded by loose vascular connective tissue, forming testicular lobules. The coiled seminiferous tubules were lined with multilayered spermatogenic cells at different developmental stages and sustentacular (Sertoli)

185

cells (Fig. 4b). There was a decrease in the number of spermatogenic cells in the seminiferous tubules. In contrast, experimental testis sections showed restoration of spermatogenesis in the seminiferous tubules (Fig.
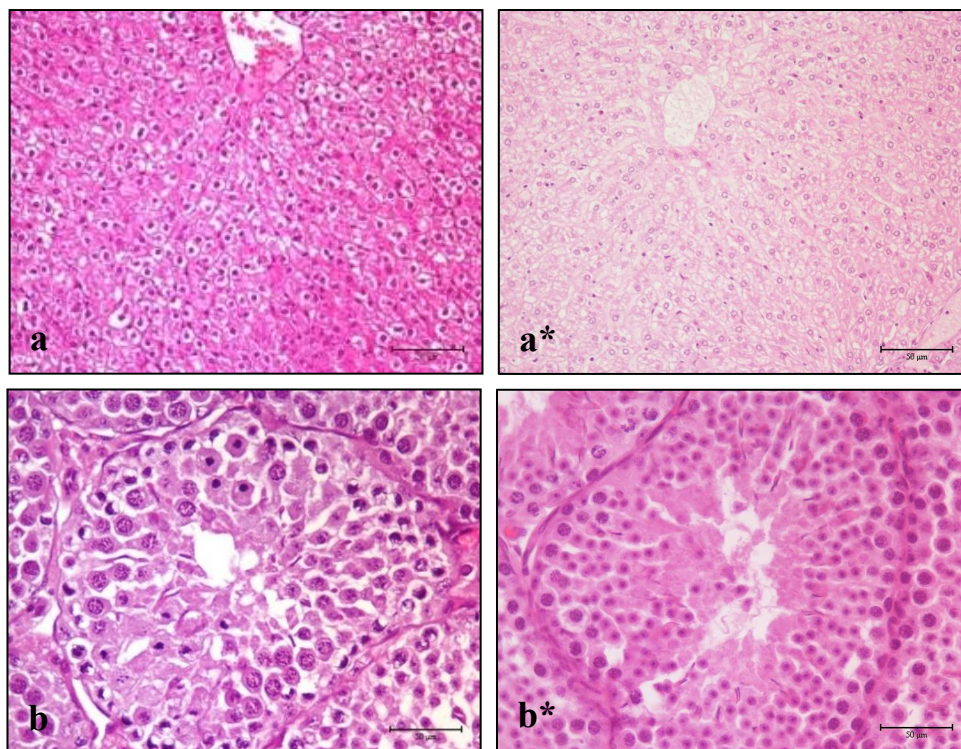

Fig. 4. Photomicrographs of paraffin sections of control (a) and experimental (a*) liver, and control (b) and experimental (b*) testis of guinea pigs. (a) Intensely stained normal hepatic lobule with hepatocytes, sinusoids, and some macrophages. (a*) Pale-stained experimental hepatic lobule with clearly visible sinusoids, some intensely stained Kupffer cells, and apoptotic figures. (b) Control testis section showing round-oval seminiferous tubules with multilayered spermatogenic cells and sustentacular cells. (b*) Experimental testis section showing a similar picture (b*), with an increase in spermatogenic cells. Staining: Hematoxylin and eosin, x20 (a, a*) and x40 (b, b*). 4b*). The quantity of spermatogonia, primary seminiferous tubule was filled with developing spermatozoa.spermatocytes, and spermatids increased, and the lumen of the seminiferous tubule was filled with developing spermatozoa.

Discussion

Our study delves into the medicinal efficacy of kuchala (*Arum korolkowii* Regel) tuber, a traditional remedy with roots in Asian folk medicine, particularly among the Kyrgyz people. By exploring its effects on male potency and reproductive health, we bridge the gap between traditional knowledge and modern scientific inquiry.

The traditional use of kuchala tubers in preparing kumys, a revered fermented mare's milk drink, underscores its significance in Kyrgyz culture [11,12]. References to ancient texts, such as those of Avicenna, further highlight its purported benefits in stimulating sexual desire and cleansing the kidneys. Despite its longstanding use, detailed recipes for kuchala-based remedies remain somewhat elusive, adding an aura of mystique to its therapeutic potential.

Utilizing guinea pigs as our experimental model offers several advantages [17-18]. Their morphofunctional similarities to humans, particularly in reproductive physiology [19-25], make them invaluable for studying male potency and related disorders. Furthermore, hematological and serum biochemical analyses provide valuable insights into physiological changes induced by experimental interventions [15,26].

Our study's focus on adult male guinea pigs, mirroring age-related declines in testosterone levels akin to those observed in aging men, strengthens the relevance of our findings to human health. The observed increases in testosterone levels and improvements in spermatogenesis among experimental animals treated with kuchala tuber tincture tunderscore its potential as a natural remedy for enhancing male reproductive function.

However, the presence of aberrant hematological parameters raises concerns regarding potential toxic effects of the tincture. This highlights the importance of dosage optimization and careful consideration of administration protocols to minimize adverse outcomes. The observed alterations in liver microstructure and

serum biochemistry parameters further emphasize the need for cautious experimentation and thorough assessment of safety profiles.

Our study contributes to the scientific validation of traditional remedies while also advocating for further research to refine dosage regimens and elucidate mechanisms underlying therapeutic effects. By bridging traditional knowledge with modern scientific inquiry, we pave the way for the development of safer and more effective treatments for male reproductive disorders.

In conclusion, our findings underscore the potential of kuchala tuber tincture as a promising avenue for enhancing male potency and reproductive health. Through rigorous experimentation and collaborative efforts between traditional healers and modern researchers, we can unlock the full therapeutic potential of natural remedies like kuchala, enriching our understanding of human health and well-being.

Conclusion

Our study demonstrates that the administration of a 10% kuchala (Arum korolkowii Regel) tuber tincture in 70% ethanol has a beneficial impact on enhancing the sexual potency of aged male guinea pigs. This effect is attributed to the increased production of testosterone and enhanced spermatogenesis observed in the experimental group.

Furthermore, our findings suggest that any potential toxic effects of this tincture on the animal organism can be mitigated by reducing the dosage. Future research endeavors will focus on determining the optimal concentration of the drug, specifically the tincture in ethanol, to maximize its therapeutic benefits while minimizing adverse effects.

These results provide valuable insights into the potential use of kuchala tuber tincture as a natural remedy to address age-related sexual potency issues in male guinea pigs. Further exploration in this direction holds promise for the development of novel therapeutic interventions for similar conditions in humans.

Competing interest
The authors declare that they have no conflict of interests.

References

1. Suleman S. et al. Treatment of malaria and related symptoms using traditional herbal medicine in Ethiopia. J. Ethnopharmacol., vol. 213, pp. 262–279, 2018. https://doi.org/10.1016/j.jep.2017.10.034.
2. Soelberg J. and Jäger A.K. Comparative ethnobotany of the Wakhi agropastoralist and the Kyrgyz nomads of Afghanistan," J. Ethnobiol. Ethnomed., vol. 12, no. 1, pp. 1–24, 2016. https://doi.org/10.1186/s13002-015-0063-x.
3. Miara M.D., Bendif H., Hammou M.A. and Teixidor-Toneu I. Ethnobotanical survey of medicinal plants used by nomadic peoples in the Algerian steppe. J. Ethnopharmacol., vol. 219, pp. 248–256, 2018. https://doi.org/10.1016/j.jep.2018.03.011.
4. Sõukand R.and Pieroni A. The importance of a border: Medical, veterinary, and wild food ethnobotany of the Hutsuls living on the Romanian and Ukrainian sides of Bukovina. J. Ethnopharmacol., vol. 185, pp. 17–40, 2016. https://doi.org/10.1016/j.jep.2016.03.009.
5. Tulobaev A.Z. Range of Medicinal Plants Used in Folk Veterinary Medicine in Kyrgyzstan. Manas J. Agric. Vet. Life Sci., vol. 9, no. 2, pp. 91–98, 2019. Available: https://dergipark.org.tr/tr/pub/mjavl/issue/51057/638745.
6. Wang Guo-Qiang, Huang Lu-Qi, Xie Dong-Mei. [Introduction of traditional medicinal plants in Kyrgyzstan]. Zhongguo Zhong Yao Za Zhi, vol. 39, no. 3, pp. 391–396, 2014. Available: https://pubmed.ncbi.nlm.nih.gov/24946536/.
7. Eisenman S.W., Zaurov D.E. and Struwe L. Medicinal plants of Central Asia: Uzbekistan and Kyrgyzstan. 2013. Available: https://link.springer.com/book/10.1007/978-1-4614-3912-7.
8. Haigh A. et al. Arum korolkowii Regel. Plants of the World online, 2011. Available: http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:86049-1.
9. Guo R. et al. Botany, Phytochemistry, Pharmacology and Toxity of Strychnos nux-vomica L.: A Review. Am. J. Chin. Med., vol. 46, no. 1, pp. 1–23, 2018. https://doi.org/10.1142/S0192415X18500015.
10. Patel K., Laloo D., Singh G.K., Gadewar M. and Patel D.K. A review on medicinal uses, analytical techniques and pharmacological activities of Strychnos nux-vomica Linn.: A concise report. Chin. J. Integr. Med., no. 221005, pp. 1–13, 2017. https://doi.org/10.1007/s11655-016-2514-1.
11. Orozbakov S. Manas IV [Манас IV]. 1997. Available: https://new.bizdin.kg/kniga/manas-eposu-4-kitep.
12. Mamai J. Semetey Zhusup Mamais variant [Семетей Жусуп Мамайдын айтуусунда]. 2017, p. 573. Available: http://erkindik.net/forum/topic/690-семетей-жусуп-мамайдын-айтымында/.
13. Institute for the Laboratory Animal Research. Guide FCULA (for the care and use of laboratory animals), 8th ed. Washington (DC): National Akademies Press 2011.
14. du Sert NP, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol 2020; 16(242) :1-7. https://doi.org/10.1371/journal.pbio.3000410.
15. Williams W.R. and Kendall L.V. Blood collection in the guinea pig (Cavia porcellus). Lab Anim. (NY)., vol. 44, no. 6, pp. 207–208, 2015. https://doi.org/10.1038/laban.787.
16. Kopteva K.E. et al. The technique of autopsy and removal of organs in laboratory animals [Техника вскрытия и удаления

органов у лабораторных животных]. Lab. Anim. Sci. Res., vol. 2, 2019. https://doi.org/10.29296/2618723X-2019-02-05.

17. Padilla-Carlin D.J., McMurray D.N. and Hickey A.J. The guinea pig as a model of infectious diseases. Comp. Med., vol. 58, no. 4, pp. 324–340, 2008. Available: https://pubmed.ncbi.nlm.nih.gov/18724774/.

18. Noonan D.E. The Guinea Pig (Cavia porcellus). Handb. Genet., no. September 1994, pp. 275–307, 1975, [Online]. https://www.researchgate.net/publication/235936317_The_Guinea_Pig_Cavia_porcellus.

19. Gradela A. et al. Morphological and morphometric study of the prostate of guinea pigs (Cavia porcellus, Linnaeus, 1758) during postnatal development. Biotemas, vol. 26, no. 4, pp. 221–231, 2013. https://doi.org/10.5007/2175-7925.2013v26n4p221.

20. Rodríguez-Casuriaga R., Geisinger A., Santiñaque F.F., López-Carro B. and Folle G.A. High-purity flow sorting of early meiocytes based on DNA analysis of guinea pig spermatogenic cells. Cytom. Part A, vol. 79 A, no. 8, pp. 625–634, 2011. https://doi.org/10.1002/cyto.a.21067.

21. Nunes A.K.R. et al. Morphological development of the testicles and spermatogenesis in Guinea pigs (Cavia porcellus Linnaeus, 1758). J. Morphol. Sci., vol. 34, no. 3, pp. 143–151, 2017. https://doi.org/10.4322/jms.107816.

22. Acosta S., Dizeyi N., Feinstein R., Pierzynowski S., Abrahamsson P-A. Long-term testosterone stimulation induces hyperplasia in the guinea-pig prostate. Prostate Cancer Prostatic Dis., vol. 7, no. 3, pp. 227–231, 2004. https://doi.org/10.1038/sj.pcan.4500744.

23. Nunes AKR et al. Morphological and functional analysis of spermatogenesis in guinea pigs (Cavia porcellus) from pre-puberty to post-puberty. Pesq. Vet. Bras., vol. 33, pp. 1–7, 2013. Available: https://paperity.org/p/186129324/morphological-and-functional-analysis-of-spermatogenesis-in-guinea-pigs-cavia-porcellus.

24. Simões L.S. et al. The quantification of testicular cells during the postnatal development in two Caviomorph rodents: The guinea pig (Cavia porcellus) and the cutia (Dasyprocta agouti). An. Acad. Bras. Cienc., vol. 89, no. 3, pp. 1745–1751, 2017. https://doi.org/10.1590/0001-3765201720170038.

25. Uppal V., Bansal N., Pathak D. and Kumar A. Histomorphochemical studies on the epididymis of guinea pig. Indian J. Anim. Sci., vol. 79, no. 8, pp. 809–812, 2009. Available: https://www.researchgate.net/publication/271647135_Histomorphochemical_studies_on_testis_of_guinea_pig.

26. Genzer S.C., Huynh T., Coleman-McCray J.A.D., Harmon J.R., Welch S.R. and Spengler J.R. Hematology and clinical chemistry reference intervals for inbred strain 13/N Guinea pigs (Cavia porcellus). J. Am. Assoc. Lab. Anim. Sci., vol. 58, no. 3, pp. 293–303, 2019. https://doi.org/10.30802/AALAS-JAALAS-18-000118.

Estimation of Climate Change Parameters for Agricultural Economy Efficiency with Machine Learning Methods

Abdullah Erdal Tümer[1]* (ID) **, Esra Kabaklarlı[2]** (ID)

ABSTRACT

Climate change threatens economies worldwide by disrupting food and water supplies, necessitating complex statistical models to forecast crop yields. Turkey, heavily reliant on agriculture, requires economic analyses of the intricate links between climate variability and resource availability to mitigate climate change impacts through effective policies. Recent predictive modeling incorporating meteorological data demonstrates the feasibility of anticipating monthly precipitation in Türkiye. The study demonstrates the effectiveness of using monthly relative humidity and average temperature data from 1970 to 2021 for precise precipitation predictions by applying artificial neural networks. The study's conclusions have important ramifications for raising agricultural output. Accurate monthly precipitation estimates enable stakeholders to make well-informed decisions on the development of grain crops, improving agricultural practices and raising sector productivity overall.

Introduction

The growing strain on the planet's limited resources emphasizes how important it is to have long-term plans for anticipating climate change, especially when it comes to promoting economic expansion. These days, climate data is essential to everything from international relations to hydrology to economic planning, healthcare, especially in industrialized countries, and agricultural and resource management [1]. As a result, there's an increasing need to use cutting-edge approaches, including machine learning techniques, to create prediction models meant to reduce the dangers related to climate change. These predictive models serve as invaluable tools, elucidating the requirements of both producer and user communities, thereby facilitating informed decision-making processes.

These forecasting models are quite useful since they clarify the needs of the production and user communities, which helps with well-informed decision-making. About 80 million people in Turkey depend on agriculture for their fundamental necessities, making it an essential sector of the national economy. That's why many studies have been done on agricultural economics [2-4].The extensive use of machine learning in predictive analytics has led to a revolution in recent years [5, 6].

Many methodologies fall under this umbrella, such as random forest, artificial neural networks, naive bayes, multi linear regression, and K-nearest neighbor. These advanced analytical procedures enable identifying historical rainfall patterns that are predictive of drought. The drought of 2021 caused a concerning 17.6% slump in wheat production in Turkey, from 20.5 million tons in 2020 down to 17.6 million tons. This alarming downward trend points to significant declines in broader grain production as well, with overall output dramatically plunging 14.3% year-over-year to just 31.9 million tons in 2021, according to data from the Turkish Statistical Institute [7].

Machine learning has become a powerful tool for accurate prognostication across a variety of domains, including health [8], animal husbandry [9], agriculture [10], sports [11], economy [12], and industry [13].

This research sets out on a critical exploration, proving the effectiveness of machine learning in predicting rainfall, a critical component determining the incidence of droughts.

Using a dataset that includes 624 data points from the Konya General Directorate of Meteorology [14] covering Turkey's 12-month relative humidity, average temperature, and precipitation records from 1970 to 2021, this study uses three different machine learning techniques: radial basis function, multiple linear regression, and

---

[1] Necmettin Erbakan University, Faculty of Engineeing, Department of Computer Egineering, Konya / Turkey
[2] Selcuk University, Faculty of Economics and Administrative Sciences, Konya / Turkey
*Corresponding Author: Abdullah Erdal Tümer, e-mail: aetumer@gmail.com

artificial neural networks. Among the models that have been studied, artificial neural networks stand out as the most effective in making predictions.

The rest of the paper is organized as follows: in the next section we describe the effect of clmate change on agriculture and economy. In Section 3 we introduce preceding related work. In section 4 we expose the research method followed. In Section 5 and 6, we present the used software and performance criteria and finding obtained, respectively. The paper ends with a conclusions.

The Effect Of Climate Change On Agriculture And Economy

Rising sea levels, altered precipitation, and increasing temperatures signify climate change, posing a major threat to Earth. More frequent and intense extreme weather also surfaces in the short-term from unpredictable weather patterns. Greenhouse gas emissions, chiefly from burning fossil fuels, livestock production, rice cultivation, and nitrogen fertilizers, drive climate change. Like a greenhouse, these gases trap solar radiation in the atmosphere [15]. Figure 1 shows agriculture's outsized role, generating nearly half of all emissions. Industry and transportation also contribute substantially as the other major greenhouse gas emitters at 23% and 17% of total emissions, respectively.
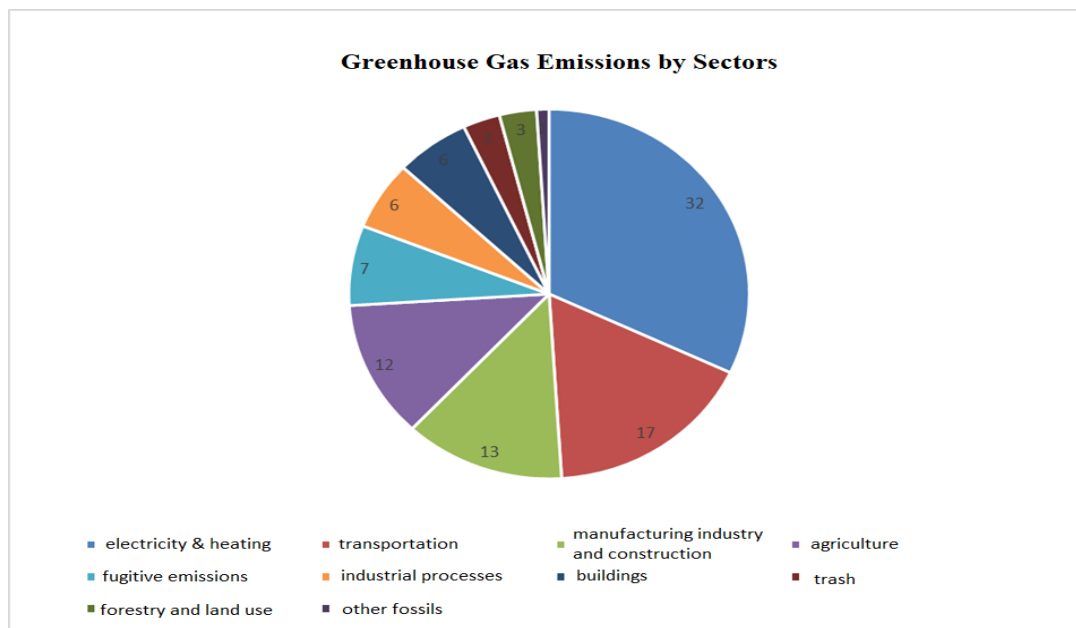

Fig 1 Greenhouse Gas Emissions by Sectors (2019) [10]

The average sea level has risen due to the melting of glaciers in Greenland and the Arctic, but within the past 100 years, weather patterns have gotten more unpredictable and extreme weather events have been more destructive, raising the sea level by 4.10–20 centimeters.

Although in 2011 the average amount of greenhouse gas emissions per person in high-income countries was ten times that of poor countries, in 2013 China surpassed the European Union (EU) by 6.8 tons with 7.2 tons of carbon per person [16].

Digital technology integration has promise for reducing the impact of climate change. Energy management techniques can be updated with the use of robotics, artificial intelligence (AI), sensors, and Internet of Things (IoT) devices. This will increase energy efficiency in all industries. To achieve the UN's sustainable development goals, it is especially important to recognize the possible negative effects of these technologies on the labor force and the production of electronic waste.

Figure 4 shows observations that show a partial decline in wheat production in Turkey throughout time, with the drought in 2021 being especially noteworthy. Therefore, in addition to deploying sensor technology for optimal irrigation techniques, it is necessary to use cutting-edge approaches like artificial intelligence and machine learning for precise rainfall and temperature projections.

Production quantity graphs also show the price pattern suggested by the spider web theory, which is that prices vary according to supply quantities from prior years. Turkey's annual production of barley varies from year to year, however it has been declining since 2019.
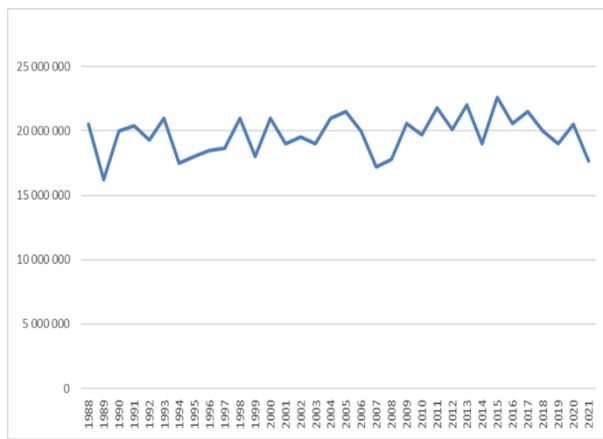
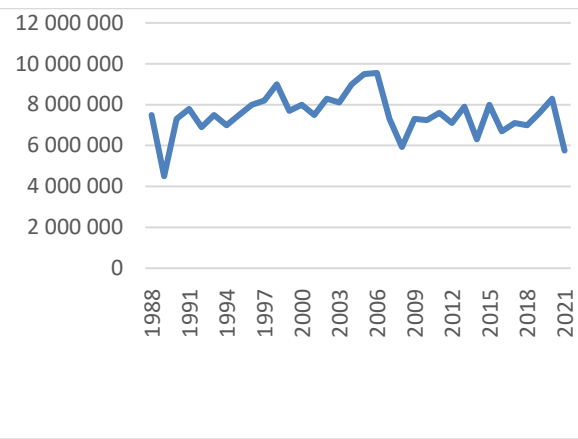**Fig 2** Türkiye Wheat Production (Ton) [17]

Fig 3 Türkiye Barley Production (Ton) [17]

The General Directorate of Meteorology's analysis of relative humidity statistics from 1970 to 2021 reveals a consistent drop in humidity levels over time, as seen in Figure 4. Simultaneously, analysis of temperature data for Türkiye from 1970 to 2021 shows a significant 1-degree rise in average temperature, from 13.5 to 14.5 degrees Celsius (Figure 5).
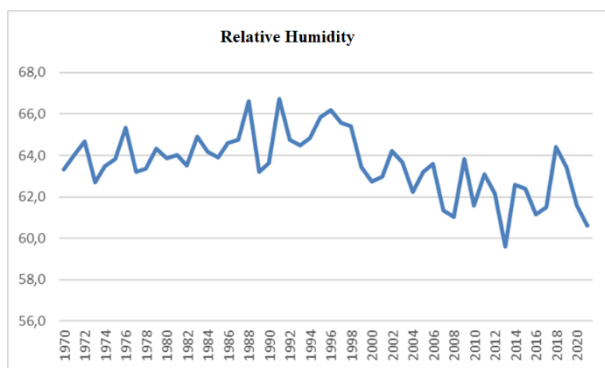

Fig 4 Annual Average of Monthly Humidity Values in Türkiye (1970-2021)
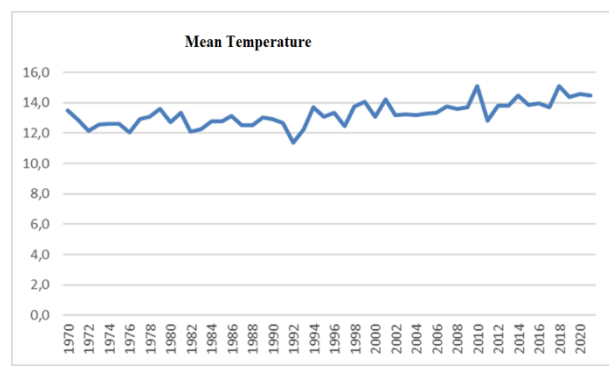

Fig 5 Annual Average of Monthly Temperature Values in Türkiye (1970-2021)

The observed patterns of decreasing humidity levels, rising average temperatures, and notable variations in maximum and lowest temperatures across the study period highlight the importance of precisely assessing climate change parameters. These kinds of discoveries play a critical role in shaping policy interventions and adaptive solutions meant to lessen the negative effects of climate change.

Digital technologies can lead to climate change mitigation. Energy management and energy efficiency can be raised in all industries with the use of sensors, the Internet of Things, robotics, and artificial intelligence. However, when considering the United Nations sustainable development goals, its detrimental effects on the workforce and the quantity of electronic waste it produces also have an adverse effect.

Literature Review

In the study [18], the Penman-Monteith method combined with an Artificial Neural Network (ANN) predicted the impact of climate change on evaporation rates in Cyprus's Kyrenia and Larnaca regions through 2050. The results show ANN's efficiency in forecasting future evaporation, with R2 coefficients ranging from 0.8959 to 0.9997 for Kyrenia and 0.8633 to 0.9996 for Larnaca.

Previous research, including [19], demonstrates ANN's superiority in predicting energy demand over simpler methods like linear regression. However, these studies didn't employ optimization methods to assess climate change's effects on hydropower and energy demand [20].

[21] found that ANN provides accurate predictions for energy efficiency, with a determination coefficient of 60.99%, indicating reliable results.

The study on California's energy demand [22] showed that climate change, particularly temperature variations, could significantly affect electricity consumption and costs.

[23] explored climate change's impact on hydroelectric energy, energy demand, and the supply-demand balance using an ANN and the Enhanced Electromagnetic Field Optimization (IEFO) algorithm. Their research forecasts a notable decrease in hydroelectric production due to temperature rises and changing precipitation patterns, by up to 14,765 MW in future scenarios.

In [24], the authors have developed artificial intelligence models with rapid decision-making ability to understand the impact of climate change on water resources. The study involves predicting evaporation in the Karaidemir Dam in Turkey with artificial neural networks (ANN). Daily meteorological data covering the irrigation season are provided for a 30-year reference period. Bayesian Regularization (BR), Levenberg-Marquardt (L-M) and Scalar Conjugate Gradient (SCG) learning algorithms were used. The results obtained revealed that the ANN model has statistically high performance in prediction with few input parameters.

The authors made the following claims in [25]:

i. Global drylands, where 38% of the world's population resides, present significant challenges such as low water productivity and scarcity.

ii. There are fields that are over- or under-irrigated due to incorrect irrigation techniques.

iii. Smart irrigation models can be developed that take into account soil characteristics, climate change, plant responses to water scarcity and weather changes. vi. Algorithms such as artificial intelligence and deep learning (fuzzy logic, expert systems, artificial neural networks and hybrid intelligent systems) can be used for models.

v. Model predictive irrigation systems will offer high water use efficiency.

The literature makes clear that various machine learning algorithms have been used to create smart models incorporating climate change characteristics for a variety of uses. They made an effort to forecast how evaporation rates, energy consumption, and water supplies will be affected by climate change. On the other hand, no research on agricultural economic efficiency has been located. In order to maximize agricultural economic efficiency, this research focuses on using machine learning algorithms to estimate monthly rainfall.

Methods

Artificial neural network

A neural network is a computational structure inspired by the study of biological neural processing. The purpose of Artificial Neural Networks (ANN) is to find the relationship between input and output values by performing internal calculations [26]. ANN operates in two stages: training and testing. During training, input and output data sets are used to adjust the network's parameters. Artificial neural networks typically comprise three layers: input layer, output layer, and one or more hidden layers, each containing numerous interconnected neurons (Figure 6)
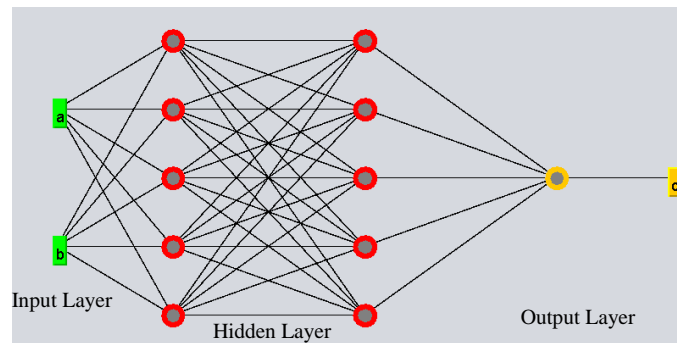
Fig 6 Architecture of Artificial Neural Network

Radial basic function

The function utilized in a radial basis neural network describes any real-valued function whose output relies solely on the distance of its input from an origin. The radial basis neural network (RBF) shares structural similarities with a multilayer perceptron. However, the RBF is constrained to feature precisely one hidden layer (see Figure 7). Within artificial neural networks, a radial basis function serves as the activation function. The Gaussian variation of this function is a widely adopted alternative. A Gaussian formula with one-dimensional input can be expressed as:

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \tag{1}$$

Here, x denotes the input, μ represents the mean or center of the Gaussian, and σ is the standard deviation controlling the width of the function's peak. The Gaussian function assigns higher weights to inputs closer to the center, gradually decreasing as the distance from the center increases, capturing the radial nature of the function.

This activation function enables the radial basis neural network to effectively model complex relationships within data, making it suitable for various tasks such as function approximation, classification, and regression.

Additionally, its single hidden layer architecture contributes to faster training and simpler optimization compared to deeper neural networks.

Fig 7 Architecture of Radial Basic Function

Multi linear regression

Multiple linear regression (MLR) refers to a statistical technique that uses two or more independent variables (input) to predict the outcome of a dependent variable (output). Its purpose is to model the linear relationship between inputs and output(s). It is expressed by equation 2.
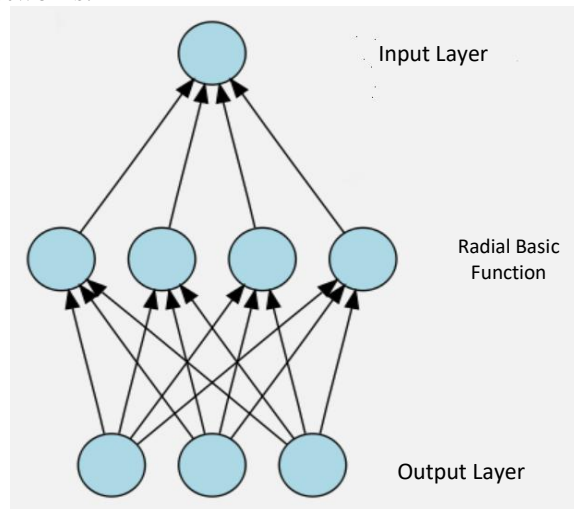
$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_{3+} \dots . b_n x_{n+} \epsilon \qquad (2)$$

Here, y is the predicted variable, b_0 is constant term, b_i 's (i=1,2…n) are the regression coefficients and ϵ is the model's error term.

Data collecting

The Konya General Directorate of Meteorology provided the data used in the models. Sixty-four data points covering the twelve-month period from 1970 to 2021 are included in total. These data, which were gathered monthly over a 52-year period, include measures of relative humidity, average temperature, and precipitation amounts. Given the comprehensive nature of these data, which cover all seasons over this extended period, they were deemed sufficient for capturing potential seasonal variations. Relative humidity and average temperature serve as the input parameters, while precipitation amount serves as the output parameter. The training and simulating subsets of the proportion were 75–25%, respectively. The training data-set was divided into three subsets (training 70%, testing 15% and validation 15%). Table 1 presents a detailed overview of the input and output parameters along with their respective statistics.

Table 1 Data ranges and statistic

Parameters	Data Statistic		
	Ranges	Mean ± S.D.	Unit
Input Layer			
Average Relatively Humidity	2.00 – 70.00	21.71 ± 11.05	gr/m^3
Average Temperature	-26.5 – 15.40	-0.93 ± 8.86	^{0}C
Output Layer			
Average Rainfall	0.00 – 24	27.57 ± 27.39	mm

Used software and performance criteria

The models developed in this study were tested and simulated using Weka 3.8.5 software, developed by the University of Waikato in Hamilton, New Zealand. Various configurations of layers and neuron parameters were explored to determine the optimal architecture for the developed ANN models. Several methods exist for evaluating the performance of models generated through different machine learning techniques. As certain performance criteria are commonly employed across various studies[27]), they were likewise adopted in this

research. Specifically, Root Mean Squared Error (RMSE) and Coefficient of Determination (R^2) were utilized as performance metrics. The formulations for these performance measures are provided below:

$$RMSE = \sqrt{\frac{1}{n}\sum_{1}^{n}(Y_{exp,i} - Y_{prd,i})^2} \qquad (3)$$

$$R^2 = 1 - \frac{\sum_{1}^{n}(Y_{prd,i} - Y_{exp,i})^2}{\sum_{i=1}^{n}(Y_{prd,i} - Y_m)^2} \qquad (4)$$

Y_{prd} is the predicted data, Y_{exp} is measured data and Y_m is the the total number of data.

For RMSE, the sum of the squares of the differences (i.e. errors) between the Actual Values and Predicted Values is divided by the number of observations and then the square root is taken. When adding errors, errors are squared to prevent positive and negative values from canceling each other out. However, to prevent swelling caused by this process and to make more accurate measurements, square roots are taken.

RMSE is used to quantify the difference between a machine learning model's anticipated and actual value. The range of the RMSE value is 0 to ∞. When the RMSE value is 0, it means that the model has successfully memorized the dataset and has made no mistakes. The model is considered more successful the closer the RMSE is near 0.

In a similar vein, the closer the R^2 value is to 1 (100%), the more successful the prediction is considered. However, a score of 100% indicates that the model might be overfitting the data, which compromises the model's dependability when R^2 is used as the machine learning performance criterion.

Findings

In this study, 624 datasets—the statistical details of which are given in Table 1—were used in an attempt to determine the quantity of rainfall that occurs on average each month in Türkiye. These datasets were acquired from the Konya General Directorate of Meteorology and span the years 1970–2021. Three different machine learning methods were employed for this purpose.

Artificial neural network findings

Various ANN models were created with different hidden layers and neuron numbers to predict Turkey's monthly average rainfall. The feedforward backpropagation algorithm, featuring a single hidden layer and neuron, demonstrated the best efficiency in estimating the average rainfall. Figure 8 displays the most suitable ANN model of graph comparing the estimated values of the model with the actual values.

Figure 8 displays the most suitable ANN model that comparing the estimated values of the model with the actual values.
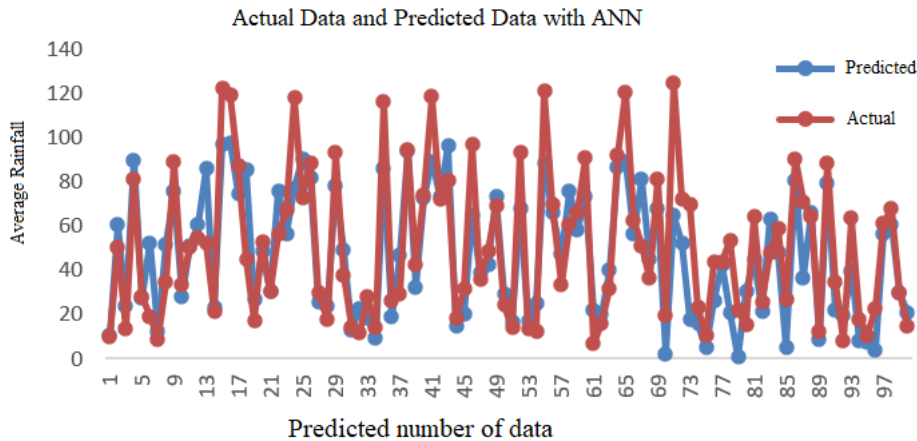
Fig 8 Actual Data and Predicted Data with ANN

The performance criterion results of the ANN model are presented in Table 2.

Table 2 Performance of ANN

Model	RMSE	R^2
ANN	18.20	0.67

Radial basis function findings

Observations indicate that the findings of RBF do not outperform those of artificial neural networks. The R^2 value for ANN is 0.67, whereas for RBF it is 0.54. This means that while ANN achieved a 67% success rate in prediction, RBF's prediction success rate was 54%. Figure 9 displays a graph comparing RBF prediction results with actual values, while Table 3 presents RBF performance based on performance criteria.
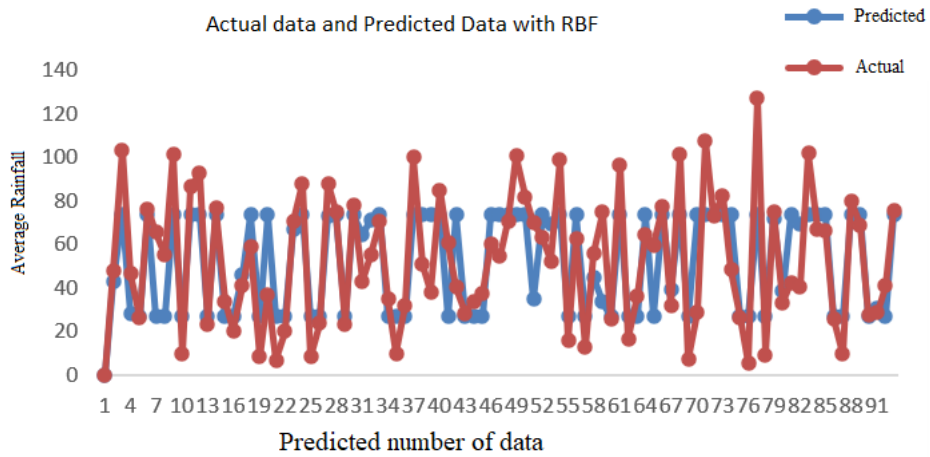
Fig 9 Actual Data and Predicted Data with RBF

Table 3 Performance of RBF

Model	RMSE	R^2
RBF	20.29	0.54

Multiple linear regression findings

MLR findings showed similar success to ANN performance. The coefficient of determination was 0.67 in both machine learning methods. The graph comparing the prediction results of the MLR with the actual data is presented in Figure 9. The performance criteria table is also shown in table 4. Figure 9 displays a graph comparing the prediction results of the MLR with the actual data, while Table 4 presents the performance criteria.

Fig 9 Actual Data and Predicted Data with MLR

Table 4 Performance of MLR

Model	RMSE	R^2
MLR	17.25	0.67

Conclusion

Climate change, resulting from increased levels of greenhouse gases, is the most critical global environmental issue today. This phenomenon occurs over many centuries. The results show that calculating climate change characteristics is important, as evidenced by the considerable changes in maximum and lowest temperatures, the 1 deg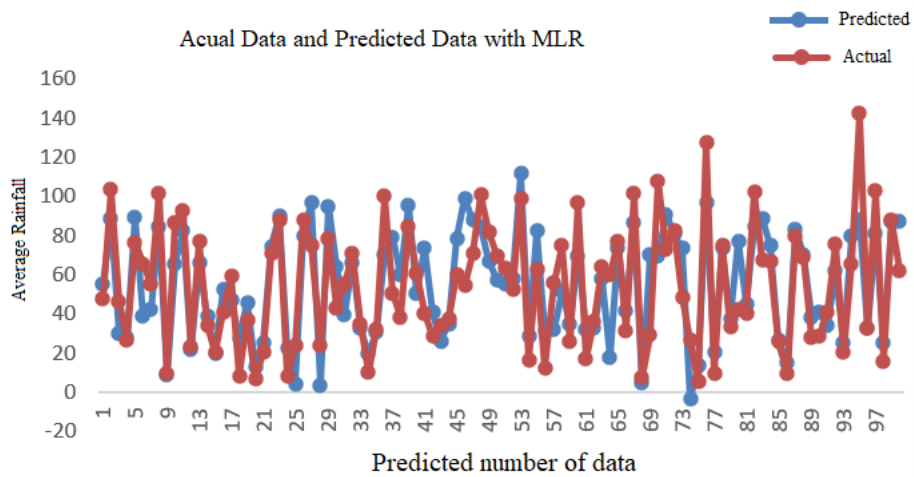ree increase in average temperature, and the decrease in humidity between 1970 and 2021. The root mean square error (RMSE) and coefficient of determination (R^2) between the measured and predicted outcome variables were used as performance metrics to assess the study's outcomes. R2 for the MLR and ANN models is 0.67. Because of this, it was discovered that the ANN and MLR models created in this study were more successful and palatable than the RBF model. The average amount of rainfall may be successfully estimated using the ANN and MLR approaches. It was determined that grain growers and decision makers may utilize it as a valuable tool for evaluating performance. Also the model we built can be made more accurate and more broadly applicable by using data that can be gathered from various places and climate conditions. To improve prediction accuracy and dependability, a variety of machine learning techniques, including the deep learning algorithm, can be applied. Furthermore, models that use real-time data have the ability to produce more dynamic predictions and can respond swiftly to sudden changes in the climate through the integration of various sensors within the framework of the Internet of Things. The energy, water resource management, aviation and transportation, and healthcare industries can all benefit from using climate change characteristics. Planning public health initiatives and creating disease control plans can be aided by forecasting the production of renewable energy sources (solar, wind), managing water better during droughts, maximizing agricultural water use, minimizing the impact of weather on flight safety and efficiency, and anticipating the spread of diseases brought on by climate change (e.g., malaria, dengue fever).

References

1. Jury, M.R., Economic impacts of climate variability in South Africa and development of resource prediction models. Journal of Applied Meteorology and Climatology, 2002. 41(1): p. 46-55.
2. Mızırak, Z. and A. Ceylan, 100. YILINDA TÜRKİYE'DEKİ TARIM POLİTİKALARININ YAPISAL DEĞİŞİMİ. Necmettin Erbakan Üniversitesi Siyasal Bilgiler Fakültesi Dergisi. 5(Özel Sayı): p. 131-147.
3. ERDİNÇ, Z., TÜRKİYE'DE UYGULANAN TARIM POLİTİKALARININ YENİDEN YAPILANMASI. Anadolu Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi, 2000. 16(1): p. 327-348.
4. Hisarlı, A., TARIM SEKTÖRÜNÜN EKONOMİK GELİŞMEYE ÜRÜN KATKISI. Anadolu Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi, 1989. 7(2): p. 241-248.
5. Karakurt, H.U., et al., Evaluation of Differences of Fast and High Accuracy Base Calling Models of Guppy on Variant Calling Using Low Coverage WGS Data. International Journal of Life Sciences and Biotechnology, 2023. 6(3): p. 276-287.
6. AYNA, Ö.F. and Ş.F. ARSLANOĞLU, Anadolu coğrafyasında yayılış gösteren Berberis türleri ve geleneksel kullanımı. International Journal of Life Sciences and Biotechnology, 2019. 2(1): p. 36-42.
7. TUIK. Bitkisel Üretim İstatistikleri. 2021 [cited 11 Sebtember 2022 11 Sebtember 2022]; Available from: https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-Istatistikleri-2021-37249.
8. Rajula, H.S.R., et al., Comparison of conventional statistical methods with machine learning in medicine: diagnosis, drug development, and treatment. Medicina, 2020. 56(9): p. 455.
9. Neethirajan, S., The role of sensors, big data and machine learning in modern animal farming. Sensing and Bio-Sensing Research, 2020. 29: p. 100367.
10. Sharma, A., et al., Machine learning applications for precision agriculture: A comprehensive review. IEEE Access, 2020. 9: p. 4843-4873.
11. Tümer, A.E. and S. Koçer, Prediction of team league's rankings in volleyball by artificial neural network method. International Journal of Performance Analysis in Sport, 2017. 17(3): p. 202-211.
12. Tümer, A.E. and A. Akkuş, Forecasting gross domestic product per capita using artificial neural networks with non-economical parameters. Physica A: Statistical Mechanics and its Applications, 2018. 512: p. 468-473.
13. Usuga Cadavid, J.P., et al., Machine learning applied in production planning and control: a state-of-the-art in the era of industry 4.0. Journal of Intelligent Manufacturing, 2020. 31: p. 1531-1558.
14. Meteoroloji. 2023 [cited 13 September 2023 13 September 2023]; Available from: https://www.mgm.gov.tr/?il=Konya.
15. Poschen, P., Decent work, green jobs and the sustainable economy: Solutions for climate change and sustainable development. 2017: Routledge.
16. Olhoff, A., Emissions Gap Report 2021: The Heat Is On–A World of Climate Promises Not Yet Delivered. 2021.
17. Statista. Distribution of greenhouse gas emissions worldwide in 2020, by sector. 2020; Available from: https://www.statista.com/statistics/241756/proportion-of-energy-in-global-greenhouse-gas-emissions.
18. Abdullahi, J. and G. Elkiran, Prediction of the future impact of climate change on reference evapotranspiration in Cyprus using artificial neural network. Procedia computer science, 2017. 120: p. 276-283.
19. Razmjooy, N. and V.V. Estrela, Applications of image processing and soft computing systems in agriculture. 2019: IGI Global.
20. Hu, A. and N. Razmjooy, Brain tumor diagnosis based on metaheuristics and deep learning. International Journal of Imaging Systems and Technology, 2021. 31(2): p. 657-669.
21. Khalil, A.J., et al., Energy efficiency prediction using artificial neural network. 2019.
22. Franco, G. and A.H. Sanstad, Climate change and electricity demand in California. Climatic Change, 2008. 87(Suppl 1): p. 139-151.

23. Guo, L.-N., et al., Prediction of the effects of climate change on hydroelectric generation, electricity demand, and emissions of greenhouse gases under climatic scenarios and optimized ANN model. Energy Reports, 2021. 7: p. 5431-5445.

24. Ahi, Y., et al., Reservoir evaporation forecasting based on climate change scenarios using artificial neural network model. Water Resources Management, 2023. 37(6): p. 2607-2624.

25. Ahmed, Z., et al., An overview of smart irrigation management for improving water productivity under climate change in drylands. Agronomy, 2023. 13(8): p. 2113.

26. Delgrange, N., et al., Neural networks for prediction of ultrafiltration transmembrane pressure–application to drinking water production. Journal of membrane science, 1998. 150(1): p. 111-123.

27. Tasdemir, S. and I.A. Ozkan, ANN approach for estimation of cow weight depending on photogrammetric body dimensions. International Journal of Engineering and Geosciences, 2019. 4(1): p. 36-44.

Nitelikli Bal Kabağı (*Cucurbita moschata* Duchesne) Hatlarının *Fusarium oxysporum* f. sp. *cucumerinum*'a Dayanıklılık Durumlarının Belirlenmesi

Fatih İpek[1] iD **, İbrahim Özkan[1]** iD **, Ahmet Balkaya[2]** iD **, İsmail Erper[2,3]** iD **, Büşra Yapici[4]** iD

ÖZET

Türkiye'de hıyar yetiştirilen bölgelerde üretimi sınırlayan önemli faktörlerden birisi de toprak kökenli patojen fungusların oluşturduğu hastalıklardır. Solgunluk hastalığına neden olan *Fusarium oxysporum* f. sp. *cucumerinum* J.H. Owen (FOC), hıyar yetiştiriciliğinde verim kaybına neden olan önemli funguslardan biridir. Bu hastalığın mücadelesinde aşılı fide kullanımı etkili bir yöntemdir. Aşılı hıyar fidesi üretiminde anaç olarak en fazla bal kabakları kullanılmaktadır. Bu araştırmada, nitelikli 43 bal kabağı hattının *F. oxysporum* f. sp. *cucumerinum*'a karşı reaksiyonlarının saptanması ve patojen ile inoküle edilmiş bal kabağı hatları ile kontrol bitkilerinin bazı vejetatif büyüme özellikleri yönünden karşılaştırılması amaçlanmıştır. Denemede, pozitif kontrol olarak Nun 9075 ticari kabak anacı ve negatif kontrol olarak Çengelköy hıyar çeşidi kullanılmıştır. Hastalık testlemesi sonucunda bal kabağı hatlarında hastalık şiddetinin %0 – 66.67 arasında değişim gösterdiği belirlenmiştir. Ayrıca bal kabağı hatlarının büyük bir çoğunluğunun patojene karşı yüksek düzeyde dayanıklı (28 hat) ve orta düzeyde dayanıklı (14 hat) oldukları saptanmıştır. Yüksek düzeyde dayanıklı bulunan bal kabağı hatlarında genel olarak bitki boyu, gövde çapı, yaprak sayısı ve toplam bitki kuru ağırlığı parametreleri yönünden azalışların hassas hatlara göre daha düşük oranlarda olduğu bulunmuştur. Çalışma sonucunda *F. oxysporum* f. sp. *cucumerinum*'a dayanıklı olarak tespit edilen ve vejetatif büyüme parametreleri yönünden de öne çıkan ümit var bal kabağı hatları, devam eden kabak anaç ıslah programında ebeveyn olarak kullanılacaktır.

Determination the the Resistance Status of Qualified Pumpkin (*Cucurbita moschata* Duchesne) Lines Against *Fusarium oxysporum* f. sp. *Cucumerinum*

ABSTRACT

One of the significant factors limiting production in cucumber growing regions in Turkey is diseases caused by soil-borne pathogenic fungi. Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cucumerinum* J.H. Owen (FOC), is one of the major fungi causing yield loss in cucumber cultivation. Using grafted seedlings is an effective method to combat this disease. Pumpkins are the most commonly used rootstocks in the production of grafted cucumber seedlings. This research aimed to determine the reactions of 43 qualified pumpkin lines against *F. oxysporum* f. sp. *cucumerinum* and to compare some vegetative growth characteristics of the inoculated pumpkin lines with the control plants. In the experiment, the commercial rootstock Nun 9075 was used as a positive control, and the Çengelköy cucumber variety was used as a negative control. The disease severity in the pumpkin lines ranged from 0% to 66.67%. Moreover, the majority of the pumpkin lines were found to be highly resistant (28 lines) or moderately resistant (14 lines) to the pathogen. In general, the highly resistant pumpkin lines showed lower reductions in plant height, stem diameter, leaf number, and total plant dry weight compared to the susceptible lines. As a result of the study, promising pumpkin lines identified as resistant to *F. oxysporum* f. sp. *cucumerinum* and prominent in terms of vegetative growth parameters will be used as parents in the ongoing pumpkin rootstock breeding program.

[1] Ondokuz Mayıs Üniversitesi, Lisansüstü Eğitim Enstitüsü, Samsun, Türkiye
[2] Ondokuz Mayıs Üniversitesi, Ziraat Fakültesi, Samsun, Türkiye
[3] Kırgızistan-Türkiye Manas Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Bişkek / Kırgızistan
[4] Petektar Tohum Tarım San. Tic. Ltd. Şti. *Corresponding Author: Fatih İpek, e-mail: fatihipekk2@gmail.com

Giriş

Kabaklar, Cucurbita cinsi içerisinde *Cucurbitaceae* familyasında yer alan önemli sebze türleridir. Cucurbita cinsi, kültüre alınmış beş türü içermektedir. Bunlardan yazlık kabak (*Cucurbita pepo* L.), kestane kabağı (*Cucurbita maxima* Duchesne) ve bal kabağı (*Cucurbita moschata* Duchesne) ekonomik yönden en önemli olanlarıdır [1, 2]. Bal kabağı türünün M.Ö. 3000'li yıllarda Peru ve M.Ö. 2000'lerde Guatemala'da kültüre alındığı bildirilmiştir [3]. Kültüre alınan kabakgil grubu sebze türlerinde meyve görünümü ve meyve ağırlığı değerleri yönünden genetik çeşitlilik oldukça yüksektir [4]. Günümüzde kışlık kabaklarda açık tozlanan çeşitler ya da yerel çeşitlerle üretim daha fazla yapılmaktadır. Hibrit çeşit ıslahı daha çok yazlık kabaklarda kullanılmaktadır. Günümüzde Cucurbita türlerinde önemli ıslah hedefleri arasında hastalık ve zararlılara dayanıklı yeni hibrit çeşitlerin geliştirilmesi ilk sıralarda gelmektedir [2]. Kabakgiller familyasında tür içi ve türler arası melezlemeler, hastalıklara karşı dayanıklılığın sağlanmasında ve aşılı fide üretimine yönelik anaç ıslahı programlarında sıklıkla kullanılmaktadır.

Dünyada ve Türkiye'de hıyar ve kavun yetiştirilen tüm bölgelerde üretimi sınırlayan en önemli faktörlerden birisi, toprak kökenli patojen fungusların oluşturduğu hastalıklardır [5, 6, 7]. Bunların içinde en önemlileri ise *Fusarium* türlerinin neden olduğu solgunluk ve kök çürüklüğü hastalıklarıdır. *Fusarium oxysporum*'un 100'ün üzerinde konukçuya özelleşmiş alt türü bulunmaktadır [8, 9]. Ekonomik öneme sahip hıyar bitkisinde pek çok patojen hastalık oluşturmasına karşın, bunlardan en önemlisi *F. oxysporum* f. sp. *cucumerinum* J.H. Owen (FOC)'un yol açtığı Fusarium solgunluğudur [10]. Ülkemizde tarla ve örtü altı hıyar yetiştiriciliği yapılan alanlarda ciddi oranda ürün kaybına neden olduğu gözlenen patojen, bitkinin herhangi bir gelişme döneminde enfeksiyon yapabilmektedir [9]. *F. oxysporum* f. sp. *cucumerinum* bitkileri önce köklerden enfekte etmekte ve daha sonra iletim demetlerinde kahverengileşme ile yapraklarda sararma şeklinde belirtiler oluşturmaktadır [11]. Yaşlı bitkilerdeki enfeksiyon, genellikle başlangıçta bir ya da birkaç dalın ve daha sonra tüm bitkinin solması ve 3-5 gün sonra bitkinin ölümü ile sonuçlanmaktadır. Solgunluk belirtileri sıcaklık, su stresi ve meyve sayısına bağlı olarak artış göstermektedir [12]. *F. oxysporum*'un hıyardan elde edilen patotipleri genellikle konukçuya özelleşmiş olmalarına rağmen FOC, hıyar bitkisinin dışında kavun bitkisinde de hastalık oluşturabilmektedir [12, 13].

Fusarium solgunluğu hastalığının mücadelesi diğer toprak kaynaklı fungal etmenlerin mücadelesinden daha zordur. Bu nedenle hastalık etmeniyle mücadelede, dayanıklı çeşitlerin kullanımı, tarlada bulaşık olan bitkilerin uzaklaştırılması, münavebe uygulanması, sulama ve toprak işlemeye dikkat edilmesi, aşırı azotlu gübrelerden kaçınılması, toprak fumigasyonu, solarizasyon, biyolojik mücadele vb. yöntemlerinin birlikte uygulanması gerekmektedir [14]. Bununla birlikte günümüzde kabakgil grubu sebze türlerinde aşılı fide kullanımı *Fusarium* solgunluğunun mücadelesinde önemli bir yer tutmaktadır [15]. Hıyarın aşılanmasında; aşılamaya en uygun nitelikli çeşitlerin (kalem) seçilmesi yanında, güçlü ve uyumlu anaçların belirlenmesi büyük bir önem arz etmektedir [16]. Aşılı hıyar fide üretiminde anaç olarak en fazla tür içi hibrit bal kabağı anaçları kullanılmaktadır [15, 17]. Ayrıca türler arası kestane kabağı x bal kabağı hibrit (*C. maxima* × *C. moschata*) anaçları; melezleme başarısı ve tohum veriminin fazla olması, hem kavun-karpuz ve hem de hıyarda kullanılabilmesi, aşı uyuşum oranının yüksek olması, bitki gelişiminin güçlü olması, verimi artırması ve genel olarak biyotik/abiyotik stres koşullarına daha dayanıklı olması gibi çok sayıda avantajları nedeniyle tercih edilmektedir [15, 18]. Ülkemizde Tohumluk Tescil Sertifikasyon Merkezi'nin 2023 yılı kayıtlarına göre aşılı fide üretiminde kullanılan 103 ticari anaç bulunmaktadır. Bu anaçların 38 tanesi (34'ü standart tohumluk kaydı bulunan ve 4'ü ticari üretim izinli) kabak anacıdır. Ticari anaçların çok büyük bir kısmı yurt dışından ithal edilmektedir. Bu durum fide maliyetinin artmasına neden olmaktadır [19]. Ülkemizde yerli sebze anaçlarının ıslahı ve geliştirilmesi hem sebzecilik ve hem de fidecilik sektörü açısından büyük bir önem taşımaktadır. Bu amaca yönelik olarak Balkaya ve Yapıcı [20], tarafından Üniversite-Özel Sektör İş birliği kapsamında yürütülen bir proje kapsamında nitelikli bal kabağı germplazmı oluşturulmuştur. Bu çalışmada, karakterizasyonu yapılmış nitelikli bal kabağı hatlarının *F. oxysporum* f. sp. *cucumerinum*'a karşı reaksiyonlarının belirlenmesi ve patojen etmeni ile inokule edilmiş bal kabağı bitkileri ile kontrol bitkilerinin bazı vejetatif büyüme özellikleri yönünden karşılaştırılması amaçlanmıştır.

Materyal ve Metot

Bu araştırma, Ondokuz Mayıs Üniversitesi (OMÜ) Ziraat Fakültesi Araştırma ve Uygulama Ünitesinde yer alan sebze çoğaltma serası ile Bahçe Bitkileri Bölümü bitki fizyoloji laboratuvarında yürütülmüştür. Denemede genetik materyal olarak farklı ıslah kademelerinde (S4-S5 generasyonu) saflaşmış 43 bal kabağı hattı kullanılmıştır (Tablo 1). Çalışmada ilk aşamada, gen havuzunda yer alan nitelikli bal kabağı hatlarının *F.*

oxysporum f. sp. *cucumerinum*'a reaksiyonları tespit edilmiştir. Pozitif kontrol olarak "Nun 9075" ticari kabak anacı ve negatif kontrol olarak "Çengelköy" hıyar çeşidi kullanılmıştır.

Araştırmada hastalık etmeni olarak virülensi yüksek *F. oxysporum* f. sp. *cucumerinum* (FOC) izolatı kullanılmıştır. İzolat, Batı Akdeniz Tarımsal Araştırma Enstitüsünden (BATEM) temin edilmiş ve OMÜ Ziraat Fakültesi Bitki Koruma Bölümü Fitopatoloji laboratuvarında kültüre alınmıştır. İnokulum hazırlama işlemi için izolat, 9 cm çaplı Petri kaplarındaki Patates Dekstroz Agar (PDA: Oxoid) besi yerinde 25 ºC'de 10 gün süreyle geliştirilmiş ve bu kültürün üzerine saf su eklenerek spatül yardımıyla kazınmıştır. Daha sonra belirtilen hastalık etmeninin konidilerinin suya geçmeleri sağlanmıştır. Hazırlanan süspansiyondaki sporlar, Thoma lamında (hemocytometre) sayılmış ve konsantrasyonları 1×10^6 konidi/mL olarak ayarlanmıştır [21, 22].

Denemede klasik hastalık testlemesi için incelenen bal kabağı hatlarının tohum ekimi viyollere yapılmıştır. Yetiştirme ortamı olarak 2:1 (v:v) oranında torf ve perlit karışımı kullanılmıştır.

Tablo 1 Bal kabağı hatlarının kayıt bilgileri
Table 1 Accession information of pumpkin lines

No	Kod	Hat No	No	Kod	Hat No
1	FB1	22ANC020-1-1	23	FB24	23ANC145-2-1
2	FB2	22ANC036-1-1	24	FB25	23ANC147-4
3	FB3	22ANC058-1-1	25	FB26	23ANC150-2
4	FB4	22ANC058-2-1	26	FB27	23ANC151-1-1
5	FB5	22ANC058-3-1	27	FB28	23ANC193-4-3
6	FB6	22ANC062-2-1	28	FB29	23ANC194-1-1
7	FB7	22ANC062-3-1	29	FB30	23ANC196-1-1
8	FB8	22ANC073-1-1	30	FB31	23ANC197-3-1
9	FB9	22ANC073-1-2	31	FB32	23ANC199-1-1
10	FB10	22ANC077-1-1	32	FB33	23ANC200-1-1
11	FB11	22ANC083-1-1	33	FB34	23ANC203-3-1
12	FB12	22ANC085-1-1	34	FB35	23ANC204-5-1
13	FB14	23ANC123-3-2	35	FB36	23ANC206-1
14	FB15	23ANC124-1	36	FB37	23ANC215-2-1
15	FB16	23ANC125-2	37	FB38	23ANC216-2
16	FB17	23ANC128-4	38	FB39	23ANC219-5-1
17	FB18	23ANC130-3	39	FB40	23ANC223-3-1
18	FB19	23ANC132-1	40	FB41	23ANC242-1-2
19	FB20	23ANC134-4	41	FB42	23ANC271-2
20	FB21	23ANC136-3	42	FB43	23ANC273-2-2
21	FB22	23ANC138-4-1	43	FB44	23ANC278-2
22	FB23	23ANC144-3-1			

Bal kabağı hatlarına ait fideler, 24 °C sıcaklık değerinde (± 2 °C) iklim odasında 3-4 gerçek yapraklı döneme kadar yetiştirilmiştir. Çalışmada, hastalık testlemesi için bal kabağı fidelerinde "kök daldırma yöntemi" uygulanmıştır [23]. Bu amaçla, fide kökleri musluk suyu ile yıkandıktan sonra steril makas yardımıyla tıraşlama işlemi yapılarak belirgin yara dokuları açılmıştır. Bu işlemden sonra fide kökleri daha önceden hazırlamış olduğumuz konidi süspansiyonuna (1×10^6 konidi mL) batırılarak 10 dakika süreyle bekletilmiştir [24]. Denemede kontrol uygulaması olarak yer alan bal kabağı fideleri ise yine 10 dakika süreyle saf su içinde tutulmuştur. Tüm fideler, belirtilen uygulamalardan sonra 2:1 oranında torf-perlit karışımı ile doldurulmuş plastik saksılara (26×19 cm çapında) her bir saksıda 1 bal kabağı bitkisi olacak şekilde dikilmiştir. Deneme tesadüf parselleri deneme desenine göre kurulmuştur. Hastalık bulaştırılan ve bulaştırılmayan (kontrol) her bir bal kabağı hattından toplam 24 bitki (8 bitki x 3 tekerrür) dikilmiştir. Belirtilen uygulamalardan sonra sıcaklık kontrollü serada 24 ± 2 °C'de bal kabağı bitkileri 3 hafta süreyle yetiştirilmiştir. Bal kabağı bitkilerinde morfolojik olarak ortaya çıkan belirtiler 21. gün sonunda 0-3 skalası kullanılarak hastalık şiddeti yönünden değerlendirilmiştir [12, 21]. Buna göre sırasıyla 0: Belirti yok, 1: Hafif veya orta derecede solgunluk, kökte hafif renk değişikliği, 2: Şiddetli solgunluk, gövdede ve iletim demetlerinde renk değişikliği 3: Ölü bitki, şeklinde değerlendirme yapılmıştır. Çalışma sonuçlarından elde edilen veriler yüzdelik değerler olduğundan, varyans analizi yapmak için SPSS istatistik paket programında arcsin $\sqrt{x}$ dönüşümü kullanılmıştır. Ardından, dayanıklılık seviyelerinin oranları Duncan çoklu karşılaştırma testi ile istatistiksel olarak analiz edilip, farklılıklar belirlenmiştir [21]. Bal kabağı hatlarında ortaya çıkan % hastalık şiddetleri, Townsend-Heuberger formülüyle hesaplanmıştır [25].

Hastalık şiddetleri: $\sum n \times v / V \times N \times 100$

n: Aynı değerdeki örnek adeti **v:** Skala değeri

V: En yüksek skala değeri **N:** Toplam örnek sayısı

Ayrıca çalışmada Martyn ve McLaughlin [26]'e göre; FOC dayanıklılık seviyesi yönünden gruplandırmalar yapılmıştır. Buna göre

I: %0-20: Yüksek düzeyde dayanıklı (HR)

II: %21-50: Orta düzeyde dayanıklı (MR)

III: %51-80: Düşük düzeyde dayanıklı (SR)

IV: %81-100: Duyarlı (S) olarak değerlendirilmiştir.

Çalışmanın ikinci aşamasında, hastalık etmeni ile inoküle edilen ve inoküle edilmeyen (kontrol) bal kabağı hatları ile Çengelköy ve Nun 9075 çeşitlerine ait bitkilerde 21. gün sonunda vejetatif büyüme parametreleri yönünden değerlendirme yapılmıştır. Bu amaçla bitki boyu (cm), gövde çapı (mm), yaprak sayısı / bitki (adet) ve toplam bitki kuru ağırlığı (70 °C'de 72 saat süreyle bitkiler etüvde kurutulmuş ve hassas terazide (0.001 g) tartılmıştır) parametreleri yönünden incelemeler yapılmıştır [21]. Vejetatif büyüme parametrelerine ilişkin verilerin istatistiksel analizinde, JUMP 5.01 paket programı kullanılmıştır.

Bulgular ve Tartışma

Bal kabağı hatlarının *F. oxysporum* f. sp. *cucumerinum*'a dayanıklılık reaksiyonları

Çalışmada yer alan 43 bal kabağı hattının virülent *F. oxysporum* f. sp. *cucumerinum* izolatına karşı gösterdiği dayanıklılık seviyeleri Tablo 2'de verilmiştir. Negatif kontrol olarak kullanılan Çengelköy hıyar çeşidinde hastalık oranı %100 olarak belirlenmiştir. Pozitif kontrol olarak kullanılan ticari kabak anacında (Nun 975) ise hastalık etmenin oluşmadığı ve yüksek düzeyde dayanıklılığın bulunduğu tespit edilmiştir. Araştırmada bal kabağı hatları ve kontrol olarak kullanılan çeşitler (Çengelköy ve Nun 9075) üzerinde FOC etmenine ait izolatın oluşturduğu hastalık şiddetleri arasında istatistiksel olarak önemli düzeyde farklılıkların olduğu saptanmıştır (P≤0,05) (Tablo 2). Klasik hastalık testlemesi sonucunda incelenen bal kabağı hatlarında hastalık şiddetinin %0-66.7 arasında değişim gösterdiği belirlenmiştir. Negatif kontrol ile kıyaslandığında bal kabağı hatlarının büyük bir çoğunluğunun yüksek düzeyde dayanıklı (28 hat) ve orta düzeyde dayanıklı (14 hat) oldukları saptanmıştır. Sadece FB35 nolu bal kabağı hattına ait bitkilerde hastalık şiddeti oranının %66.7 olduğu ve düşük düzeyde dayanıklı olduğu belirlenmiştir (Tablo 2). Bu sonuç, bal kabağı hatlarının büyük bir çoğunluğunun hastalık etmenine dayanım yönünden nitelikli genitörler olduklarını göstermektedir. Yine Tablo 2 incelendiğinde 20 bal kabağı hattında hastalık etmeninin hiç hastalık oluşturmadığı belirlenmiştir.

Birçok araştırmada bal kabağı anaçlarının Fusarium solgunluğuna dayanıklı olduğu bildirilmiştir [15, 27, 28, 29]. Aşılı hıyarda yapılan bir çalışmada *F. oxysporum* f. sp. *radicis-cucumerinum* (FORC)'a dayanıklılıkta bal kabağı anaçlarının aşısız uygulamaya göre daha iyi performans sergilediği belirlenmiştir [30]. Bu nedenle yaptığımız çalışma sonucunda hastalık şiddeti %0 olan ve yüksek düzeyde dayanıklı olarak belirlenen bal kabağı hatlarının gelecekte gerek hibrit çeşit ıslahında ve gerekse hibrit anaç ıslah programlarında nitelikli ebeveyn olarak kullanımı büyük bir önem taşımaktadır.

F. oxysporum f. sp. *cucumerinum*'un bal kabağı hatlarının bazı vejetatif büyüme özellikleri üzerine etkileri

Hastalık testlemeleri sonunda incelenen bal kabağı genotiplerinde vejetatif büyüme yönünden etkilenme durumları belirlenmiştir. Araştırmada FOC izolatı ile enfekte edilen ve edilmeyen uygulamalarda bitki boyu yönünden istatistiksel olarak çok önemli düzeyde farklılıklar olduğu saptanmıştır (Tablo 3). Hastalık etmeni ile bulaşık bal kabağı genotipleri arasında en yüksek bitki boyu FB21 nolu hatta 81.1 cm ölçülmüştür. En kısa bitki boyu ise sırasıyla FB10 (14.2 cm) ve FB7 (15.1 cm) nolu bal kabağı hatlarında belirlenmiştir (Tablo 3). Kontrol uygulamasında en yüksek bitki boyu FB21 nolu hatta (110.2 cm) ve en kısa bitki boyu ise FB39 (26.3 cm) nolu bal kabağı hattında ölçülmüştür. İncelenen bal kabağı hatlarında kontrol bitkilerinde ortalama bitki boyu 47.4 cm olarak bulunmuştur. Hastalık etmeni ile bulaşık bal kabağı hatlarında bitki boyu ortalaması 28.7 cm olarak belirlenmiştir. Buna göre enfekteli bitkilerde bitki boyunda yaklaşık %39,5 oranında belirgin bir azalışın olduğu tespit edilmiştir.

Araştırma sonucunda, Düşük düzeyde dayanıklı olduğu belirlenen FB35 nolu bal kabağı hattında bitki boyunda yaklaşık %60 oranında belirgin bir azalış olduğu tespit edilmiştir. Hastalık etmenine dayanıklı olarak belirlenen bal kabağı hatlarında genotiplere göre değişmekle birlikte bitki boyundaki azalışların daha düşük oranlarda olduğu bulunmuştur (Tablo 3).

Tablo 2 Bal kabağı hatlarının *Fusarium oxysporum* f. sp. *cucumerinum*'a karşı gösterdiği hastalık indeksi, hastalık şiddeti ve dayanıklılık seviyeleri
Table 2 Disease index, disease severity, and resistance levels of pumpkin lines against *Fusarium oxysporum* f. sp. *cucumerinum*

Kod	Hastalık İndeksi*	Hastalık şiddeti (%)	Dayanıklılık seviyesi**
FB1	0.67 de	22.22	MR
FB2	0.00 f	0	HR
FB3	0.00 f	0	HR
FB4	0.67 de	22.22	MR
FB5	0.00 f	0	HR
FB6	0.33 ef	11.11	HR
FB7	0.00 f	0	HR
FB8	0.33 ef	11.11	HR
FB 9	0.00 f	0	HR
FB10	0.67 de	22.22	MR
FB11	0.33 ef	11.11	HR
FB12	1.00 cd	33.33	MR
FB14	1.33 c	44.44	MR
FB15	0.00 f	0	HR
FB16	1.00 cd	33.33	MR
FB17	0.00 f	0	HR
FB18	1.00 cd	33.33	MR
FB19	0.33 ef	11.11	HR
FB20	0.00 f	0	HR
FB21	0.33 ef	11.11	HR
FB22	0.00 f	0	HR
FB23	0.00 f	0	HR
FB24	0.00 f	0	HR
FB25	0.00 f	0	HR
FB26	1.00 cd	33.33	MR
FB27	1.00 cd	33.33	MR
FB28	0.00 f	0	HR
FB29	0.00 f	0	HR
FB30	0.33 ef	11.11	HR
FB31	1.00 cd	33.33	MR
FB32	0.67 de	22.22	MR
FB33	0.00 f	0	HR
FB34	1.00 cd	33.33	MR
FB35	2.00 b	66.67	SR
FB36	1.00 cd	33.33	MR
FB37	0.00 f	0	HR
FB38	0.00 f	0	HR
FB39	1.00 cd	33.33	MR
FB40	0.33 ef	11.11	HR
FB41	0.00 f	0	HR
FB42	0.33 ef	11.11	HR
FB43	0.00 f	0	HR
FB44	0.00 f	0	HR
Çengelköy	3,00 a	100	S
Nun9075	0,00 d	0	HR
P	< 0,05	< 0,05	

* 0: Belirti yok, 1: Hafif veya orta derecede solgunluk, kökte hafif renk değişikliği, 2: Şiddetli solgunluk, gövdede ve iletim demetlerinde renk değişikliği, 3: Ölü bitki
** I: %0-20: Yüksek düzeyde dayanıklı (HR), II: %21-50: Orta düzeyde dayanıklı (MR), III: %51-80: Düşük düzeyde dayanıklı (SR), IV: %81-100: Duyarlı (S)

Denemede pozitif kontrol olarak kullanılan Nun 9075 kabak anacının kontrol bitkilerinde bitki boyu 27.7 cm iken enfekteli bitkilerde 16.3 cm olarak ölçülmüştür. Negatif kontrol olarak yer alan Çengelköy hıyar çeşidinde ise tüm bitkilerin öldüğü ve bitki boyunun en kısa (0.001 cm) olduğu bulunmuştur (Tablo 3). Yapılan çalışmalarda Fusarium solgunluğu etmeni ile bulaşık bitkilerde hastalık belirtileri, fide aşamasında hipokotilde zararlanma ve çökerten belirtisi olarak kendini göstermekte, ilerleyen büyüme ve gelişme dönemlerinde ise bitkide solgunluk semptomu ortaya çıkmaktadır [31]. Bu durumda solgunluk, bitkide büyümenin durmasına ya da bitki boyunun kısalmasına neden olmaktadır. Kabak anaçlarının aşılı hıyar yetiştiriciliğinde vejetatif büyüme üzerine etkilerinin belirlenmesi üzerinde yapılan bir çalışmada bal kabağı hatlarında benzer şekilde bitki boyunda değişen oranlarda azalışların olduğu saptanmıştır [32]. Araştırıcılar kabak anaçları ile aşılı hıyar bitkilerinin aşısız hıyar bitkilerine göre daha fazla vejetatif aksam oluşturduklarını bildirmişlerdir.

Çalışmada FOC izolatı ile inokule edilen bal kabağı hatlarında, gövde çapı değerlenin 3.9-8.1 mm ve kontrol uygulamasında ise 5.3-9.4 mm arasında değişim gösterdiği belirlenmiştir (Tablo 3).

Tablo 3 Bal kabağı hatları ile Çengelköy ve Nun 9075 çeşitlerinde *Fusarium oxysporum* f. sp. *cucumerinum* ile enfekteli olan (+) ve enfekteli olmayan (-) bitkilerdeki bitki boyu (cm) ve gövde çapları (mm)
Table 3 Plant height (cm) and stem diameter (mm) results in infected (+) and non-infected (-) plants with *Fusarium oxysporum* f. sp. *cucumerinum* in pumpkin lines

Hatlar	Bitki boyu		Gövde çapı	
	Bitki Enfekte Edilme Durumu			
	+	-	+	-
FB1	40.83 c-ı	75.10 e	5.21 b-j	7.33 z
FB2	53.97 b-c	62.60 k	5.80 b-j	6.28 ll
FB3	28.3 f-1	32.20 h1	6.31 a-h	7.58 u
FB4	21.3 k-ı	43.20 s	5.75 b-j	7.71 q
FB5	17.53 k-m	16.20 p1	6.04 a-j	6.48 j1
FB6	20.83 k-ı	42.70 t	7.6 a-b	7.92 o
FB7	15.1 l-m	46.90 p	6.11 a-j	8.16 m
FB8	20.27 k-ı	36.30 b1	7.06 a-f	7.25 b1
FB 9	28.30 f-l	38.20 w	5.72 b-j	6.27 m1
FB10	14.27 l-m	32.30 g1	6.67 a-g	7.63 t
FB11	16.30 l-m	52.40 n	6.43 a-h	6.65 g1
FB12	24.47 ı-l	65.40 j	6.55 a-h	8.35 j
FB14	17.67 k-m	37.50 x	4.2 z-b1	6.74 e1
FB15	27.97 g-1	34.70 d1	6.79 a-f	8.62 d
FB16	25.77 ı-1	28.20 j1	6.65 a-g	7.48 w
FB17	20.8 a1-h1	29.90 11	4.93 f-j	7.29 a1
FB18	47.03 b-e	83.20 d	4.93 f-j	7.56 v
FB19	45.83 b-g	92.40 b	5.10 e-j	5.28 r1
FB20	45.47 b-h	66.20 ı	6.59 a-g	6.72 f1
FB21	81.10 a	110.20 a	6.03 a-j	8.52 f
FB22	60.77 b	70.30 g	7.50 a-c	7.69 s
FB23	46.27 b-f	67.50 h	6.65 a-g	6.99 c1
FB24	51. I7 b-d	72.10 f	6.75 a-f	7.36 y
FB25	40.33 c-j	49.20 o	5.24 c-j	5.42 p1
FB26	44.87 b-h	58.80 m	4.45 g-j	5.32 q1
FB27	22.47 j-1	61.50 l	3.99 ı-j	6.32 k1
FB28	20.93 k-1	34.20 e1	7.38 a-e	8.32 k
FB29	28.40 f-1	40.50 v	7.11 a-f	8.48 h
FB30	27.53 h-1	33.00 f1	7.47 a-d	8.44 ı
FB31	28.30 f-1	36.50 z	6.42 a-h	7.71 r
FB32	26.67 ı-1	42.30 u	7.40 a-d	7.42 x
FB33	29.80 e-1	37.20 y	8.10 a	8.58 e
FB34	19.53 k-1	38.20 w	7.37 a-e	8.26 l
FB35	34.97 d-k	86.70 c	3.89 j	5.99 o1
FB36	20.13 k-1	27.80 m1	7.03 a-f	8.88 c
FB37	27.97 g-1	34.70 d1	6.73 a-g	8.51 g
FB38	22.13 k-1	36.40 a1	7.27 a-e	7.88 p
FB39	18.57 k-1	26.30 o1	6.36 a-h	9.40 b
FB40	22.30 j-1	30.70 k1	6.31 a-h	6.87 d1
FB41	19.37 k-1	31.40 ı1	6.28 a-h	6.60 ı1
FB42	21.83 k-1	40.50 v	6.26 a-ı	8.08 n
FB43	28.13 g-1	35.30 c1	5.95 a-j	6.22 n1
FB44	21.90 k-1	44.20 r	7.29 a-e	7.69 r
Çengelköy	0.001 m	45.10 q	0.00 k	6.65 h1
Nun-9075	16.27 l-m	27.70 n1	7.30 a-e	10.10 a
P	< 0001		< 0001	

Araştırmada düşük düzeyde dayanıklı olduğu belirlenen F35 nolu bal kabağı hattının enfekteli bitkiler içerisinde en düşük gövde çapının olduğu ve kontrol bitkilere göre gövde çapında yaklaşık %35.1 oranında belirgin azalışın meydana geldiği saptanmıştır. Bal kabağı hatlarında kontrol bitkilerinde ortalama gövde çapı

değeri 7.4 mm olarak tespit edilmiştir. Hastalık etmeni ile inokule edilen bal kabaklarında ise gövde çapı ortalama 6.1 mm olarak belirlenmiştir.

Buna göre enfekteli bitkilerde gövde çapında yaklaşık %17,3 oranında azalışın olduğu tespit edilmiştir. Ticari olarak kullanılan Nun 9075 kabak anacında gövde çapında azalış miktarı %27,7 olarak bulunmuştur. Denemede dayanıklı olarak belirlenen bal kabağı hatlarında gövde çapındaki azalış miktarının, ticari anaçtaki gövde çapının azalış miktarından daha düşük veya benzer oranlarda olduğu saptanmıştır. Bu sonuç bal kabağı hatlarının gelecekte ıslah programlarında kullanımı yönünden önemli bir sonuç olduğu düşünülmektedir

Bitkilerde en önemli vejetatif büyüme parametrelerinden birisi de oluşan mevcut yaprak sayısıdır. Bal kabağı hatlarında hastalık etmeni ile bulaşık bitkilerde ve kontrol bitkilerinde yaprak sayısı yönünden çok önemli düzeyde farklılıkların olduğu belirlenmiştir (Tablo 4). Fusarium solgunluğu etmeni ile bulaşık bal kabağı hatlarında en fazla yaprak sayısının ortalama 6.7 adet (FB32 nolu hat) ve kontrol bitkilerinde ise 8.0 adet (FB6, FB18, FB32) olduğu tespit edilmiştir (Tablo 4). Araştırma sonucunda incelenen bal kabağı hatlarında kontrol uygulamasında ortalama yaprak sayısı 6.2 adet olarak bulunmuştur. Hastalık etmeni ile bulaştırılan bal kabağı hatlarında ise ortalama yaprak sayısı 4.7 olarak belirlenmiştir. Buna göre araştırmada enfekteli bal kabağı bitkilerinde de yaprak sayısının %23.3 oranında azalış gösterdiği saptanmıştır (Tablo 4). Karaağaç [21], farklı kabak anaçları üzerine Crisby karpuz çeşidini aşılamış ve ortalama yaprak sayılarının 22.3-64.3 adet arasında olduğunu belirlemiştir. Kobal ve ark. [32], farklı kabak anaçları ile aşılı hıyar bitkilerinde dikimden 40 gün sonra ortalama yaprak sayılarının 14.7-24.0 adet arasında değiştiğini tespit etmişlerdir.

Araştırmada kantitatif analiz sonucunda kontrol bal kabağı hatlarında bitki kuru ağırlığının 1.9 g ile 11.5 g arasında değişim gösterdiği belirlenmiştir (Tablo 4). Hastalık etmeni ile bulaşık bal kabağı hatlarında ise bu değer 1.2 g ile 9.6 g arasında tespit edilmiştir. Araştırmada düşük düzeyde dayanıklı olduğu belirlenen FB35 nolu bal kabağı hattında, bitki kuru ağırlığı yönünden yaklaşık %37.1 oranında azalış olduğu saptanmıştır. Tüm bal kabağı hatlarında kontrol bitkilerinde ortalama bitki kuru ağırlığı 6.39 g olarak bulunmuştur. Hastalık etmeni patojenle bulaştırılan bal kabağı hatlarında ise bitki kuru ortalaması 3.2 g olarak belirlenmiştir. Buna göre enfekteli bitkilerde, toplam bitki kuru ağırlığı değerlerinde belirgin azalışların olduğu tespit edilmiştir. Nun 9075 ticari kabak anacında kontrol bitkilerde bitki kuru ağırlığı ortalaması 3.2 g ve patojen ile bulaşık olan bitkilerde ise 1.9 g olarak belirlenmiştir. Bitki kuru ağırlığındaki azalış miktarı %41.3 olarak bulunmuştur. Araştırma sonucunda özellikle yüksek düzeyde dayanıklı olan ve bitki kuru ağırlıkları yönünden azalış miktarının en düşük olduğu hatlar, yakın gelecekte devam eden ıslah programlarında ümitvar ebeveynler olarak değerlendirilecektir. Literatürde birçok çalışma sonuçları, kabakgil grubu sebzelerde aşılama ile birlikte bitkilerde kuru madde miktarının arttığını göstermiştir [33, 34, 21, 35, 36, 37]. Dolayısıyla bu araştırma sonucunda öne çıkan hatlarla yapılacak melez kombinasyonlarının anaç olarak kullanımı ve doğru nitelikli bir kalem (hıyar çeşidi) ile aşılanması sonucunda hem *F. oxysporum* f. sp. *cucumerinum* etmenine dayanıklı ve hem de vejetatif büyüme özellikleri yönünden güçlü aşılı hıyar bitkileri elde edilmiş olacaktır.

Sonuç

Kabakgiller (*Cucurbitacea*) familyasına ait bir sebze türü olan hıyar, dünyada ve ülkemizde yaygın olarak yetiştirilen ve ekonomik değeri olan önemli sebze türlerindendir. Ülkemizde farklı ürün segmentlerinde hıyar yetiştiriciliğinde kullanılan F_1 hıyar çeşitlerinin sayısı ve kullanımı her geçen gün artış göstermektedir. Hibrit çeşitlerin kullanım oranının artış göstermesine rağmen, özellikle seralarda yoğun üretim nedeniyle toprak kaynaklı hastalıklar, zararlılar, tuzlu veya alkali toprak koşulları ve toprak yorgunluğu gibi problemler, yetiştiricilikte istenilen verim ve kaliteye ulaşılmasını olumsuz yönde etkilemektedir [38]. Bu sorunların azaltılması ve çözümü için çevreye dost bir uygulama olarak aşılı fide ile hıyar yetiştiriciliği önerilmektedir. Günümüzde kabakgil grubu sebze türlerinde aşılı fide kullanımı, toprak kökenli Fusarium solgunluğu hastalığının mücadelesinde önemli bir yer tutmaktadır [15]. Ancak, başarılı bir aşılı hıyar yetiştiriciliği için öncelikle aşılamada kullanılan anacın niteliği (kök yapısı, hastalık ve zararlılara dayanıklılık durumu, aşı uyuşumu vb.) oldukça önemlidir. Dünyada ve ülkemizde fide işletmelerinde aşılı hıyar fidesi üretiminde anaç olarak daha çok tür içi bal kabağı (*C. moschata*) hibrit anaçları yada kestane kabağı ve bal kabağı hibritleri (*C. maxima* × *C. moschata*) kullanılmaktadır.

Fide üretim tesislerinde aşılı hıyar, karpuz ve kavun fidesi üretiminde kullanılan anaçların büyük bir kısmı yurt dışından ithal edilmektedir. Ülkemizde son yıllarda sayıları az da olsa özel sektör ve üniversiteler tarafından yerli anaç geliştirilmesine yönelik yürütülen anaç ıslah programları vardır. Üniversite-Sanayi işbirliği kapsamında gerçekleştirilen proje (Aşılı Hıyar Fidesi Üretimi İçin Yerli Kabak Anaçlarının Bitkisel Özelliklerinin İncelenmesi, Fenotipik Kabak Seleksiyonu ve Kök Kanopilerinin Belirlenmesi) kapsamında bitki özellikleri yönünden oldukça heterojen, anaçlık potansiyeli yüksek niteliklere sahip olan nitelikli bal kabağı gen havuzu oluşturulmuştur.

Tablo 4 Bal kabağı hatları ile Çengelköy ve Nun 9075 çeşitlerinde *Fusarium oxysporum* f. sp. *cucumerinum* ile enfekteli olan (+) ve enfekteli olmayan (-) bitkilerde yaprak sayısı (adet) ve toplam bitki kuru ağırlığı (g) sonuçları
Table 4 Leaf number (count) and total plant dry weight (g) results in infected (+) and non-infected (-) plants with *Fusarium oxysporum* f. sp. *cucumerinum* in pumpkin lines

Hatlar	Yaprak sayısı		Bitki kuru ağırlığı	
	Bitki enfekte edilme durumu			
	+	-	+	-
FB1	4.67 b-g	6.00 c	2.00 e-ı	4.05 fl
FB2	5.67 a-d	7.00 b	3.07 c-ı	3.56 kl
FB3	4.67 b-g	5.00 d	2.55 d-ı	4.48 al
FB4	3.67 e-g	6.00 c	4.63 b-g	4.76 y
FB5	4.67 b-g	6.00 c	1.81 f-ı	2.97 ol
FB6	4.33 c-g	8.00 a	1.91 eı	4.32 cl
FB7	3.33 f-g	5.00 d	1.54 g-ı	4.11 el
FB8	4.33 c-g	5.00 d	4.29 b-h	5.17 x
FB 9	5.67 a-d	7.00 b	2.03 e-ı	4.20 dl
FB10	3.67 e-g	5.00 d	2.44 d-ı	6.18 q
FB11	3.67 e-g	5.00 d	2.044 e-ı	5.32 w
FB12	4.33 c-g	7.00 b	9.59 a	11.48 a
FB14	3.67 e-g	6.00 c	2.92 c-ı	8.18 f
FB15	6.00 a-c	6.00 c	4.04 b-h	6.37 p
FB16	5,33 a-e	7.00 b	2.93 c-ı	4.45 bl
FB17	4.33 c-g	5.00 d	2.29 d-ı	3.75 ıl
FB18	5.67 a-d	8.00 a	3.24 c-h	4.04 gl
FB19	5,33 a-e	7.00 b	1.45 g-ı	2.85.ql
FB20	5.00 a-f	5.00 d	2.13 d-ı	3.35 ll
FB21	6.33 a-b	7.00 b	2.72 d-ı	5.80 t
FB22	5.00 a-f	7.00 b	2.57 d-ı	3.20 nl
FB23	5.67 a-d	7.00 b	3.85 b-h	8.20 e
FB24	5,33 a-e	7.00 b	3.03 c-ı	7.95 h
FB25	4.67 b-g	6.00 c	2.18 d-ı	2.94 pl
FB26	5.00 a-f	6.00 c	3.57 c-h	5.91 s
FB27	4.00 d-g	5.00 d	2.12 d-ı	7.22 l
FB28	4.00 d-g	5.00 d	5.04 b-e	8.58 d
FB29	5.67 a-d	6.00 c	5.25 b-d	6.72 o
FB30	6.00 a-c	7.00 b	3.52 c-h	5.45 v
FB31	5.67 a-d	7.00 b	4.03 b-h	8.81 b
FB32	6.67 a	8.00 a	6.02 b-c	8.66 c
FB33	6.00 a-c	7.00 b	3.63 c-h	6,05 r
FB34	5.00 a-f	6.00 c	6.88 a-b	7.20 m
FB35	3.00 g	6.00 c	1.22 h-ı	1.94 sl
FB36	5.00 a-f	5.00 d	3.29 c-h	4.67 z
FB37	5.00 a-f	6.00 c	4.74 b-f	6.84 n
FB38	5,33 a-e	7.00 b	4.15 b-h	8.09 g
FB39	4.67 b-g	7.00 b	3.16 c-ı	7.46 k
FB40	5.00 a-f	6.00 c	2.68 d-ı	3.65 jl
FB41	4.33 c-g	6.00 c	2.50 d-ı	7.50 j
FB42	4.00 d-g	6.00 c	3.07 c-ı	7.77 ı
FB43	5.00 a-f	5.00 d	4.02 b-h	5.75 u
FB44	4.00 d-g	5.00 d	2.39 d-ı	3.86 hl
Çengelköy	0.00 h	6.00 c	0.00 ı	2.79 rl
Nun-9075	4.00 d-g	5.00 d	1.89 e-ı	3.32 ml
P	< 0001		< 0001	

Bu araştırma ile de mevcut bal kabağı germplazmının *F. oxysporum* f. sp. *cucumerinum*'a karşı reaksiyonları belirlenmiştir. Çalışma sonucunda bal kabağı hatlarında hastalık şiddetinin %0-66.7 arasında değişim gösterdiği, 28 bal kabağı hattının yüksek düzeyde dayanıklı ve 14'ünün ise orta düzeyde dayanıklı olduğu belirlenmiştir. Mevcut germplazm içerisinde sadece FB35 nolu bal kabağı hattına ait bitkilerin düşük düzeyde dayanıklı olduğu tespit edilmiştir. Bu sonuç gen havuzunda yer alan diğer bal kabağı hatlarının halen devam eden kabak anaç ıslah programında ümit var ebeveynler olarak kullanılabileceklerini göstermiştir. Ayrıca bu araştırma bulguları, gelecekte bal kabağı yetiştiriciliğinde hastalıkla mücadele stratejilerinin geliştirilmesine de olumlu yönde katkı sağlayabilir. Çalışmada vejetatif büyüme özellikleri yönünden yapılan kantitatif analiz

sonuçlarına göre dayanıklı olarak belirlenen bal kabağı hatlarında genel olarak oranlar değişmekle birlikte bitki boyu, gövde çapı ve yaprak sayısı yönünden belirlenen azalışların hassas bitkilere göre oldukça düşük düzeyde olduğu tespit edilmiştir. Araştırma sonuçları ayrıca bal kabağı hatlarının vejetatif büyüme özellikleri yönünden ticari kabak anaçlarına benzer düzeylerde performansa sahip olmaları nedeniyle anaç adayı olma potansiyellerinin de oldukça yüksek olduğunu göstermiştir. Araştırma sonrasında yerli kabak anaçlarının geliştirilmesi, aşılı hıyar fidesi üretiminde yerli anaç tohumu kullanımının artmasına, fideliklerde kaliteli aşılı fide elde edilmesine ve gelecekte aşılı hıyar yetiştiriciliğinin yaygınlaşması ile tarımsal sürdürülebilirliğin sağlanmasına katkı sağlayacaktır.

Data Availability statement / Veri Kullanılabilirliği bildirimi
The author confirms that the data supporting this study are cited in the article.
Yazar, bu çalışmayı destekleyen verilere makalede atıfta bulunulduğunu onaylamaktadır.

Compliance with ethical standards / Etik standartlara uyum
Conflict of interest / Çıkar çatışması
The author declare no conflict of interest.
Yazar herhangi bir çıkar çatışması beyan etmemektedir.

Ethical standards / Etik standartlar
The study is proper with ethical standards.
Çalışma etik standartlara uygundur**.**

Authors' contributions / Yazar katkıları
The authors declare that they have contributed equally to the article.
Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Kaynakça

1. Robinson, R.W., and D.S. Decker-Walters, Cucurbits. CAB International New York, 1997. NY USA.
2. Balkaya, A., E.S. Kurtar, and O. Karaağaç, Kabak Islahı. In: K., Abak, A., Balkaya, Ş.Ş. Ellialtıoğlu., E. Düzyaman, (Eds.) 2021, Sebze Islahı Cilt 2: Cucurbitaceae (Kabakgiller), pp. 19-91. Gece Kitaplığı s. 332, ISBN: 978-625-7478-49-6, Ankara.
3. Smith, B.D., Reassessing coxcatlan caveand the early history of domesticated plants in Mesoamerica. Proceedings of the National Academy of Sciences, 2005. 102 (27): 9438- 9445.
4. Balkaya A., M. Özbakır, and O. Karaağaç, Karadeniz bölgesinden toplanan balkabağı (*Cucurbita moschata* Duchesne) populasyonlarının karakterizasyonu ve meyve özelliklerindeki varyasyonun değerlendirilmesi. Tarım Bilimleri Dergisi, 2010. 16: 17–25.
5. Kurt, S., et al., Physiologic races of *Fusarium oxysporum* f. sp. *melonis* in the Southeastern Anatolia Region of Turkey and varietal reactions of races of the pathogen. Phytoparasitica, 2002. 30(4):395-402.
6. İncekara, R., Hıyar kök ve kök boğazı çürüklüğü etmeni *Fusarium oxysporum* f.sp. *radicis-cucumerinum*'a karşı bitki uçucu yağlarının anti fungal etkinlikleri. 2011. Mustafa Kemal Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi.
7. Kotan, R., *Fusarium oxysporum* f.sp. *radicis-cucumerinum*'a karşı etkili bakteri içerikli biyolojik ürün geliştirilmesi. 2020. Atatürk Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi.
8. Booth, C., The Genus Fusarium. Commonwealth Mycological Institute, 1971. Kew, Surrey, United Kingdom.
9. Tok, F.M., Kavun ve hıyar patojen *Fusarium oxysporum* izolatlarının patojenisite, ırk, vejetatif uyum grubu ve AFLP teknikleriyle karakterizasyonu ve dağılımları. 2011. Mustafa Kemal Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi.
10. Vakalounakis, D.J., Diseases and pests of vegetable crops and their control. Technological Education Institute, 1988. Heraklio, Greece.
11. Ünlü, M., A. Ünlü, and A. Kabaş, Bazı yerel kavun genotiplerinin *Fusarium oxysporum* f.sp. *melonis*'e reaksiyonlarının tespiti. VIII. Sebze Tarımı Sempozyumu Van, 2010. p:85.
12. Vakalounakis, D.J., Root and stem rot of cucumber caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum*. Plant Disease, 1996. 80:313-316.
13. Vakalounakis, D.J., and G.A. Fragkiadakis, Genetic diversity of *Fusarium oxysporum* isolates from cucumber: differentation by pathogenisity, vegetative compatibility, and RAPD fingerprinting. Phytopathology, 1999. 89:161-168.

14. Boyraz, N., and K.B. Baştaş, Kavun Fusarium solgunluğuna bazı biyotik ve abiyotik uyarıcıların etkileri. Selçuk Üniversitesi Ziraat Fakültesi Dergisi, 2005. 19(37):106-112.

15. Yücel, Ş., et al., Aşılı Fide Üretiminde Kullanılan Anaçlar. In: Yetişir, H., Ellialtıoğlu, Ş.Ş.(Eds.) Sebzelerde Fide Yetiştiriciliği-2, pp. 399-490. Gece Kitaplığı, 2022, ISBN: 978-625-430-538-2.

16. Yıldız, S., and A. Balkaya, Tuza tolerant kabak anaçlarının hipokotil özellikleri ve hıyarla aşı uyuşum durumlarının belirlenmesi. Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, 2016. 26(4):538-546.

17. Balkaya, A., Aşılı sebze üretiminde kullanılan anaçlar. TÜRKTOB Türkiye Tohumcular Birliği Dergisi, 2014. 106, 4-7.

18. Sakata, Y., T. Ohara, and M. Sugiyama,. The history and present state of the grafting of cucurbitaceous vegetables in Japan. Acta Horticulturae, 2007. 731, 159-170.

19. Kandemir, D., et al., Dünyada ve Türkiye'de Fide Sektörü. In: Yetişir, H., Ellialtıoğlu, Ş.Ş.(Eds.) 2022, Sebzelerde Fide Yetiştiriciliği-1, pp. 45-68. Gece Kitaplığı, ISBN: 978-625-430-537-5.

20. Balkaya, A., and B. Yapıcı, Aşılı Hıyar Fidesi Üretimi İçin Yerli Kabak Anaçlarının Bitkisel Özelliklerinin İncelenmesi, Fenotipik Kabak Seleksiyonu ve Kök Kanopilerinin Belirlenmesi. 2022. OMÜ TTO.ZRT.ULAP.22.516 Nolu Proje Sonuç Raporu.

21. Karaağaç, O., Karadeniz bölgesi'nden toplanan kestane kabağı (*C. maxima*) ve bal kabağı (*C. moschata*) genotiplerinin karpuza anaçlık potansiyellerinin belirlenmesi. 2013. Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi.

22. Sarıbaş, H.Ş., Aşılı patlıcan üretiminde genetik kaynakların anaç ıslah programında değerlendirilmesi ve yerli hibrit anaçların geliştirilmesi. 2019. Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi.

23. Biles, C., and R. Martyn, Local and systemic resistance induced in watermelons by formae speciales of *Fusarium oxysporum*. Phytopathology, 1989. 79:856-860.

24. Altınok, H.H., First report of fusarium wilt of eggplant caused by *Fusarium oxysporum* f. sp. *melongenae* in Turkey. Plant Pathology, 2005. 54(4): p.577.

25. Swiader, M., M. Pronczuk, and K. Niemirowicz-Szczyt, Resistance of Polish lines and hybrids of watermelon [*Citrullus lanatus* (Thunb.) Matsum et Nakai] to *Fusarium oxysporum* at the seedling stage. Journal of Applied Genetics, 2002. 43 (2):161-170.

26. Martyn, R.D., and R.J. McLaughlin, Effects of inoculum concentration on the apparent resistance of atermelon to *Fusarium oxysporum* f. sp. *niveum*. Plant Disease, 1983. (67):493-495.

27. Kim, H., et al., Characteristics of *Cucurbita* spp. for use as cucumber rootstock. RDA Journal of Horticulture Science, 1997. 39(2):8-14.

28. Göçmen, M., et al., Kabak (*Cucurbita* spp.) Genetik Kaynaklarının Hıyar (Cucumis sativus L.) Anaç Islah Programında Değerlendirilmesi ve Yerli Hibrit Anaçların Geliştirilmesi. 2014. TUBİTAK-TEYDEB Proje No: 3110194.

29. Reyad, N.E.H.A., S.F. El-Sayed, and S.N. Azoz, Evaluation of grafting using cucurbit interspecific hybrids to control Fusarium wilt in cucumber. Plant Cell Biotechnology and Molecular Biology 2021. 22(37–38):50-63.

30. Pavlou, G.C., D.J. Vakalounakis, and E.K., Ligoxigakis, Control of root and stem rot of cucumber, caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, by grafting onto resistant rootstocks. Plant Disease, 2002. 86(4):379-382.

31. Egel, D.S., and R.D. Martyn, Fusarium wilt of watermelon and other cucurbits. Plant Health Instructor, 2007. (10)1094.

32. Bekar, N. K., A. Balkaya, and M. Göçmen, Kabak anaçlarının aşılı hıyar yetiştiriciliğinde vejetatif büyüme üzerine etkilerinin belirlenmesi. Selçuk Tarım Bilimleri Dergisi, 2017. 3(2):280-290.

33. Yetişir, H., Karpuzda aşılı fide kullanımının bitki büyümesi, verim ve meyve kalitesi üzerine etkileri ile aşı yerinin histolojik açıdan incelenmesi. 2001. Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Yayınlanmamış Doktora Tezi.

34. Fita, A., B. Picó, and F. Nuez, Melon roots under stress: melon vine decline. Plant Stress, 2007. 1(93):104.

35. Balkaya, A., Aşılı sebze üretiminde kullanılan anaçlar. TÜRKTOB Türkiye Tohumcular Birliği Dergisi, 2014. 106:4-7.

36. Yıldız, S., Aşılı hıyar fidesi üretiminde anaç olarak kullanılacak bazı kabak (*Cucurbita* spp.) genetik kaynaklarının tuzluluğa tolerans seviyelerinin belirlenmesi. 2014. Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi.

37. Güngör, B., and A. Balkaya, Yerli kabak anaç çeşit adaylarının aşılı mini karpuzun vejetatif büyümesi üzerine kantitatif etkilerinin incelenmesi. Bahçe Özel Sayı, VII. Ulusal Bahçe Bitkileri Kongresi Bildirileri 2, 2016. 21-26.

38. Yoldaş, F., et al., Farklı bal kabağı anaç adayları ile aşılı hıyar çeşitlerinin küçük menderes havzasındaki verim ve kalite performanslarının belirlenmesi. Ege Üniversitesi Ziraat Fakültesi Dergisi, 2019. 56(3):319-326.

Endemik *Astragalus davisii* (Fabaceae) Tohumunun Liyofilize Su Özütünün Antioksidan Aktivitesi

Deniz İrtem Kartal[1] , Zafer Yaren[2]*

ÖZET

Bu çalışmada, Van ilinde yayılış gösteren endemik *Astragalus davisii Chamb. & Matthews* bitkisinin tohumlarından elde edilen liyofilize su özütünün, antioksidan aktivitelerinin belirlenmesi amaçlanmıştır. Antioksidan aktivite çalışmaları kapsamında, DPPH radikal süpürme aktivitesi deneyi, toplam fenolik madde miktarı, toplam flavonoid madde miktarı ve metal şelatlama aktivitesi deneyleri uygulandı. Bitki özütünden %8.68 verim elde edildi. *A. davisii* su özütünün DPPH radikal süpürme aktivitesi % 28.80 bulundu. Ayrıca IC_{50} değeri 33.78±0.64 mg/ml; toplam fenolik ve toplam flavonoid madde miktarları sırasıyla 34.52±0.04 µg GA/mg kuru özüt GA eşdeğeri ve 58.33±0.012 µg QE/mg kuru özüt kuersetin eşdeğeri ve 7.71±0.005 µg CE/mg kuru özüt kateşin eşdeğeri; metal şelatlama aktivitesinin inhibisyon değeri % 67.79, IC_{50} değeri ise 3.97±0.05 mg/ml olarak tespit edildi. Elde edilen verilere göre, *A. davisii* tohumunun su özütünün düşük antioksidan aktivite, yüksek şelatlama gücüne sahip olduğu sonucuna varılmıştır.

Antioxidant Activity of Lyophilized Water Extract of Endemic *Astragalus davisii* (Fabaceae) Seed

ABSTRACT

In this study, it was aimed to determine the antioxidant activity of lyophilized water extract obtained from the seeds of the endemic *Astragalus davisii* Chamb. & Matthews plant distributed in Van province. Within the scope of antioxidant activity studies, DPPH radical scavenging activity assay, total phenolic content, total flavonoid content and metal chelating activity assays were performed. A yield of 8.68% was obtained from the plant extract. DPPH radical scavenging activity of *A. davisii* water extract was 28.80%. In addition, IC_{50} value was 33.78±0.64 mg/ml; total phenolic and total flavonoid substance amounts were 34.52±0.04 µg GA/mg dry extract GA equivalent and 58.33±0.012 µg QE/mg dry extract quercetin equivalent and 7.71±0.005 µg CE/mg dry extract catechin equivalent; inhibition value of metal chelating activity was 67.79% and IC_{50} value was 3.97±0.05 mg/ml, respectively. According to the data obtained, it was concluded that the water extract of *A. davisii* seed has low antioxidant activity and high chelating power.

Giriş

Eski çağlardan beri tıbbi bitkilerin tedavi edici etkilerinin olduğu inancı olup her toplumda hastalıkların tedavisi için bir iyileştirme aracı olarak kullanılmışlardır. Sahip olduğu coğrafi konumdan dolayı biyoçeşitlilik bakımından zengin bir ülke konumunda olan Türkiye, yaklaşık 12 bin bitki türü içeren bir floraya sahiptir ve bunlardan 500 kadarı ise tıbbi amaçlı olarak kullanılmaktadır[1]. Böylece bitkilerin halk arasında geleneksel olarak sıkça kullanılması da oldukça yaygındır [2]. Tıbbi bitkilerde bulunan farklı kimyasal bileşenler, insan sağlığını hastalıklardan koruyabilecek biyolojik aktivitelere sahiptir [3]. Bu bitkilerin belirli ölçüde hazırlanmış ve kök, yaprak, çiçek, meyve ve tohum gibi kurutulmuş kısımlarından faydalanılmaktadır Tıbbi bitkilerin tedavi edici kullanımlarının yanı sıra gıda, parfüm, ilaç, kozmetik endüstrilerinde de kullanımları yaygındır [4]. Fabaceae (Baklagiller), dünya genelinde 700 cins ve yaklaşık 21 bin tür ile Orchidaceae ve Asteraceae'den sonra üçüncü büyük çiçekli bitki ailesidir. Bu ailenin tohumları

[1,2]Van Yüzüncü Yıl University, Department of Molecular Biology and Genetics, Van/ Turkey
*Correspondence Author: Zafer Yaren, e-mail: zfryrn.65@gmail.com

yüksek protein içeriğine sahiptir. Belirli türlerinden elde edilen ilaçlar, geleneksel ve modern tıpta halk arasında kullanılmaktadır [5]. İçerdiği 3270 takson ile Astragalus L. cinsi, Fabaceae ailesinin en önemli üyesidir. Astragalus'un dünya genelinde oldukça yaygın bir dağılımı vardır. Tıbbi bitki olarak kullanılan birçok türü mevcuttur [6]. Astragalus cinsine ait bazı bitkilerin farmakolojik özellikleri, sahip oldukları hepatoprotektif, immüno-uyarıcı, antiviral, antikanser ve antioksidan aktiviteleri ile karakterize edilmektedir. Bu cinsin, çiftlik hayvanları ve yabani hayvanlar için yem olarak kullanılmasının yanı sıra, cinse ait bazı türlerin gıdalarda, ilaç sanayinde, kozmetikte ve bitkisel sakız kaynağı olarak kullanıldığı da bildirilmiştir [7]. Tedavi amaçlı kullanılan bitkilerin tedavi edici özellikleri içerdikleri fitokimyasallardan kaynaklanmaktadır. Bitkiler, zararlılara karşı kendilerini savunmak ve nesillerini sürdürmek için fitokimyasal üretirler [8]. Ancak son araştırmalar, insanları hastalıklara karşı koruyabileceklerini göstermektedir. Bitkiler tarafından üretilen fitokimyasalların antimikrobiyal ve antioksidan özellikleri son yıllarda kapsamlı bir şekilde araştırılmıştır [9]. Bu doğrultuda, Fabaceae familyasının 3000'den fazla türü içeren en büyük cinsi olan Astragalus'un çeşitli türlerinin özellikleri üzerine çeşitli araştırmalar yapılmış, bu türlerin sahip olduğu biyoaktif bileşiklerin güçlü antioksidan kapasitesi nedeniyle gıda ve ilaç endüstrilerinde yaygın olarak kullanıldığı bildirilmiştir [10]. Birçok tıbbi bitki alkaloidler, fenolik bileşikler ve terpenler gibi anti-enflamatuar özelliklere sahip fitokimyasal bileşikler içerir [11]. Bitkilerde en fazla bulunan kimyasal gruplardan biri fenolik bileşiklerdir. Fenolik bileşikler, hücreleri serbest radikallere karşı koruyan güçlü antioksidanlardır [12]. Bitkilerin kök, gövde, yaprak, meyve ve tohum gibi çeşitli kısımlarından izole edilen 8 bine yakın fenolik bileşiğin 4 binden fazlasını flavonoidler oluşturmaktadır [13]. Flavonoidlerin antioksidan, anti-kanser, anti-diyabet, anti-aging, hepatoprotektif ve nöroprotektif gibi birçok etkilerinin olduğu bildirilmiştir [3].

Ülkemizde, 425-450 arası Astragalus türü bulunmaktadır. Bunlardan 201-224'ünün endemik olduğu ve önemli bir kısmının da Doğu Anadolu Bölgesi'nde yayılış gösterdiği bildirilmiştir [14]. Van ili Astragalus türlerinin yayılış gösterdiği önemli bir yaşam alanıdır. Astragalus davisii, Van'da yayılış gösteren Astragalus cinsine ait endemik bir bitkidir. Bu çalışmadaki amaç, A. davisii tohumunun liyofilize su özütünün, antioksidan aktivite, metal şelatlama gücü, toplam fenolik ve toplam flavonoid madde miktarlarını belirlemektedir.

Materyal ve Yöntem

Bitki materyalinin toplanması ve teşhisi

Çalışmada kullanılan *A. davisii* bitkisi, Van'ın Bahçesaray İlçesi'nde, türün endemik olması göz önünde bulundurularak olabildiğince titizlikle toplandı (38º08^{I}16.05^{II} N 42º51^{I}18.60^{II} E, 2275 m). Arazide toplanan bitkiler Van Yüzüncü Yıl Üniversitesi (VANF) herbaryumuna getirildi. Bitkilerin tür teşhisi, Van Yüzüncü Yıl Üniversitesi Moleküler Biyoloji ve Genetik Anabilim Dalı Öğretim Üyesi Prof. Dr. Fevzi Özgökçe tarafından yapıldı. Burada bitkilerin herbaryum örnekleri alınarak bitkilere kayıt numaraları (F15514) verildi (Şekil 1).

Şekil 1 *Astragalus davisii* genel görünüşü
Fig 1 General appearance of *Astragalus davisii*

Bitki materyalinin kurutulması ve öğütülmesi

A. davisii'nin tohum kısmı çıkarıldı. Kurutma kâğıdının üzerine yerleştirilerek direk güneş ışığına maruz bırakılmadan gölgede havadar ortamda kurumaya bırakıldı. Bir öğütücü yardımıyla iyice öğütülerek toz haline getirildi. Özütleme işlemi yapılana kadar -20 °C'de saklandı (Şekil 2).

Şekil 2 *A. davisii* tohumunun kurutulması ve öğütülmesi
Fig 2 Drying and grinding *A. davisii* seed

Su özütünün hazırlanması

25 gr *A. davisii* tohumu tartıldı ve üzerine 250 ml deiyonize su (dH$_2$O) eklendi. 40º C ve 650 rpm'e ayarlanmış ısıtıcılı manyetik karıştırıcıda 48 saat karıştırıldı. Süre sonunda şişede bulunan su özütü filtre kâğıdı ile ayrı bir şişeye süzüldü. Süzüntü rotary evaporatörde 18 atm basınçta 37ºC'de uçuruldu ve kalan yoğunlaştırılmış özüt, falkonlara 10 ml olacak şekilde paylaştırıldı. Falkonlar -80ºC'ye alınarak burada bir gece bırakıldı. Ertesi gün liyofilizatörde kurutma işlemi gerçekleştirildi. Liyofilizasyon işleminden sonra kuru özüt tartıldı ve elde edilen özütün verimi hesaplandı. Liyofilize su özütü deneylerde kullanılıncaya kadar kadar -20°C'de muhafaza edildi.
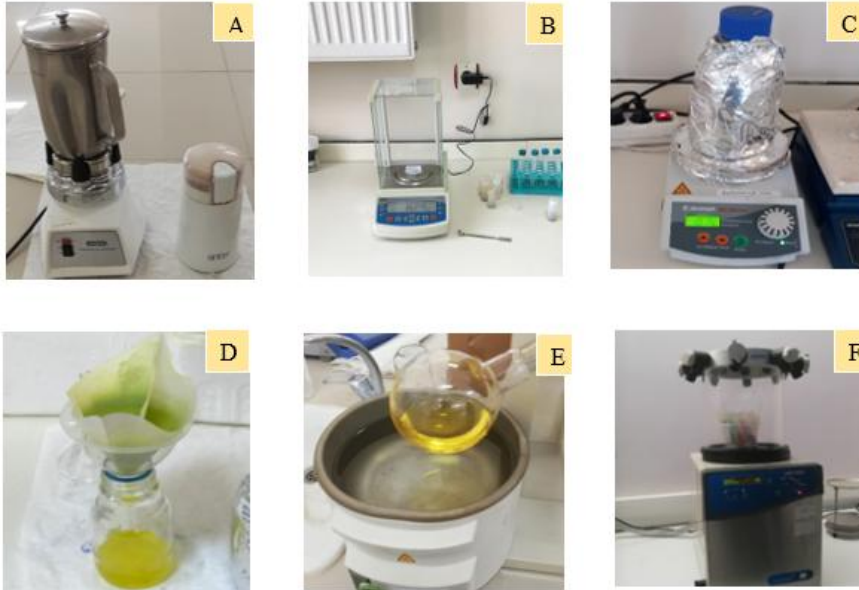
Şekil 3 Ekstraksiyon aşamaları. a) öğütme b) tartma c) özütleme d) süzme e) evaporasyon   f) liyofilizasyon
Fig 3 Extraction stages. a) grinding b) weighing c) extraction d) filtration e) evaporation f) lyophilization

Antioksidan aktivite çalışmaları

DPPH radikal süpürme aktivitesi

DPPH serbest radikal giderme aktivitesinin belirlenmesi, Blois metodunun (1958) modifiye edilmesi ile yapıldı. DPPH (:2,2-difenil-1-pikril hidrazil) giderme aktivitesinin ölçümünde standart olarak kuersetin,

(7.812 µg/ml – 125 µg/ml) ve değişik konsantrasyonlarda *A. davisii* (1 mg/ml-30 mg/ml) ekstraktları hazırlandı. İşlemlerin tamamı 3 tekerrür olarak yapılmıştır.

Toplam fenolik madde miktarı

Toplam fenolik madde miktarları, Singleton ve Rossi (1965)' nin uyguladığı yöntemin modifiye şekline göre belirlendi. Standart olarak gallik asit (GA) (25 µg/ml - 250 µg/ml) ve değişik konsantrasyonlarda *A. davisii* özütü (1 mg/ml - 6 mg/ml) kullanıldı. Absorbans 750 nm'de ölçüldü [15]. Testler 3 tekerrür halinde yapılmıştır.

Toplam flavonoid madde miktarı

Mevcut çalışmada toplam flavonoid miktarı, Zhishen ve arkadaşlarının (1999) modifiye edilmiş yöntemine göre belirlendi. Toplam flavonid madde içeriğinin belirlenmesin standart olarak kuersetin ve kateşin kullanıldı. Farklı konsantrasyonlarda kuersetin ve kateşin (25 µg/ml - 250 µg/ml) ve değişik konsantrasyonlarda (1 mg/mL - 6 mg/ml) liyofilize özütler kullanıldı. 415 ve 510 nm'de absorbans değerleri okundu. Her bir test en az üç kez tekrar edildi.

Metal Şelatlama Aktivitesi

Metal şelatlama aktivitesi modifiye Dinis (1994) metodu ile ölçüldü. Çok güçlü şelatlama aktivitesine sahip olan EDTA pozitif kontrol olarak kullanıldı 562 nm'de absorbans okuması gerçekleştirildi. IC_{50} değeri ile % inhibisyon değeri hesaplandı. Her bir test en az üç kez tekrar edildi.

Bulgular ve Tartışma

Bu çalışmada kullanılan *A. davisii* bitkisinin tohumlarının su özütü verimi % 8.68 olarak tespit edildi (Tablo 1).

Tablo 1 *A. davisii* tohumlarının % özüt verimi
Table 1 % extract yield of *A. davisii* seeds

	Özüt	%Verim
Su özütü	Tohum	8.68 g/g

Tanrıöver [16] yaptığı çalışmada, *Astragalus neurocarpus* BOISS ve *Astragalus. elongatus subsp. nucleiferus* Wılld taksonlarının toprak üstü su özütlerinin verimlerini sırasıyla % 7.67 ve % 6.42 olarak bildirmiştir. Başka bir çalışmada *Astragalus tokatensis*'in toprak üstü su özütünün verimi % 9.50 olarak hesaplanmıştır [17]. Elde ettiğimiz verilerle bu çalışmalardaki veriler, birbirine yakın olmakla birlikte farklılık göstermektedir. Bitkilerin yayılış gösterdiği habitatlar, bitkilerin fitokimyasal bilişimlerine etki ettiğinden dolayı farklı alanlarda yayılış gösteren bitkilerin benzer deney sonuçları farklı çıkabilmektedir.

A. davisii bitki özütünün radikal süpürme aktivitesinin belirlenmesinde DPPH metodu kullanıldı. DPPH, mor renkli bir bileşiktir ve hidrojen (H) atomu verebilen bir maddenin çözeltisi ile karıştırıldığında indirgenerek soluk sarı bir renge dönüşür. Bu metod antioksidanların radikalleri temizleme kapasitesinin spektrofotometrik ölçümlerine dayanmaktadır. Bitkisel özütlerin veya fenolik bileşiklerin antioksidan kapasitelerinin değerlendirilmesinde yaygın olarak kullanılmaktadır [18]. Bu çalışmada *A. davisii* özütünün konsantrasyona bağlı serbest radikal süpürme aktivitesi, yüzde inhibisyon olarak verilmiştir (Şekil 4). Bitki özütünün konsantrasyonu arttıkça DPPH üzerindeki radikal süpürme aktivitesinin de arttığı tespit edildi. *A. davisii* tohumlarının liyofilize su özütünün maksimum konsantrasyonda (20 mg/ml) serbest radikal süpürme aktivitesi % 28.80 olarak tespit edildi.
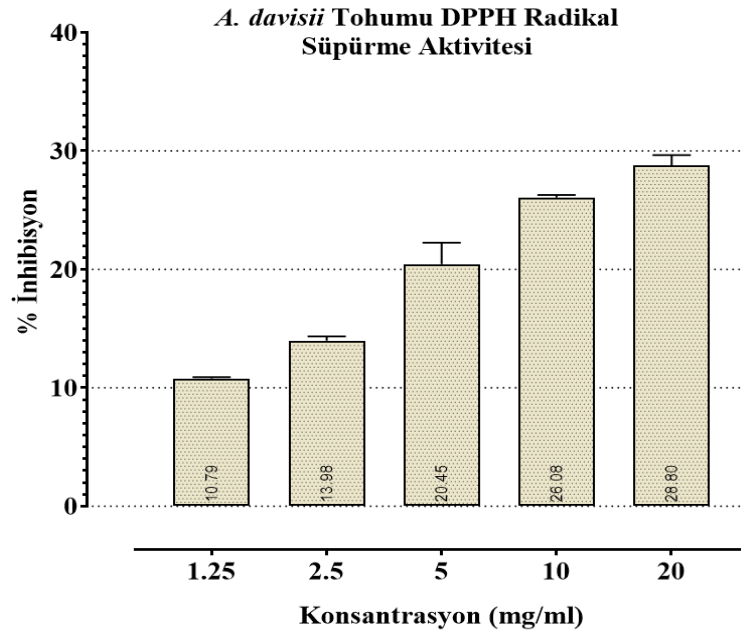
Şekil 4 *A. davisii* liyofilize su özütünün DPPH radikal süpürme aktivitesi
Fig 4 DPPH radical scavenging activity of *A. davisii* lyophilized water extract

Lim ve ark. (2021) yaptıkları bir çalışmada, *A. sinicus* L. tohumunun aseton özütünün antioksidan aktivitesi *in vitro* olarak değerlendirmiş ve 10mg/ml konsantrasyonda radikal süpürme aktivitesini %95 bulmuşlardır [19]. Başka bir çalışmada *Astragalus. tenuifoliosus* tohumunun metanol özütünün radikal süpürme aktivitesi %13 olarak tespit edilmiştir [20]. Başka bir çalışmada ise *Astragalus. compactus* Lam. bitkisinin kök, çiçek ve yapraklarından elde edilen metanol özütlerinin radikal süpürme aktiviteleri 50-300 µg/ml aralığında bildirilmiştir [21].

A.davisii özütünün %50 inhibisyon konsantrasyonuna karşılık gelen IC_{50} değeri Tablo 2'de verilmiş ve 33.78±0.64 bulunmuştur.

Tablo 2 *A. davisii* su özütünün DPPH radikal süpürme aktivitesinin IC_{50} değeri
Table 2 IC_{50} value of DPPH radical scavenging activity of *A. davisii* water extract

| Bitki materyali	IC_{50} konsantrasyonu (mg/ml)
A. davisii su özütü	33.78±0.64
Kuersetin	0.238±1.06

Astragalus cinsine ait taksonların DPPH radikal süpürme değeri birçok çalışmada bildirilmiştir. Bunlar: *A. gombiformis* tohumunun bütanol özütünün DPPH radikal süpürme değeri IC_{50}:244±1.94 µg/ml [22]; Erez ve ark.(2019) *A. guzelsuensis*'in toprak üstü su özütünün IC_{50} değeri 22.2 ± 3.9 mg/ml [23] ve başka bir çalışmada ise *A. tokatensis*'in su özütünün IC_{50} değeri 58,97 mg/L olarak tespit edilmiştir [17]. Elde edilen bu farklı sonuçlar, kullanılan çözücülerin türü, kullanılan farklı bitkiler, kullanılan bitki kısımlarının farklılığı, bitkilerin yetişme koşulları, kullanılan analiz yöntemleri gibi nedenlerden kaynaklanmış olabilir.

Bitkilerde yer alan ve biyolojik olarak aktif olan bileşikler, antioksidan aktivitelerin gerçekleşmesinde önemli bir rol oynamaktadır. Bitkilerin sahip olduğu antioksidan özellikler, içerdikleri fenolik ve flavonoid bileşenler gibi biyoaktif bileşenlerden kaynaklanmaktadır [24]. Bu çalışmada *A. davisii* özütünün toplam fenolik madde miktarının belirlenmesinde Folin-Ciocalteu yöntemi kullanıldı. Standart olarak gallik asit kullanıldı ve gallik asit standart eğrisi oluşturuldu (Şekil 5). *A. davisii* özütünün toplam fenolik madde miktarları 34.52±0.04 µg GA/mg kuru özüt GA eşdeğeri olarak belirlendi.

Bitkilerde doğal olarak bulunan ve fenolik bileşikler grubunda yer alan flavonoidlerin saptanmasında farklı yöntemler vardır. Bu çalışmada flavonoid madde içeriği, alüminyum klorür kolorimetrik yöntemi ile belirlendi. *A. davisii* bitki özütündeki flavonoid madde miktarının belirlenmesinde standart olarak kuersetin ve kateşin eşdeğerleri temel alınarak hesaplandı. Kuersetin ve kateşin standart grafikleri oluşturuldu (Şekil 6). Çalışmamızda *A. davisii* özütünün toplam flavonoid madde miktarı 58.33±0.012 µg QE/mg kuru özüt ve 7.71±0.005 µg CE/mg kuru özüt olarak tespit edildi (Tablo 3).
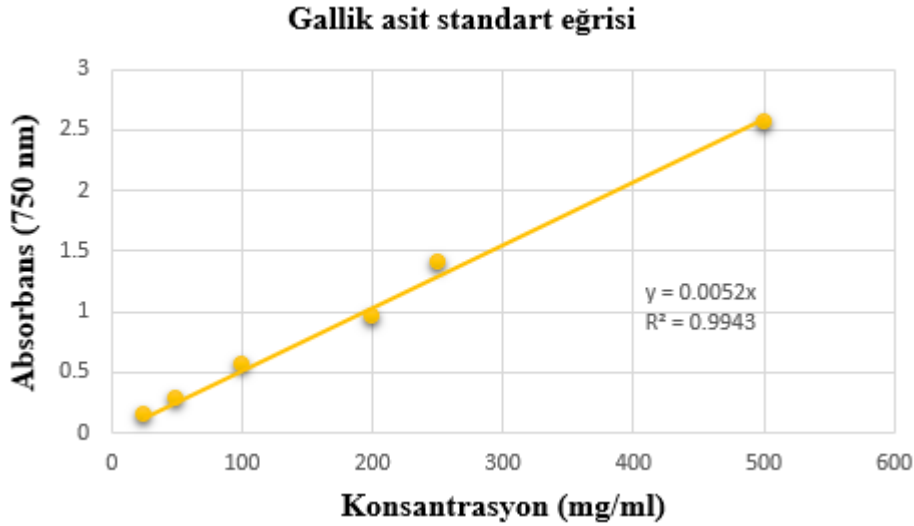
Şekil 5 Gallik asit standart grafiği
Fig 5 Gallic acid standard graph
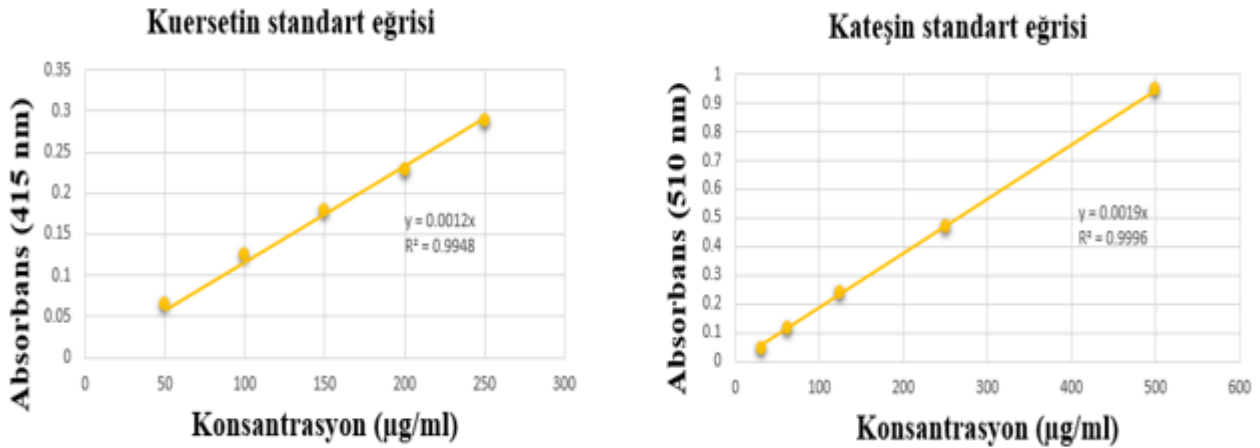
Şekil 6 Kuersetin ve kateşin standart grafikleri
Fig 6 Quercetin and catechin standard graphs

Tablo 3 *A. davisii* su özütünün toplam fenolik ve toplam flavonoid madde içerikleri
Table 3 Total phenolic and total flavonoid content of *A. davisii* water extract

Bitki özütü	TFM(µg GAE/mg)	TFQ (µg QE/mg)	TFC (µg CE/mg)
Tohum su	34.52±0.04	58.33±0.012	7.71±0.005

*TFM (Toplam fenolik madde), TFQ (kuercetin eşdeğeri toplam flavonoid), TFC (kateşin eşdeğeri toplam flavonoid)

Elde edilen sonuçlara dayanarak, liyofilize su özütünün toplam fenolik madde miktarı gallik asitten hazırlanan kalibrasyon eğrisine göre (y = 0,0052x, R^2 = 0,994) hesaplanırken, toplam flavonoid madde

miktarı kuersetinden (y = 0,0012x, R^2 = 0,994) ve kateşinden (y = 0,0019×, R^2 = 0,999) hazırlanan kalibrasyon eğrisine göre hesaplanmıştır. Toplam fenolik ve flavonoid madde miktarlarının belirlendiği bir çalışmada *Astragalus gombiformis* tohumunun bütanol özütünün toplam fenolik ve toplam flavonoid madde miktarları sırasıyla 52, 23 ± 2, 29 µg GAE/ mg ve 33,47 ± 2,65 µg QE/mg bulunmuştur [25]. Başka bir çalışmada *Astragalus armatus* tohumunun petrolyum eter özütünün toplam fenolik ve flavonoid madde miktarları sırasıyla 6.5 ± 0.01 mg GAE/ g kuru özüt ve 5.15 ± 0.52 mg QE/g kuru özüt bulunmuştur [26]. Başka bir çalışmada da *Astragalus sinicus* L. tohumunun su özütünün toplam fenolik ve toplam flavonoid madde miktarları sırasıyla 67.8± 3.0 mg GAE/ g ve 51.7±1.6 mg QE/g bildirilmiştir [19]. Literatür çalışmaları ile çalışmamızdan elde edilen sonuçlar arasındaki farklılıklar, çalışılan bitkilerin farklı türlerde olması, bitkilerin yetişme koşulları, kullanılan analiz yöntemi, bitki türlerinin farklı miktarlarda etken madde içermeleri, kullanılan çözücü vb. gibi nedenlerden kaynaklanmış olabilir.

Metal şelatlama yeteneği, lipid peroksidasyonunda katalitik etki gösteren metal konsantrasyonunu düşürdüğü için son derece önemlidir. Ayrıca, metal şelatlama ajanları redoks potansiyelini düşürdükleri ve böylece oksitlenmiş metal iyonlarını stabilize ettikleri için ikincil antioksidanlar olarak kabul edilirler. Antioksidanların, metal bağlama gerçekleştiren fonksiyonel grupları nedeniyle etkili bir Fe bağlama yeteneğine sahip oldukları bildirilmiştir [27]. Bu çalışmada *A. davisii*'nin farklı konsantrasyonlarındaki (0.5 mg/ml-15mg/ml) özütünün metal şelatlama aktivitesi ölçüldü ve elde edilen sonuçlar % inhibisyon olarak gösterildi. Konsantrasyon artışına bağlı olarak metal şelatlama aktivitesinin arttığı tespit edildi (Şekil 7). % inhibisyon değeri maksimum konsantrasyonda (10 mg/ml) % 67.79 bulundu. Ayrıca *A.davisii* özütünün %50 inhibisyon konsantrasyonuna karşılık gelen IC$_{50}$ değeri hesaplandı ve 3.97±0.06 bulundu. Standart EDTA'nın IC$_{50}$ değeri ise 0.234±0.54 olarak hesaplandı (Tablo 4).
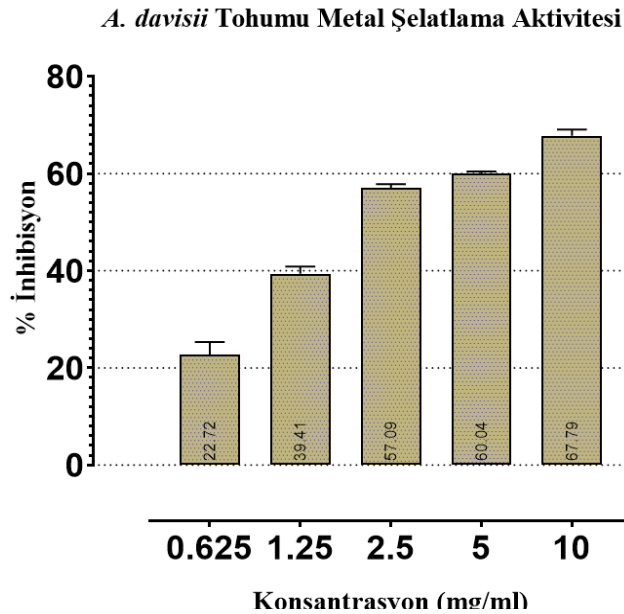
Şekil 7 *A. davisii* tohum özütünün metal şelatlama aktivitesi
Fig 7 Metal chelating activity of *A. davisii* seed extract

Lim ve ark. (2011)'de yaptığı bir çalışmada A.sinicus L tohumunun su özütünün metal şelatlama aktivitesini % 55 bulmuştur [19]. Başka bir çalışmada A. argaeus'un toprak üstü metanol özütünün metal şelatlama aktivitesi %55.19 bulunmuştur [28]. Metal şelatlama aktivitesinin IC$_{50}$ değerinin incelendiği bir çalışmada A. macrocephalus subsp. finitimus bitkisinin yaprak metanol özütünün IC$_{50}$ değeri 1.65 ± 0.05 bulunmuştur [29]. Başka bir çalışmada ise A. brachycalyx'ın etanol özütünün IC$_{50}$ değeri 5.095 ± 0.043 bulunmuştur [27].

Tablo 4. *A. davisii* su özütünün metal şelatlama aktivitesinin IC$_{50}$ değeri
Table 4 IC$_{50}$ value of metal chelating activity of *A. davisii* water extract

Bitki materyali	IC$_{50}$ konsantrasyonu (mg/ml)
A. davisii su özütü	3.97±0.05
EDTA	0.234±0.54

Yukarıdaki çalışmalarda, *Astragalus*'un farklı türlerinin farklı kısımlarından, farklı çözücüler kullanılarak birbirinden farklı sonuçlar elde edilmiştir. Buradan bitkilerin farklı kısımlarının farklı metal şelatlama aktivitesi gösterdiği söylenebilir. Ayrıca farklı çözücülerin kullanılması elde edilen farklı sonuçların nedeni olabilir.

Sonuç

Türkiye'de doğal olarak yayılış gösteren bitkilerin biyolojik etkinliklerinin, tıbbi amaçlarla potansiyel kullanımlarının belirlenmesi büyük bir önem taşımaktadır. Bu sebeple, bu bitkilerdeki aktif bileşenlerin analiz edilmesi, tespit edilen bileşenlerin ilaç hammaddesi olarak farmasötik sektörde kullanılması ve nihayetinde bitkisel tedavi seçeneklerinin geliştirilmesi önem arz etmektedir. Eski çağlardan beri tıbbi bitkiler, hastalıkların tedavisi için bir iyileştirme aracı olarak kullanılmışlardır. Tıbbi bitkilerin kullanımı, ülkelerin gelişmişlik düzeyine göre farklılık gösterse de oldukça yaygın bir kullanım göze çarpmaktadır. Tıbbi bitkiler üzerinde daha fazla çalışmalar, çeşitli kimyasal bileşiklerin sonsuz bir rezervuarı olarak hizmet eden bitkilerdeki yeni biyoaktif ürünlerin keşfedilmesine olanak sağlayacaktır. Türkiye, biyoçeşitlilik bakımından zengin bir ülke konumunda olup birçok tıbbi bitkiye ev sahipliği yapmaktadır. Bu tıbbi bitkilerden bazıları Fabaceae (Baklagiller) ailesinin en önemli üyesi olan *Astragalus* cinsine ait taksonlardır. *Astragalus* cinsine ait bazı taksonların farmakolojik özellikleri, immüno-uyarıcı, antiviral, antikanser ve antioksidan aktiviteleri ile karakterize edilmektedir. Bu çalışma, Van ilinde yayılış gösteren endemik *A. davisii* tohumunun su özütünün radikal süpürme aktivitesi, toplam fenolik madde miktarı, toplam flavonoid madde miktarı ve metal şelatlama aktivitesinin belirlenmesi için yapılan ilk çalışmadır. Çalışmada elde edilen verilere göre, *A. davisii* tohumunun su özütünün, karşılaştırılabilir seviyede antioksidan aktiviteye sahip olduğu tespit edildi. *A. davisii* tohumunun su özütünün antioksidan aktivitesinin literatür çalışmalarına göre düşük olması, *A. davisii* tohumlarının su özütünün fenolik madde miktarı bakımından düşük olmasından kaynaklanmaktadır. Bitki özütünün metal şelatlama aktivitesi yüksek bulunmuştur. *A. davisii* tohumunun su özütü, iyi bir metal şelatlayıcı ajan olarak kabul edilebilir. Bu çalışmanın *Astragalus* tohumları ile ilgili yapılacak ileriki çalışmalara ışık tutacağı düşünülmektedir.

Data Availability statement / Veri Kullanılabilirliği bildirimi
The author confirms that the data supporting this study are cited in the article.
Yazar, bu çalışmayı destekleyen verilere makalede atıfta bulunulduğunu onaylamaktadır.
Compliance with ethical standards / Etik standartlara uyum
Conflict of interest / Çıkar çatışması
The author declare no conflict of interest.
Yazar herhangi bir çıkar çatışması beyan etmemektedir.
Ethical standards / Etik standartlar
The study is proper with ethical standards.
Çalışma etik standartlara uygundur.
Authors' contributions / Yazar katkıları
All authors contributed equally to the article. All authors have reviewed and approved the manuscript.
Makalede adı geçen tüm yazarlar makaleye eşit oranda katkı yapmışlardır. Tüm yazarlar makaleyi incelemiş ve onaylamışlardır.

Kaynaklar

1. Göktaş, Ö. ve Gıdık, B, Tıbbi ve Aromatik Bitkilerin Kullanım Alanları. Bayburt Üniversitesi Fen Bilimleri Dergisi, 2019. 2(1): s. 145–151
2. Kültür, Ş. et al., Türkiye'de mide ülserinde kullanılan tıbbi bitkiler. Marmara Pharm J, 2018. 22 (1): p. 1–14.

3. Li, Y. et al., The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol. Biochem, 2020. 148: p.80-89

4. Temel, M. et al., Dünyada ve Türkiye'de Tıbbi -Aromatik Bitkilerin Üretimi ve Ticareti," Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Derg, 2018. 21: p. 198–214.

5. Koçak, S., Cytisus (Fabaceae) cinsinde yer alan taksonların karyolojileri üzerine bir çalışma. Yüksek lisans tezi, 2022. Necmettin Erbakan Üniversitesi, Fen Bilimleri Enstitüsü, Konya.

6. Klichkhanov, N.K and M. N. Suleimanova, Chemical Composition and Therapeutic Effects of Several Astragalus Species (Fabaceae). Doklady Biological Sciences, 2024. 518 (4): p. 172-186.

7. Linnek, J. et al., Cycloartane glycosides from three species of Astragalus (Fabaceae). Helv. Chim. Acta, 2011. 94(2): p. 230-237.

8. Mulabagal, V. and H. Tsay, Plant Cell Cultures - An Alternative and Efficient Source for the Production of Biologically Important Secondary Metabolites. International Journal of Applied Science and Engineering, 2004. p. 29–48.

9. Kashani, H. et al., Pharmacological properties of medicinal herbs by focus on secondary metabolites. Life Science Journal, 2012. 9(1): p. 509-520.

10. Lekmine, S. et al., "Preliminary Investigation of Astragalus arpilobus subsp. hauarensis: LC-MS/MS Chemical Profiling, In vitro Evaluation of Antioxidant, Anti-Inflammatory Properties, Cytotoxicity, and In Silico Analysis against COX-2," Antioxidants, 2024. 13(6).

11. Kadi, K. et al., Evaluation of the anticoagulant activity of margins from olives extraction in the Khenchela region. Journal of Fundamental and Applied Sciences, 2020. 12(2): p. 634-649.

12. Kulbat, K., Biotechnology and Food Sciences The role of phenolic compounds in plant resistance.Biotechnol Food Sci, 2016. 80 (2): p. 97–108.

13. Kaya, O., Bazı Astragalus L. taksonlarının fenolik bileşik ve biyoaktivitelerinin karşılaştırmalı incelenmesi.Yüksek lisans tezi, 2016. Erciyes Üniversitesi,Türkiye.

14. Başbağ, M. et al., Türkiye florasında yer alan endemik Astragalus taksonları, 2018. In International Congress on Agriculture and Animal Sciences, Antalya.

15. Vuran, N., D.İ. Kartal, and İ. Çelik, Antiproliferative activity of Malus sylvestris Miller against HepG2 cell line with their antioxidant properties and phenolic composition. Turkish Journal of Biochemistry, 2023. 48(3): p. 272-281.

16. Tanrıöver, C., Bazı Astragalus türlerinin fitokimyasal içeriklerinin ve antioksidan aktivitelerinin belirlenmesi,sağlıklı ve kanserli hücre hatları üzerinde antikanser ve antiproliferatif kapasitelerinin araştırılması. Yüksek lisans tezi, 2019. Kilis 7 Aralık Üniversitesi, Fen Bilimleri Enstitüsü, Kilis.

17. Kök, Ö., Bazi Endemik Bitki Türlerinin Farklı Ekstraktlarinin Biyolojik Aktivitelerinin İncelenmesi. Yüksek lisans tezi, 2022. Karamanoğlu Mehmetbey Üniversitesi, Fen Bilimleri Enstitüsü, Karaman.

18. Gulcin, I. and S. H. Alwasel, DPPH Radical Scavenging Assay. Processes, 2023. 11(8).

19. Lim, D.H. et al., Effect of Astragalus sinicus L. seed extract on antioxidant activity, J. Ind. Eng. Chem, 2011. 17 (3): p. 510-516.

20. Mahmoudi, M. et al., Phytochemical profile, antioxidant properties and protein contents of Astragalus tenuifoliosus seeds. Journal of the Mexican Chemical Society, 2023. 67(1): p. 24-32.

21. Naghiloo, S. et al., Ontogenetic variation of total phenolics and antioxidant activity in roots, leaves and flowers of Astragalus compactus Lam. (Fabaceae)," BioImpacts, 2012. 2 (2): p. 105-109.

22. Lekmine, S. et al., Investigation of photoprotective, anti-inflammatory, antioxidant capacities and lc–esi–ms phenolic profile of astragalus gombiformis pomel, Foods, 2021. 10 (8): p. 1-15.

23. Erez, M.E. et al., Comprehensive appraisement of antioxidant potential and phytochemical profile of native botanicals from Turkey. Journal of Food Measurement and Characterization, 2019. 13: 3230-3241.

24. İşbilir, Ş. and E. Çelik, Edirne ilinde yetişen dut ağacı yapraklarının antioksidan ve antidiyabetik aktivitesi üzerine bir çalışma. Journal of Advanced Research in Natural and Applied Sciences, 2021. 7(3): p. 319-332.

25. Lekmine, S. et al., A comparative study on chemical profile and biological activities of aerial parts (stems, flowers, leaves, pods and seeds) of Astragalus gombiformis. Biocatalysis and Agricultural Biotechnology, 2020. 27, 101668.

26. Mahmoudi, M. et al., Characterization of lipids, proteins, and bioactive compounds in the seeds of three Astragalus species. Food Chemistry, 2021. 339, 127824.

27. Kızıltaş, H. et al., LC-HRMS profiling of phytochemicals, antidiabetic, anticholinergic and antioxidant activities of evaporated ethanol extract of Astragalus brachycalyx Fischer. J. Chem. Metrol, 2021. 15(2): p. 135-151.

28. Albayrak, S. and O.Kaya, Antioxidant, antimicrobial and cytotoxic activities of endemic Astragalus argaeus Boiss. from Turkey. Hacettepe Journal of Biology and Chemistry, 2019. 47(1): p. 87-97.

29. Sarikurkcu. C. and G. Zengin, Polyphenol profile and biological activity comparisons of different parts of Astragalus macrocephalus subsp. finitimus from Turkey. Biology, 2020. 9(8): p. 231.

İşgal Altındaki Vakıfbelören Köyü Merası İçin Tahsis Amacı Değişikliği İsteminin Vejetasyon ve Sosyolojik Etkileri Bağlamında İrdelenmesi

Ferat Uzun[1*] (ID) **, Fatih Gürel[2]** (ID) **, Fatih Kumbasar[3]** (ID) **, Ömer Faruk Uzun[4]** (ID)

ÖZET

Çalışma ile kadimden beri mera olarak kullanılan Vakıfbelören köyü merasının Orman Genel Müdürlüğü tarafından işgali ve mevcut fiili durumu bir şekilde meşru hale getirme isteği irdelenmiştir. Elde edilen bulgulara göre 181/5 numaralı mera parselinin tamamı, 181/7 numaralı parselin ise % 50'si işgal altında olup, üzerindeki tüm bitki örtüsü tahrip edilmiş, mevcut haliyle mera olarak kullanım imkânı kalmamıştır. Tapu ve Kadastro ve Tarım İl Müdürlüğü'nün kayıtlarına göre arazinin niteliği mera, Orman Genel Müdürlüğü'nün E-Harita Uygulaması'nda ise "ağaçsız orman alanı"dır. Orman Müdürlüğü'nün bu bakış açısına göre, 4342 sayılı mera kanunu kapsamında mera olarak tescillenen alanların neredeyse tamamı, potansiyel olarak gelecekte orman sınırları içerisine alınabilecek hedef alanlardır. Bu anlayış nedeniyle meralar, on yıllardır süreklilik arz eden bir şekilde Orman Müdürlüğü tarafından şu veya bu şekilde bu çalışma konusunda olduğu gibi orman sınırları içerisine alınmaktadır. İşgal edilmeyen mera alanında lup yöntemiyle yapılan vejetasyon etüdü çalışması bulgularına göre azalıcı bitki türlerinin oranı % 27.00, çoğalıcı türlerin ise % 7.50'dir. Buna göre mera durumu "orta" dır. Bunun anlamı meranın bitki örtüsünün kalitesi, asgari olması gereken miktarın üzerinde olup verimliliği, çeşitli amenajman düzenlemeleriyle artırılabilme potansiyeline sahiptir.

Discussion of the Request for Change of Allocation for the Occupied Vakıfbelören Village Rangeland

ABSTRACT

The study examines the occupation of the Vakıfbelören village rangeland, which has been used as rangeland since ancient times, by the General Directorate of Forestry and the desire to somehow legitimize the current de facto situation. According to the findings obtained, the entire rangeland parcel numbered 181/5 and 50% of the 181/7 were occupied, all vegetation on them has been destroyed, and there was no possibility of using them as rangeland in their current state. According to the records of the Land Registry and Cadastre and the Provincial Directorate of Agriculture, the nature of the land is rangeland, and in the E-Map Application of the General Directorate of Forestry, it is "treeless forest area". According to this perspective of the Forestry Directorate, almost all of the areas registered as rangelands within the scope of the rangeland law numbered 4342 are target areas that could potentially be included within the borders of the forestry in the future. Due to this perspective, rangelands have been included within the forest land by the Forestry Directorate for decades, as in the subject of this study. According to the findings of the vegetation study conducted with the loop method in the unoccupied rangeland area, the rate of decreaser plant species were 27.00% and the increasers were 7.50%. Accordingly, the rangeland condition was "medium". This means that the quality of the rangeland's vegetation is above the minimum required amount and its productivity has the potential to be increased with various management arrangements.

[1] Ondokuz Mayıs Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü, Samsun, Türkiye
[2] Tarım ve Orman İl Müdürlüğü, Kastamonu, Türkiye
[3] Karadeniz Tarımsal Araştırma Enstitüsü Müdürlüğü, Samsun, Türkiye
[4] Sinop Üniversitesi, Boyabat Meslek Yüksekokulu, Sinop, Türkiye
*Corresponding Author: Ferat Uzun, e-mail: fuzun@omu.edu.tr

Giriş

Orman Bakanlığı'nın olmadığı zamanlarda hayvan otlatmak suretiyle kullanılan ve ancak bitki süksesyonu sebebiyle bitki örtüsü içerisinde belirli oranlarda çalı-ağaç varlığına sahip olan çayır ve mera alanları, 1969 yılı itibarıyla Orman Bakanlığı'nın kuruluşuna müteakip mera olmaktan çıkarılarak orman-fundalık sahası kayıtlarına geçmiştir. Bu süreçte bitki varlığı olarak hiçbir değişikliğe uğramayan bu alanlar, sadece arazi sınıflamasından ileri gelen değişiklikle Orman Bakanlığı'na verilmiştir. Orman alanları, o günden bu yana çayır ve meralardan alan kazanımıyla sürekli artış göstermiştir. Orman Genel Müdürlüğü verilerine göre ülkemizin orman varlığı 1973 yılı itibarıyla 20199296 ha iken, 2023 yılı itibarıyla 23363071 ha'a ulaşmıştır. Orman alanlarında son 50 yıl içerisinde 3045704 ha'lık bir artış meydana geldiği görülmektedir [1].

Mülga Köy Hizmetleri Genel Müdürlüğü'nün 1970 yılı kayıtlarında 21698400 ha olarak bildirilen ülkemiz çayır ve mera alanları, Mera Kanunu çerçevesinde yapılan çalışmalardan elde edilen verilere göre 2024 yılı itibarıyla 13147701 ha'a düşmüştür [2]. Bu rakamlara göre çayır ve mera alanları geçen 53 sene zarfında yaklaşık % 40 oranında azalmıştır.

Ülkemiz çayır ve mera alanlarının hukuki vasfının değiştirilerek orman sınırları içerisine alınması suretiyle orman varlığında sağlanan bu artışta, 2 temel uygulamadan söz etmek mümkündür. Bunlardan birincisi erozyona maruz kalan ve ıslahının mümkün olmadığı kanaatine varılan mera alanlarında erozyonun önlenmesi amacıyla toprak muhafaza amaçlı ağaçlandırma çalışmaları nedeniyle 4342 sayılı Mera Kanunu'nun 3/14. Maddesi [Değişik:3/7.2005-5403/27 md.] ve Mülga Gıda Tarım ve Hayvancılık Bakanlığı ile Orman ve Su İşleri Bakanlığı arasında "Ağaçlandırma Seferberliği" kapsamında yapılacak ortak çalışmalara ilişkin 2012 yılında imzalanan protokol gereğince "tahsis amacı değişikliği yapılarak bir kısım mera alanlarının orman kayıtlarına geçmesidir [3]. İkincisi ise, kadimden beri mera olarak kullanılan ve ancak mera tespiti çalışmaları esnasında bildirimde ihmal edilen ve hatta bazılarının Osmanlıca evrakları olduğu halde bu evraklar okunamadığından mera olarak kayıtlara alınmada eksik kalan mera alanlarının Orman Genel Müdürlüğü'nce orman kayıtlarına geçirilmesidir [4, 5, 6, 7]. Mera Kanunu'nun 14. maddesi gereğince çeşitli sebeplerle başka amaçlarla kullanılmak üzere diğer kurumlara verilmek suretiyle de mera alanları kayıp yaşamaktadır. Ancak en büyük kayıp orman sınırları içerisine alınmak suretiyle yaşanmaktadır. Bu nedenle mera hayvancılığı yapan insanlarımız veya Tarım İl Müdürlükleri tarafından Orman Müdürlüğü'nün bu türlü tasarrufları zaman zaman mahkemelere taşınmakta, zaman zaman da Orman Müdürlüğü meralarda otlayan keçi sayısının azalması ve diğer bazı sebeplerle üzerinde ağaç varlığı artan meraları, sınırlarına katmak için mahkemelere başvurmaktadır. Ancak istatistiklerden anlaşıldığı kadarıyla mera kayıplarının önüne bir türlü geçilememektedir. Mera Komisyonu kararlarında ve mahkemelere intikal etmiş davalarda, meralara ilişkin bir takım mevzuat eksiklikleri yanında, meralara oranla kamuoyunun daha çok ilgisine mazhar olan ormanların lehine olacak şekilde pozitif ayrımcılığın da bunda etkisi olduğu söylenilebilir.

Sonuç olarak son 50 yıllık süreçte mera alanları şu ya da bu sebeplerle -istisnalar hariç- sürekli orman alanlarına dahil edilmiş ve oldukça yüksek bir kayıp yaşamıştır. Bu kayıp ta tüm ülkemiz genelinde mera hayvancılığında gerileme olarak kendini göstermiştir.

Bu çalışmanın amacı, kadimden beri mera olarak kullanılan dolayısıyla mera olarak tescil edilen, ancak üzerine betonarme bina yapımı ve odun deposu olarak kullanmak suretiyle Orman Müdürlüğü tarafından işgal edilen mera parsellerinin, Mera Kanunu'nun 14/c maddesindeki "Kamu yatırımları yapılması için gerekli bulunan" ifadesine atfen mevcut fiili durumuna meşruiyet kazandırma istemini irdelemektir.

Materyal ve Metot

Orman Müdürlüğü tarafından tahsis amacı değişikliği istenilen mera alanı, Kastamonu ili, Taşköprü ilçesi, Vakıfbelören köyüne tahsisli 181/5 ve 181/7 numaralı parsellerdir. 181/5 numaralı parselin tapu alanı 99 da ve pafta numarası: E32d25c'dir. 181/7 numaralı parselin tapu alanı ise 129.23 da'dır [8]. Parsellerin ortalama rakım değeri 650 metredir. İşgal altındaki 181/5 ada/parsel numaralı parsel üzerine Orman Müdürlüğü tarafından betonarme bina yapılmış ve kalan kısmı ise odun deposu olarak kullanmak suretiyle toprak yüzeyindeki bitki örtüsü tamamen tahrip edilmiş, ne otsu ne de çalı-ağaç türleri adına hiçbir bitkisel varlık kalmamıştır. 181/7 numaralı parselin ise yaklaşık % 50'si işgal altında olup onun da yüzeyindeki tüm bitki örtüsü tahrip edilmiş mera olarak kullanım imkânı kalmamıştır. 181/7 numaralı parselin kalan % 50'lik kısmı üzerinde 15.05.2024 tarihinde tekerlekli lup aleti kullanılarak 3 farklı hat üzerinde 400 okuma yapılarak yaprak alanı esasına göre vejetasyon etüdü yapılmıştır. Etüt neticesinde tespit edilen bitki türleri azalıcılar, çoğalıcılar ve istilacılar şeklinde sınıflandırılmıştır. Azalıcı ile çoğalıcı bitki türlerin oranları toplanarak mera durumu belirlenmiştir [Şekil 1, Tablo 1] [9].
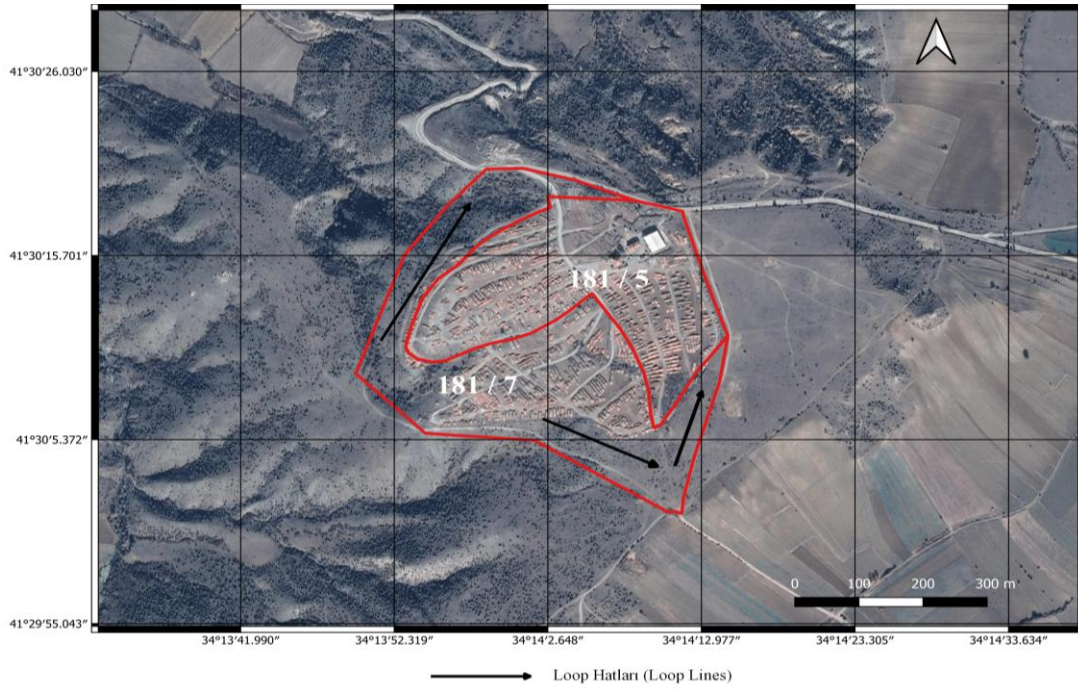
Şekil 1 Çalışılan mera alanının ve loop hatlarının görüntüsü
Fig 1 Satellite image and loop lines of the studied area

Vakıfbelören köyüne tahsisli 181/5 ve 181/7 numaralı parsellerin işgal edilmemiş kısımlarından 0-30 cm derinliğinden mera alanını temsil edecek şekilde 20 adet toprak numunesi alınmıştır. Numunelerin Kastamonu İl Özel İdaresi, toprak laboratuvarında yapılan analiz sonuçlarına göre; pH değeri 7.3 ile nötr civarında, toplam tuz [%0.01] ve organik madde [%0.79] miktarı ise çok azdır. Yarayışlı fosfor [P_2O_5] [0.22 kg/da] çok az, yarayışlı potasyum [K_2O] [49.08 kg/da] çok yüksek, killi-tınlı [Saturasyon %55.99] bünyelidir. Parseller ortalama %3-5 eğimli, toprak derinliği sığ ya da çok sığdır [10].

Bulgular ve Tartışma

Vejetasyon etüdü çalışması

181/7 numaralı mera parselinin işgal edilmemiş kısmının zemininde yapılan vejetasyon etüdü çalışması neticesinde elde edilen verilere göre toprak yüzeyi % 85.5 oranında bitki ile örtülü, % 14.5'u ise çıplak alandır. Bu değer toprağın her türlü aşındırıcı etkiye karşı yeterince mukavemete sahip olduğunun ifadesidir [11]. Otlatma takvimine uyulması ve meranın kapasitesine dikkat edilerek otlatılması ve gübreleme gibi uygulamalar ile örtü derecesinin daha da artırılması imkân dâhilindedir. Vejetasyonda 34 adet bitki türüne rastlanılmıştır [Tablo 1]. Vejetasyonunda belirlenen bitki türlerinin kalite derecelerine göre dağılımına göre; 6 adedi [% 27.00] azalıcı, 3 adedi [% 7.50] çoğalıcı ve 25 adedi [% 65.50] istilacıdır. Tablo 1'de verilen sınıflamaya göre parselin işgal edilmeyen bu kısmı "Orta" kalitede mera durumunu ifade eden bitki örtüsüne sahiptir [9]. Çalışılan alanda bitki örtüsü içerisindeki oranları itibarıyla öne çıkan azalıcı türler sırasıyla *Chrysopogon gryllus* [% 17.25] ve *Bothriochloa ischaemum* [% 7.25] olurken, çoğalıcı türler ise sırasıyla *Festuca ovina* [% 5.50] ve *Teucrium polium* [% 1.25] olmuştur. Alanda tespit edilen otsu yabancı otlar içerisinde en fazla rastlanılanları *Blysmus compressus* [% 10.50], *Brachypodium distachyon* [% 9.50] ve *Pilosella echioides* [% 5.50] olmuştur [Tablo 1].

Elde edilen verilere göre meranın; otlatma takvimine uyma, doğal tohumlama amacıyla münavebeli otlatma, temizlik biçimi, keçi ile istilacı bitkiler ile biyolojik mücadele ve gübreleme ile verimliliği artırılabilir. Özellikle vejetasyonda bulunan azalıcı ve çoğalıcı bitki türlerinin daha çok çiçek dalı ve tohum oluşturmalarını teşvik edebilecek gübreleme programı ile mera üretkenliği artırılabilir [12, 13, 14].

Tapu ve Kadastro Genel Müdürlüğü, Orman Genel Müdürlüğü ve Tarım ve Orman Bakanlığı'na göre mera parselleri

Kastamonu Tarım İl Müdürlüğü kayıtlarında çalışılan meranın 5/a kaynaklı, yani kadimden beri mera olarak kullanılan alan olarak tescil edilmiştir [15]. Şekil 2'de Tapu ve Kadastro Genel Müdürlüğü'nün "Parsel Sorgu" uygulaması [8] ile Şekil 3'te Avrupa Çevre Ajansının belirlediği kriterleri dikkate alarak Tarım İl

Müdürlüğü'nün yürüttüğü proje kapsamında yapılan "arazi örtüsü/kullanımı sınıflandırması"nda [16] Vakıfbelören köyüne hayvan otlatmaları amacıyla tahsis edilen bu alanlar mera olarak gösterilmiştir.

Tablo 1 181/7 numaralı mera parselinin işgal edilmemiş kısmındaki bitki örtüsünü oluşturan türler [%]
Table 1 Species in the vegetation in the unoccupied part of the rangeland parcel number 181/7 [%]

Azalıcı Bitki Türleri [%]			
Chrysopogon gryllus	17.25	*Sanguisorba minor*	1.25
Bothriochloa ischaemum	7.25	*Medicago falcata*	0.50
Dactylis glomerata	0.50	*Poa pratensis*	0.25
	Toplam		27.00
Çoğalıcı Bitki Türleri [%]			
Festuca ovina	5.50	*Teucrium polium*	1.25
Dorycnium graceum	0.75		
	Toplam		7.50
		Azalıcı + Çoğalıcı Tür Toplamı	**34.50**
İstilacı Bitki Türleri [%]			
Blysmus compressus	10.50	*Astragalus adustus*	0.50
Brachypodium distachyon	9.50	*Astragalus bicolor*	0.50
Pilosella echioides	5.50	*Astragalus canescens*	0.50
Teucrium chamaedrys	3.25	*Chondrilla juncea*	0.50
Eryngium campestre	2.50	*Convolvulus calvertii*	0.50
Helianthemum nummularium	2.50	*Euphorbia helioscopia*	0.50
Potentilla recta	2.25	*Minuartia anatolica*	0.50
Linum hypericifolium	2.00	*Plantago lagopus*	0.25
Globularia orientalis	1.75	*Juniperus communis*	8.75
Muscari armeniacum	1.75	*Rhamnus hirtellus*	3.25
Thymus longicaulis	1.75	*Rosa canina*	3.00
Trifolium hirtum	1.00	*Fumana arabica*	1.50
Veronica arvensis	1.00		
		İstilacı Türlerin Toplamı	**65.50**
		Genel Toplam	**100.00**

Bu gösterim şekli, diğer köyler adına tahsis edilen mera parselleri için de aynıdır. Bu tanımlama oldukça sorunludur. Orman Müdürlüğü'nün bu bakış açısına göre 4342 sayılı Mera Kanunu'na göre mera olarak tescillenen mera alanlarının neredeyse tamamı, potansiyel olarak gelecekte Orman Genel Müdürlüğü'nün sınırları içerisine alınabilecek hedef alanlardır. Mera Komisyonlarında da bu görüş çok bariz olarak kendini göstermekte diğer birçok kurumdan gelen üyelerin mera olarak görüş bildirdikleri birçok kararda, sadece Orman Müdürlüğü temsilcisinin karara şerh düştüğü görülmektedir. Örneğin Zonguldak İl Mera Komisyonu'nun 24.10.2014 tarihli, 2014/19 numaralı karar defterinde, Yenidoğancılar köyü 152 ada/45 numaralı parsel için kadim mera alanları için zaman aşımı uygulanamayacağına, zemindeki bitki örtüsünde yıllar itibarıyla değişiklik olsa da bu değişikliğin o yerin kadim mera vasfını kaybettirdiği anlamına gelmediği yönündeki kararı, diğer kurumlardan gelen 8 üyenin lehte, Orman Müdürlüğü temsilcisinin ise aleyhte oyları ile kabul edilmiştir. Aynı komisyonda görüşülen Yazıcık, Nizamlar, Yalnızçam köylerinin meraları için de aynı veya benzer sebeplerle Orman Müdürlüğü'nün temsilci üyesi karşı oy kullanmış ve kararlar "oy çokluğu" ile alınmıştır [5]. Kastamonu ili, İncesu köyü merasının tescil sürecinin irdelendiği makalede de Orman Müdürlüğü'nün benzer tavrına işaret edilmiştir [18].
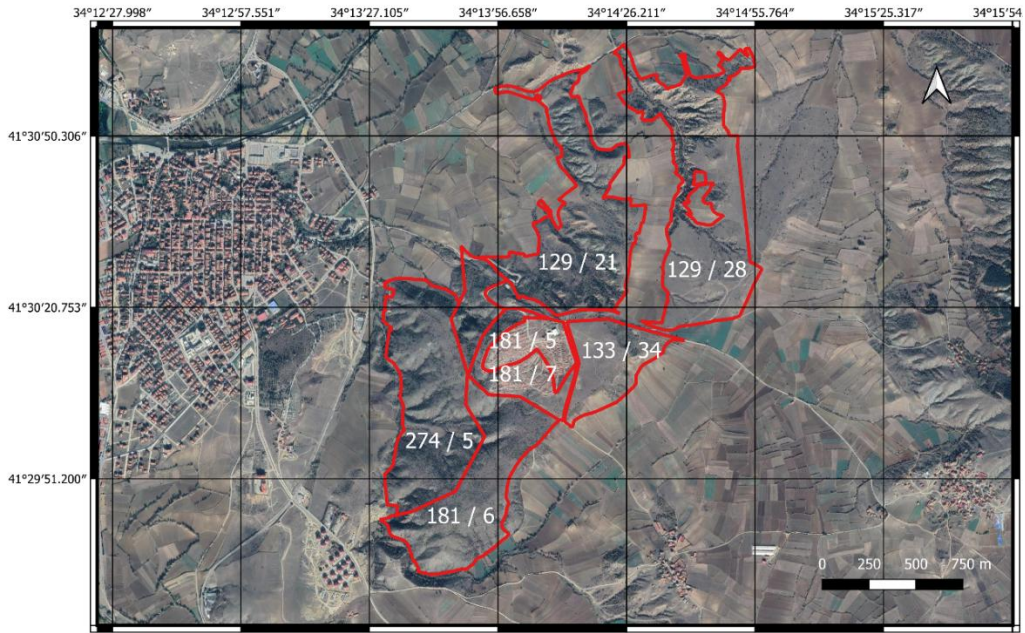
Şekil 2 Tapu ve Kadastro Genel Müdürlüğü parsel sorgu uygulamasına göre mera parsellerinin görünümü

Fig 2 Appearance of rangeland parcels according to the parcel query application of the General Directorate of Land Registry and Cadastre

Meralarımızda otlayan hayvan türlerinin azaltılıp çok büyük oranda sadece sığırlar ile otlatılması ve hatta yerli sığır ırklarından kültür ırkı sığırlara olan dönüşüm sebebiyle gittikçe azalan meraya dayalı hayvancılık sistemi nedeniyle, başlangıçta otsu bitki örtüsünün hâkim olduğu mera alanlarında da tabiatın kendi sistematiği içerisinde kol kola yürüyen toprak ve bitki süksesyonu nedeniyle her geçen gün otsu tabakanın aleyhine, çalı-ağaç tabakasının lehine bir dönüşümün olduğu bilimsel bir gerçekliktir [9, 12]. Orman Genel Müdürlüğü'nün E- Harita Uygulamasında ise adı geçen mera parselleri "ağaçsız orman alanları" olarak ifade edilmektedir [Şekil 4] [17].
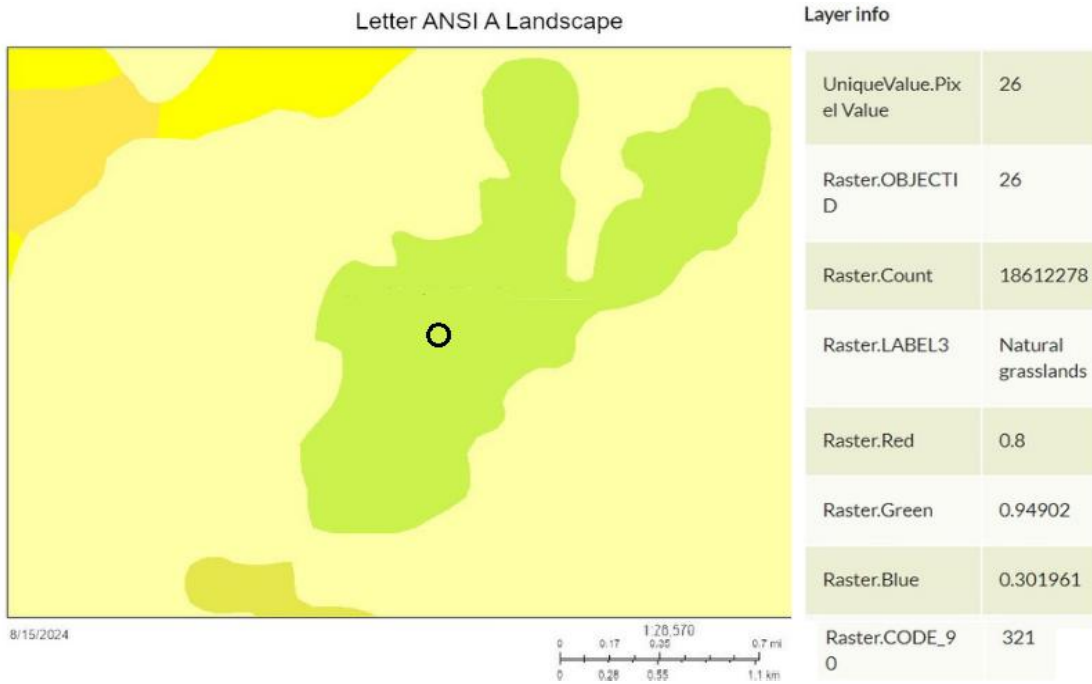
Şekil 3 Tarım ve Orman Bakanlığı'nın arazi örtüsü/kullanımı sınıflandırmasına göre mera parsellerinin görünümü ve niteliği [Natural grasslands=Doğal mera alanları, Yeşil renk ile gösterilen]

Fig 3 Appearance and character of rangeland parcels according to the land cover/use classification of the Ministry of Agriculture and Forestry [Natural grasslands, Shown in green]

Mera hayvancılığının koyun ile birlikte başrol oyuncusu keçilerin meralardan çekilmesi, süksesyonal değişimin hızlanmasındaki en önemli sebep olmuştur. Keçilerin meralardan çekilmesinde de maalesef Orman Bakanlığı'nın geçmişteki önyargılı/yanlış politikalarının rolü çok fazladır [14]. Mera hayvancılığı konusundaki hâlihazırdaki mevcut politikalardan hareketle, yakın gelecekte çalı ve ağaçların özellikle yağışlı bölgelerimizde hızlı bir şekilde artacağını öngörmek çok ta zor değildir [19]. Doğru yönetilemeyen bu değişim/dönüşüme bağlı olarak meraların hukuki statüsünde de doğru olmayan tasarruflarda bulunmak yanlışı katmerleştirmekten başka bir şeye hizmet etmeyecektir. Bu bakış açısı nedeniyle meralar, on yıllardır Orman Müdürlüğü tarafından şu veya bu şekilde bu çalışma konusunda olduğu gibi orman sınırları içerisine katılmaktadır. Mera Komisyonu da bu anlamda her ne kadar meraların hak ve hukukunu savunmak amacıyla teşekkül ettirilse de bu gidişatı durdurma anlamında maalesef yetersiz kalmakta, süreç *"meralar aleyhine, ormanlar lehine"* işlemeye devam etmektedir. Bu da mera hayvancılığı yapan insanlarımızın her geçen gün hareket alanı ve imkânlarının daralmasıyla sonuçlanmaktadır [18]. Ve gelişen süreç ile paralel olarak mera hayvancılığı her geçen gün terkedilmektedir [20]. Bu durum, diğer sebepler ile bir araya geldiğinde ülkemizi net hayvan ithalatçısı konumuna sokmaktadır [21]. Kırsal alanlardan şehirlere göçü ve dolayısıyla şehirlerdeki istihdam problemlerini artırmaya hizmet eden bu süreç de, devlet bütçesine fazladan istihdam oluşturma maliyeti yüklemektedir.

Şekil 4 Orman Genel Müdürlüğü'nün e- harita uygulamasına göre mera parsellerinin görünümü ve niteliği [Ağaçsız orman alanları]

Fig 4 Appearance and quality of rangeland parcels according to the e-map application of the General Directorate of Forestry [Treeless forest areas]

Mera Komisyonu'nun mera işgaline tavrı ve köy sakinlerinin iradesi

Mera Komisyonunun en öncelikli görevi meraların hak ve hukukunu korumaktır. 4342 sayılı Mera Kanunu'na aykırı bir şekilde on yıllardır işgal altında olan ve tüm diğer kamu kurumları tarafından mera olarak tanınan Vakıfbelören köyü merasının, işgalden kurtarılması ve asli hüviyetine döndürülmesi hususunda, bugüne kadar hiçbir tasarrufta bulunmayan Mera Komisyonu'nun olaya ilk müdahalesinde "ver ve hukuksuzluk probleminden kurtul" tarzı bir karar almıştır. Bu karar ile Mera Komisyonu'nun 181/5 ve 181/7 numaralı mera parsellerini "tahsis amacı değişikliği" ile mera olmaktan çıkararak, orman istif alanı, satış deposu, şef binası, yangın harekât binası, helikopter pisti ve garaj olarak kullanım için Orman Müdürlüğü'nün kullanımına açması asli görevi bağlamında doğru olmamıştır. Mera tecavüzleri açısından bu tavır, mera hayvancılığının geleceği açısından kötü bir emsal karar olmuştur. Tamamen kanun çerçevesinde hukuk mücadelesi veren Vakıfbelören köyü halkı yalnız bırakılmak bir yana daha da zora sokulmuştur. Milletin efendisi olduğu ifade edilen köy sakinlerinin, kadimden beri hayvan yetiştiriciliği için kullandıkları meranın aynı amaçla kullanıma devam etme isteğine dair Mera Teknik Ekibine imza vermek suretiyle oybirliği ile ortaya koydukları eksiksiz irade, kendilerine hizmet etme pozisyonundaki kişi ve kurumlar tarafından yok hükmünde sayılmıştır [22]. Sürecin en üzücü yönü; on yıllardır her anlamda talan edilen

meralara sahip çıksın diye oluşturulan Mera Komisyonu'nun aldığı bu kararın, hukuksuz bir şekilde işgal edilen meranın ve dolayısıyla burayı kullanan halkın hakkını-hukukunu korumadığı gerekçesiyle halk tarafından mahkeme kanalıyla iptal ettirilmesidir. Diğer bir anlatımla halk meralarını hem Mera Komisyonu'ndan ve hem de Orman Müdürlüğü'nden koruyabilmek amacıyla hukuk mücadelesi yürütmektedir. Bunun anlamı meraların korunması hakkında yapılan yasal düzenlemelerin tam olarak amacına ulaşmadığı, ek birtakım düzenlemelere ihtiyaç duyulduğudur. Bu çalışma özelinde sergilenen birtakım tavırlar, köy sakinlerinin kendilerini önemsiz/kıymetsiz hissetmelerine, dolayısıyla tarımdan vazgeçmeye sevk etmekte, kendilerini önemli hissettirebilecekleri işlere ve dolayısıyla şehirlere göç etmelerine sebep olmaktadır. Köyde yaşayan insanlara karşı takınılan bu tavrın hiçbir insan topluluğunda karşılığı yoktur. Bu olgu, genelde tarım sektörümüzün istenilen noktaya ulaşamamasının en büyük psikolojik sebebini oluşturmaktadır. Aslolan köylü olmalıdır. Diğer yandan Mera Komisyonu son karar mercii değildir. Şöyle ki, Samsun ilinde gerçekleşen bir tahsis amacı değişikliği isteminde Mera Komisyonu, alan için tahsis amacında değişiklik yapılması talebini kabul etmiş ve kararı onay için Tarım ve Orman Bakanlığına göndermiştir. Ancak Bakanlık; Mera Yönetmeliği'nin 8. Maddesine atıfla [Değişik: RG:12.04.2005-25784], bu tür her başvuruda olumlu görüş bildirilme gibi bir zorunluluğun olmadığını ifade ederek, bölgedeki hayvan sayısı, kaba yem açığı olup olmadığı, meranın vasfı, topografyası, toprak yapısı, vejetasyon yapısı, otlatma kapasitesi, köyün toplam merası, teknik ekibin kesin görüşü, çiftçi sayısı ve görüşü, alternatif bir alanın bulunup bulunmadığı, istenilen alanın gereğinden büyük olup olmadığı ve mera bütünlüğünün bozulup bozulmadığı gibi kriterler bakımından yeniden değerlendirilmesini istemiştir. Bunun üzerine Mera Komisyonu toplanıp, yeniden olumlu görüş bildirmiştir. Ancak Bakanlık kararı uygun bulmamış ve mera alanı Samsun Büyükşehir Belediyesi'ne tahsis edilmemiştir [23]. Bu noktadan hareketle özellikle belirtmek gerekir ki istenilen mera parseli diğer parsellerin tam ortasındadır ve Mera Yönetmeliği'nin, 8. Maddesi [Değişik: RG-12.08.2005-25784]'nde dikkate alınması gereken hususlardan sayılan "mera bütünlüğü"nü bozmaktadır.

Sonuç

Orta sınıflı bitkisel varlığıyla, bir takım ıslah ve amenajman çalışmalarıyla verimliliğinin daha da artırılabilme potansiyeli olan ve fakat hukuken işgal altında olan, bu nedenle bütün bitkisel varlığı tahrip edilmiş olan Vakıfbelören köyü merasının bir kısmının Orman Müdürlüğü'ne devrinde kamu yararı olmadığı gibi, aksine hukuk devleti olgusunu aşındırıcı ve başta Vakıfbelören köyü sakinleri olmak üzere kamu vicdanında telafisi mümkün olmayan zararlanmaya neden olacaktır. Ülkemiz mera alanlarının sürekli orman sınırları içerisine dâhil edilmeleri suretiyle yaşanan kayıplar bakımından kötü bir emsal karar olacak ve işgali aklayacaktır. Mera alanlarında mütemadiyen yaşanan bu kayıplar da mera hayvancılığının hareket alanını her geçen gün daha da daraltacak, hayvan ithalatı olgusuna bir tuğla daha koyacak, köyden kente göçü teşvik edecek, devletin sırtında büyük bir yük olan istihdam problemini daha da büyütecektir.

Kaynaklar

1. Anonim, Türkiye orman varlığı. 2024a, Tarım ve Orman Bakanlığı, https://www.ogm.gov.tr/tr/ormanlarimiz/Turkiye-Orman-Varligi, [Erişim: 10.08.2024].
2. Anonim, Türkiye geneli mera tespit, tahdit ve tahsis çalışmaları. 2024b, TC. Tarım ve Orman Bakanlığı. https://www.tarimorman.gov.tr/Konular/Bitkisel-Uretim/Cayir-Mera-ve-Yem-Bitkileri [Erişim: 22.Temmuz. 2024].
3. İspirli, K., Uzun, F. ve Ö.F. Uzun, Aşdağul beldesi merasında tahsis amacı değişikliği isteminin mevzuata uygunluk açısından incelenmesi. 7th International Conference on Agriculture, Animal Science and Rural Development. Ed. Yaşar Karadağ, Seyithan Seydoşoğlu. 2021, September 18-19. Muş, Türkiye: p. 631-644.

4. Anonim, Helvacılar köyü kadim merasının orman kayıtlarına alınması davası. Tapu iptali ve tescil. 2013, TC. Çaycuma, 2. Asliye Hukuk Mahkemesi. Gerekçeli Karar. Esas No: 2013/419, Karar No: 2013/577.

5. Anonim, Zonguldak il mera komisyon kararları. 2014a, TC. Zonguldak Valiliği İl Gıda Tarım ve Hayvancılık Müdürlüğü. Toplantı tarihi: 24.10.2014, Karar No:2014/19.

6. Anonim, Karadeniz Ereğlisi, Yenidoğancılar köyü 152/45 no'lu parselin mera olarak tespitine rağmen orman olarak kaydedilmesinin görüşülmesi. 2014b, Zonguldak İl Mera Komisyon Kararları. TC. Zonguldak Valiliği İl Gıda Tarım ve Hayvancılık Müdürlüğü. Toplantı tarihi: 4.07.2014, Karar No:2014/13.

7. Anonim, Zonguldak Orman Bölge Müdürlüğü'nün Yalnızçam köyü mera tahsis kararına itirazı. Zonguldak İl Mera Komisyon Kararları. 2014c, TC. Zonguldak Valiliği İl Gıda Tarım ve Hayvancılık Müdürlüğü. Toplantı tarihi: 4.07.2014, Karar No:2014/22.

8. Anonim, Tapu ve Kadastro Genel Müdürlüğü parsel sorgu uygulaması. 2024c, https://parselsorgu.tkgm.gov.tr/#ara/cografi/41.499449622502006/34.227390289 [Erişim:10.08. 2024].

9. Holechek, J.L., Pieper, R.D. and C.H. Herbel, Range Management: Principles and Practices [6th Edition]. 2010, Amsterdam, Netherland: Prentice Hall, One Lake Street, Upper Saddle River.

10. Anonim, Toprak ve arazi sınıflaması standartları teknik talimatı. 2005, https://www.tarimorman.gov.tr/Belgeler/Mevzuat/Talimatlar/ToprakAraziSiniflamasiStandartlariTeknikTalimativeIlgiliMevzuat_yeni.pdf [Erişim: 10.08.2024].

11. Koç, A., Gökkuş, A. ve M. Altın, Mera durumu tespitinde dünyada yaygın olarak kullanılan yöntemlerin mukayesesi ve Türkiye için bir öneri. Türkiye 5. Tarla Bitkileri Kongresi. 2003, 13-17 Ekim, Diyarbakır, Türkiye: p. 36-42.

12. Aydın, İ. ve F. Uzun, Çayır-Mera Amenajmanı ve Islahı [Ders Kitabı No: 9]. 2002, Samsun, Türkiye: Ondokuz Mayıs Üniversitesi, Ziraat Fakültesi Basımevi.

13. 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]: p. 8-14.

14. Uzun, F., Garipoğlu, A.B. ve H.B. Dönmez, Mera yabancı otlarının kontrolünde keçilerin kullanımı. Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi, 2015. 1[1]: p. 40-50. https:// dergipark.org.tr/tr/pub/ijaws/issue/ 7987/104845.

15. Anonim, Kastamonu ili, Taşköprü ilçesi, Vakıfbelören köyü mera yaylak ve kışlak alanlarına ait tahsis için ihtiyaç tespit tutanağı. 2020, Karar No: 931.

16. Anonim, Corine Land Cover 2012 [Vector/raster 100 m]. [2024d], https://corinecbs. tarimorman.gov.tr/corine [Erişim:10.08.2024].

17. Anonim, Orman Genel Müdürlüğü. E-Harita. Vakıfbelören, Taşköprü, Kastamonu. 2024e, https://cbs.ogm.gov.tr/vatandas/ [Erişim:10.08.2024].

18. Uzun, F., Gürel, F., Kumbasar, F. ve Ö.F. Uzun, Mera tescillerindeki anlaşmazlıkların giderilmesinde referans alınabilecek bazı uygulamaların irdelenmesi [Kastamonu, İncesu köyü merası örneği]. Journal of Life Sciences and Biotechnology, 2024. 7[2]: p. 102-111.

19. İspirli, K., Alay, F., Çankaya, N., Şahin, M., Uzun, Ö.F. ve F. Uzun, Nemli-mezotermal iklim kuşağında yer alan ve ağır otlanılan Hasanlar köyü merasının bazı özellikleri. Anadolu Tarım Bilimleri Dergisi, 2022. 37[3]: p. 487-504.

20. Uzun, F., Alay, F. ve K. İspirli, Rangeland characteristic of open for grazing area for long years of West Black Sea Region. International Agricultural Science Congress, 2018. 9-12 May, Van, Türkiye [Özet Basım]: p. 295.

21. Anonim, Dış Ticaret İstatistikleri. 2023, https://data.tuik.gov.tr/Kategori/GetKategori?p= Dis-Ticaret-104 [Erişim:10.08.2024].

22. Anonim, Kastamonu İl Mera Komisyon kararı. 2022, Karar No:1082.

23. Uzun, Ö.F. ve F. Alay, Meralarda kentsel dönüşüm ve gelişim amaçlı tahsis amacı değişikliği talebinin değerlendirilmesi: Doyran ve Kızılcaören örneği. International Journal of Life Sciences and Biotechnology, 2022. 5[3]: p. 602–610.

ERRATUM

This erratum addresses the article titled "Some Fruit Characteristics of Some High Albenic Apple (Malus x domestica Borkh.) Varieties Grown in Kyrgyz National Ala Archa Park Local Varieties Research Station," published in the *International Journal of Life Sciences and Biotechnology* [2024;7(2):112-119]. The article has been reviewed and republished in its corrected form in [2024;7(3):226-233].

The corrections are as follows:

1. **Funding Statement**:
   The original publication contained incomplete or incorrect information regarding funding support. The corrected version is as follows:
   Funding: This study was supported by the Kyrgyz-Turk Manas University Scientific Research Projects, project number KTMU-BAP-2024.FB.03.
2. **Corresponding Author Information**:
   The original publication omitted essential information about the corresponding author. The corrected version includes the following:
   Corresponding Author: Malika Turgunbaeva, e-mail: malika.rashidovna@gmail.com
3. **Author Affiliation Address**:
   The original publication included an incorrect address for the corresponding author. The corrected version specifies the following updated address:
   Affiliation: Kocaeli University, Faculty of Agriculture, Department of Horticulture, Kocaeli, Turkey. and Kyrgyz-Turk Manas University, Faculty of Agriculture, Department of Horticulture and Field Crops, Bishkek, Kyrgyzstan,

Authors sincerely apologize for these errors. Thank our readers for their understanding.

DÜZELTME

Bu düzeltme, *International Journal of Life Sciences and Biotechnology* dergisinde yayımlanan "Some Fruit Characteristics of Some High Albenic Apple (Malus x domestica Borkh.) Varieties Grown in Kyrgyz National Ala Archa Park Local Varieties Research Station" başlıklı makale ile ilgilidir [2024;7(2):112-119]. Makale gözden geçirilmiş ve düzeltilmiş haliyle [2024;7(3):226-233] sayfalarında yeniden yayımlanmıştır.

Düzeltmeler şu şekildedir:

1. **Fon Desteği Beyanı**:
   Orijinal yayında fon desteğiyle ilgili eksik veya hatalı bilgi yer almıştır. Düzeltilmiş versiyonu aşağıdaki gibidir:
   Fon Desteği: Bu çalışma, Kırgız-Türk Manas Üniversitesi Bilimsel Araştırma Projeleri (KTMU-BAP) tarafından desteklenmiştir, proje numarası: KTMU-BAP-2024.FB.03.
2. **Sorumlu Yazar Bilgisi**:
   Orijinal yayında sorumlu yazarla ilgili önemli bilgiler eksikti. Düzeltilmiş versiyon aşağıdaki gibidir:
   Sorumlu Yazar: Malika Turgunbaeva, e-posta: malika.rashidovna@gmail.com
3. **Yazar Kurum Adresi**:
   Orijinal yayında sorumlu yazarın adresi yanlış verilmiştir. Düzeltilmiş versiyonda güncellenmiş adres şu şekildedir:
   Kurum: Kocaeli Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Kocaeli/Türkiye. ve Kırgız-Türk Manas Üniversitesi, Ziraat Fakültesi, Bahçe ve Tarla Bitkileri Bölümü, Bişkek/Kırgızistan

Yazarlar hatalar için özür dilerler. Okurlarımızın dikkatine sunarız.

Kırgız Milli Ala Arça Parka Ait Yerli Çeşitler Araştırma İstasyonunda Yetişen Albenisi Yüksek Bazı Elma (*Malus x domestica* Borkh.) Çeşitlerinin Bazı Meyve Özellikleri

Malika Turgunbaeva[1]* ID , **Ahmet Aygün**[1,2] ID

ÖZET

Bu araştırma, Kırgızistan'nın Bişkek şehri içerisinde bulunan farklı yerlerden daha önceden toplanarak kurulmuş yerel elma çeşitlerine ait koleksiyon bahçesinde yetiştirilen bazı elma çeşitlerinin meyve özelliklerinin belirlenmesi amacıyla yürütülmüştür. Çalışmada toplam 18 adet yerel elma çeşidi incelenmiştir. Elma çeşitlerinde meyve ağırlığı 42.28-261.25 g, meyve boyu 25.39-73.31 mm, meyve eni 30.58-94.19 mm, meyve kalınlığı 31.41-91.36 mm, sap çukuru derinliği 5.22-17.58 mm, sap çukuru eni 9.88-26.42 mm, sap çukuru genişliği 8.59-31.80 mm, sap uzunluğu 13.32-32.21 mm, sap kalınlığı 1.16-9.47 mm, çiçek çukuru derinliği 5.75-28.69 mm, çiçek çukuru genişliği 8.73-22.61 mm, çiçek çukuru eni 4.14-17.46 mm, çekirdek sayısı 3.22-8.60 adet, meyve eti sertliği 2.51-11.35 kg/cm², suda çözünebilir kuru madde miktarı %3.48-16.75, pH 3.02 ile 7.40, titre edilebilir asitlik miktarı %0.21-1.86 aralığında bulunmuştur. Bu sonuçlar bölgede farklı lokasyonlardan daha önce toplanan elmalarda özellik bakımından farklılıklar olduğunu ortaya koymasının yanında bu çeşitlerin piyasada talep edilebileceği fikrini doğurmuştur. Diğer yandan bu genetik kaynakları oluşturan bahçenin yaşlanması ve bakımsız olması yüzünden bu çeşitler yok olma tehlikesi altındadır.

Some Fruit Characteristics of Some High Albenic Apple (*Malus x domestica* Borkh.) Varieties Grown in Kyrgyz National Ala Archa Park Local Varieties Research Station

ABSTRACT

This study was carried out to determine the fruit characteristics of some apple cultivars grown in the collection orchard of local apple cultivars previously collected from different places in Bishkek city of Kyrgyzstan. A total of 18 local apple cultivars were examined in the study. Fruit weight 42.28-261.25 g, fruit length 25.39-73.31 mm, fruit width 30.58-94.19 mm, fruit thickness 31.41-91.36 mm, stem pit depth 5.22-17.58 mm, stem pit width 9.88-26.42 mm, stem pit width 8.59-31.80 mm, stem length 13.32-32.21 mm, stem thickness 1.16-9. 47 mm, flower pit depth 5.75-28.69 mm, flower pit width 8.73-22.61 mm, flower pit width 4.14-17.46 mm, number of seeds 3.22-8.60, fruit flesh hardness 2.51-11.35 kg/cm², water soluble dry matter 3.48-16.75%, pH 3.02 to 7.40, titratable acidity 0.21-1.86%. These results showed that there were differences in the characteristics of the apples collected from different locations in the region, and it gave rise to the idea that these varieties could be demanded in the market. On the other hand, these varieties are under the danger of extinction due to the aging and neglect of the orchard that constitutes these genetic resources.

Giriş

Milattan öncesine dayanan elma kültürü, yüksek adaptasyon kabiliyeti ve çeşit sayısının fazlalığı nedeniyle, ılıman iklim meyve türleri arasında en çok tüketilen türdür. Orijini, Anadolu da dahil olmak üzere Güney Kafkasya'ya kadar uzanan bir coğrafyayı içerir. Bugün Dünyanın birçok ülkesinde yaklaşık 100 milyon ton elma üretilmektedir [1]. *Malus x domestica* Borkh dünyanın farklı ülkelerinden gelen 7500'den fazla çeşidi içerir, ancak sadece bu çeşitler içerisinde 20 civarında çeşit bahçecilik endüstrisinde yaygın olarak

---

[1] Kırgızistan-Türkiye Manas Üniversitesi, Ziraat Fakültesi, Bahçe ve Tarla Bitkileri Bölümü, Bişkek/ Kırgızistan
[2] Kocaeli Üniversitesi, Ziraat Fakültesi Bahçe Bitkileri Bölümü Kocaeli/ Türkiye
*Correspondence Author: Malika Turgunbaeva, e-mail:malika.rashidovna@gmail.com

yetiştirilmektedir [2]. Elma (*Malus x domestica* Borkh.) taze meyve tüketiminin yanı sıra, gıda endüstrisinde ve biyolojik olarak aktif bileşiklerin ve diğer ikincil metabolitlerin kaynağı olarak yaygın olarak kullanılmaktadır [3,4]. Günümüzde dünya üzerinde pek çok bölgede geniş elma plantasyonları bulunmaktadır. Birçok çeşit, belirli koşullar altında yetiştirme için istenen özelliklere sahiptir, ancak bunlardan sadece birkaç düzinesi ticari olarak dünya çapında yetiştirilmektedir. Her geçen gün bu çeşitlere yenileri ekleniyor ve yeni çeşitler daha iyi özellikler ile piyasaya sürülmektedir [5]. Kırgızistan Cumhuriyeti Orta Asya ülkelerinin kuzeydoğusunda yer almaktadır: kuzeyden, batıdan ve güneyden Bağımsız Devletler Topluluğu CumhuriyetleriKazakistan, Özbekistan, Tacikistan ve doğudan ve güneydoğudan Çin Ulusal Cumhuriyeti ile sınırı vardır. Tiyan-Şan Dağları'nın iç ve kısmen kuzey ve batı kısımlarının yanı sıra 390 12' ve 430 15' Kuzey enlemlerinde ve 690 16' ve 800 18' Doğu boylamlarında Türkistan-Alay dağlarını kaplar. Kırgızistan topraklarındaki iklim, rakıma bağlı olarak büyük yerel sapmalarla birlikte soğuk kış ve sıcak yaz ile karakterize edilen karasal iklime hakimdir [6]. Orta Asya'da Kırgızistan önemli bir elma orijin merkezidir. Ceviz, Antep fıstığı ve badem türlerinin yanı sıra Kırgızistan'ın farklı bölgelerinde önemli elma popülasyonları ve ormanları bulunmaktadır. Ancak bu materyallerde ciddi antropojenik kayıplar vardır ve yakın gelecekte tamamen kaybolabilirler [6]. Kırgızistan'da doğal olarak bulunan üç elma türü vardır. Bunlar *Malus sieversii*, *Malus kirghisorum* ve *Malus niedzwetzkyana*'dır. Bu türler aynı zamanda kültür elmalarının kaynağı olarak da bilinmektedir [7]. Malus sieversii, Kazakistan, Kırgızistan, Çin, Tacikistan, Özbekistan ve Türkmenistan'ın kuru ve dağlık bölgelerine doğal olarak yayılmıştır. *Malus kirghisorum* genetik olarak *Malus sieversii*'ye yakındır, ancak meyve şekli, rengi ve diğer özellikleri bakımından farklıdır. *Malus niedzwetzkyana* türlerinde, yaprak, çiçek ve meyveler üzerinde pembe-mor pigmentasyonlar vardır. Bu ülkede yetiştirilen yerel çeşitler muhtemelen bu üç türün hibrit kompleksleridir [6]. Dünya elma üretimi 93.144.358 tona ulaşmıştır. Çin 45.983.400 tonluk üretimiyle dünyada ilk sırada yer alırken, onu Türkiye (4.493.264 ton) ve ABD (4.467.206 ton) takip etmektedir [8]. Kırgızistan elma üretimini 2022 yılı verilerine göre 132.102 ton elma üretimi yapılmaktadır. Kişi başı elma üretimi ise 18.87 kilogramdır. Kırgızistan'da yumuşak ve sert çekirdekli meyve üretimi değerlendirildiğinde elma üretimi ilk sırada yer almaktadır [9]. Bu üretimin büyük bir kısmını yerli ve yabani çeşitler oluşturmaktadır. Bu üretim miktarı Dünya sıralamasında önemli bir yer almasa da elma, Kırgızistanda'da toplam tarım üretimi içerisinde önemli bir rol oynamaktadır. Yine de bu üretim miktarı Kırgızistan gibi elmanın anavatanı olan ve ekolojisi elma yetiştiriciliği için çok uygun olan bir ülke için oldukça düşük miktardadır. Üç farklı türün gen merkezi olmasına rağmen istenilen elma üretimi yapılamamaktadır. Bunun sebepleri arasında çeşit sayısının az olması, modern meyvecilikte kullanılan anaçların ülkeye yeni yeni girmesi ve meyvecilik anlayışının tam olarak oluşmaması sayılabilir. Ülke çapında elma ile ilgili farklı çalışmalar yapılmasına rağmen ıslah yönünden bir çalışmaya rastlanılmamıştır. Bu çalışma mevcut popülasyonda üstün özellikli elma çeşitlerini seçmek ve çeşitlerin özelliklerini belirlemek amacıyla yürütülmüştür.

Materyal ve Yöntem

Materyal
Kırgızistan'ın Çüy bölgesindeki Kırgız Milli Ala Arça Parkı Gen merkezinde bulunan elma genetik kaynakları bahçesinde bulunan elma genotipleri içerisinden meyve özelliklerine göre ön plana çıkan 18 adet yerel elma çeşitleri çalışmanın bitkisel materyalini oluşturmaktadır.

Yöntem
Meyve ağırlığı (g) 0.01 g duyarlı hassas terazide ayrı ayrı tartılmış ve meyve boyu, çapı, kalınlığı, çiçek çukuru derinliği, eni ve genişliği ile sap uzunluğu, kalınlığı, sap çukuru genişliği, derinliği ve eni (mm) ölçümleri dijital kumpas yardımıyla, çekirdek sayısı (adet) elle sayılmış ve bu ölçümlerin ortalaması alınarak her çeşit için belirlenmiştir. Meyvelerin kabuk ve et rengi ölçümleri Colorimeter NR200 marka renk ölçüm cihazıyla, her örneğe ait her bir meyvenin üç farklı noktasından ölçüm yapılmıştır. Meyve kabuk ve et rengi parametreleri üç farklı okuma şeklinde L*, a*, b* olarak belirlenerek L* değeri meyve renginin parlaklığındaki değişimi göstermektedir. L*: siyahtan beyaza, a*: kırmızıdan yeşile, b*: sarıdan maviye renk değişimlerini göstermektedir. Meyve eti sertliği her örneğe ait her bir meyvenin ekvatoral bölgesinde 3 farklı kısmında 1 cm² büyüklüğündeki kabuğun kaldırılmasından sonra 11.1 mm delici uç çapına sahip basınç ölçerin (penetrometre) meyve etine batırılması ile kg/cm² olarak ölçüm yapılmıştır. Meyve suyunda; suda çözünebilir toplam kuru madde (SÇKM) dijital refraktometre ile (%) ve meyve suyu pH'sı pH metre ile titre edilebilir asitlik miktarı ise 0.1N NaOH ile meyve suyunun titre edilmesi ve harcanan baz miktarına göre malik asit cinsinden hesaplanması ile % olarak belirlenmiştir.

Bulgular ve Tartışma

Gen kaynakları bahçesinde toplanmış elma çeşitleri arasından seçilen 18 adet yerli çeşitlerin meyve özellikleri incelenmiştir. Yetiştirilen elma çeşitlerinin meyve ağırlığı, meyve boyu, meyve eni ve meyve kalınlığı özellikleri Tablo 1'de verilmiştir. Yapılan ölçümler neticesinde çeşitlerin meyve ağırlığı 42.28-261.25 g aralığında değişim göstermiştir. Belirlenen yerel elma çeşitlerinden Çeşit 16 (261.25 g) en fazla meyve ağırlığına sahip olurken Çeşit 1 (162.4 g) ve Çeşit 4 (162.2 g) takip etmiştir. En az meyve ağırlığına sahip olan çeşitler ise Çeşit 12 (65.54 g), Çeşit 13 (63.71 g) ve Çeşit 14 (42.28 g) olarak belirlenmiştir. Elde edilen çeşitlerinin verilerin ortalaması dikkate alındığında meyve boyu en yüksek olanlar Çeşit 16 (73.31 mm), Çeşit 7 (62.4 mm), Çeşit 18 (60.87 mm) ve en düşük değerlere sahip olanlarda Çeşit 14 (25.39 mm), Çeşit 13 (34.54 mm), Çeşit 11 (45.15 mm) arasında değiştiği tespit edilmiştir. Meyve eni bakımından ise en yüksek Çeşit 16 (94.19 mm), Çeşit 1 (74.99 mm) ve Çeşit 7 (74.75 mm), en düşük ise Çeşit 14 (30.58 mm), Çeşit 13 (44.47 mm), Çeşit 17 (54.55 mm) aralığında değişim göstermişler. İncelenen çeşitlerin meyve kalınlığı bakımından değerlendirilenler 31.41-91.36mm aralığındadır. Çeşit 14 hariç tüm elma çeşitlerinin meyve kalınlıkları 50 mm üzerinde belirlenmiştir. Çeşitlerin çekirdek sayıları incelendiğinde 3.22-8.60 adet aralığında bir dağılıma sahip olduğu görülmüştür.

Tablo 1 Kırgız Milli Ala Arça Parka Ait Yerli Çeşitler Araştırma İstasyonunda Yetişen Elma Çeşitlerinin Pomolojik Özellikleri

Table 1 Pomological Characteristics of Apple Varieties Grown in Kyrgyz National Ala Archa Park Domestic Varieties Research Station

№	Meyve ağırlığı(g)	Meyve boyu(mm)	Meyve eni(mm)	Meyve kalınlığı(mm)	Çekirdek sayısı (adet)	Meyve eti sertliği (kg/cm²)
Çeşit1	162.40	59.61	74.99	72.94	3.22	3.85
Çeşit2	73,56	47.36	55.73	57.95	5.69	5.25
Çeşit3	97.78	51.50	61.38	62.63	6.00	5.01
Çeşit4	162.20	60.15	71.88	75.00	7.30	5.06
Çeşit5	140.10	59.02	69.60	69.80	8.60	7.75
Çeşit6	121.82	57.11	62.87	67.45	5.12	5.89
Çeşit7	155.10	62.4	74.75	76.41	3.28	4.73
Çeşit8	117.11	57.37	60.9	61.68	6.91	4.45
Çeşit9	101.33	55.12	60.83	61.73	6.94	5.71
Çeşit10	121.60	52.18	68.40	69.33	6.90	5.53
Çeşit11	85.91	45.15	61.71	61.44	4.90	5.39
Çeşit12	65.54	46.73	55.64	58.07	3.33	4.12
Çeşit13	63.71	34.54	44.47	51.39	7.94	11.35
Çeşit14	42.28	25.39	30.58	31.41	5.35	2.86
Çeşit15	97.54	51.12	62.09	62.15	8.12	5.56
Çeşit16	261.25	73.31	94.19	91.36	6.50	2.51
Çeşit17	68.40	47.24	54.55	54.26	7.04	5.50
Çeşit18	129.84	60.87	64.94	64.02	5.53	5.17

İncelenen elma çeşitlerinde meyve sap derinliği 5.22 mm ile 17.58 mm arsında değişmektedir. En yüksek değer Çeşit 6 (17.58 mm) en düşük değerler ise Çeşit 13'te (5.22 mm) belirlenmiştir. Meyve sap eni verilerin ortalamasına göre yüksek olanlar Çeşit 16 (26.42 mm), Çeşit 10 (16.49 mm), Çeşit 7 (16.28 mm) ve düşükler değere sahip olanlar ise Çeşit 3 (9.88 mm), Çeşit 14 (10.14 mm), Çeşit 6 (11.86 mm). Çeşitlerin meyve sap genişliği 8.59-31.8 mm arasında değişmektedir. Yine çeşitlerin sap uzunluğu değerleri bakımından 13.32-32.21 mm arasında bir varyasyon belirlenmiştir. Çeşitlerin meyve sap kalınlıkları arasında da büyük farklılıklar bulunmuştur. Nitekim sap kalınlığı 1.16-9.47 mm arasında değişim göstermiştir.

Meyve şeklini belirlemede bir kriter olan çiçek çukuru derinliği ölçümlerinde en yüksek değerlere Çeşit 16 (28.69 mm) sahip olurken, en düşük değere ise Çeşit 9 (5.75 mm) sahip olmuştur. Çiçek çukuru genişliği parametresi incelendiğinde değerlerin 8.73-22.61 mm aralığında değişim gösterdiği görülmüştür. Meyve çiçek çukur eni ise 4.14-17.46 mm arasında değiştiği tespit edilmiştir.

Kırgız milli Ala Arça parka ait yerli çeşitler araştırma istasyonunda yetişen bazı yerel elma çeşitlerin suda çözünebilir kuru madde miktarı, pH ve titre edilebilir asitlik özellikleri Tablo 4'te verilmiştir. İncelenen

çeşitlerde SÇKM içeriği %3.48-16.75 arasında değerler içermiştir. SÇKM içeriğinin yüksek olması bakımından öne çıkan çeşitler Çeşit 10 (%16.75), Çeşit 8 (%15.50), Çeşit 9 (%14.60) ve Çeşit 6 (%14.55) olarak bulunurken, düşük olması bakımından öne çıkan çeşitler ise Çeşit 13 (%3.48), Çeşit 15 (%10.60), Çeşit 16 (%10.87) olarak tespit edilmiştir. Meyve suyu pH'sı 3.02 ile 7.40 arsında değişmektedir. Meyve kalitesi bakımından önemli bir kriter olan titre edilebilir asitlik miktarları %0.21-1.86 aralığında dağılım göstermiştir.

Tablo 2 Kırgız Milli Ala Arça Parka Ait Yerli Çeşitler Araştırma İstasyonunda Yetişen Elma Çeşitlerinin Meyve Sap Özellikleri
Table 2 Fruit Stalk and Flower Pit Characteristics of Apple Varieties Grown in Kyrgyz National Ala Archa Park Native Varieties Research Station

№	Sap çukuru derinliği(mm)	Sap çukuru eni (mm)	Sap çukuru genişliği(mm)	Sap uzunluğu(mm)	Sap kalınlığı(mm)
Çeşit1	10.27	13.67	14.79	24.70	2.39
Çeşit2	7.39	13.91	13.96	24.85	1.85
Çeşit3	8.25	9.88	10.28	23.32	1.62
Çeşit4	8.25	16.20	16.97	16.30	9.47
Çeşit5	8.40	15.12	15.64	17.16	3.15
Çeşit6	17.58	11.86	12.19	23.31	2.25
Çeşit7	10.29	16.28	14.48	16.41	2.66
Çeşit8	7.32	12.08	13.24	25.37	2.33
Çeşit9	9.21	14.12	13.28	22.82	2.03
Çeşit10	8.08	16.49	17.38	18.62	2.21
Çeşit11	6.53	13.62	13.29	16.32	2.33
Çeşit12	9.76	12.61	13.91	29.17	1.85
Çeşit13	5.22	13.74	14.94	16.72	1.81
Çeşit14	5.92	10.14	8.59	21.34	1.16
Çeşit15	8.99	13.36	14.42	14.12	2.26
Çeşit16	15.98	26.42	31.80	13.32	3.18
Çeşit17	8.48	12.50	11.86	20.85	1.65
Çeşit18	7.70	15.82	14.82	32.21	1.66

Elma çeşitlerine ait meyve kabuk rengi ve meyve et rengi değerlerinde de büyük farklılıklar tespit edilmiştir. L değeri -6.79 ile 68.03 aralığında, a değeri -18.41 ile 5.17 aralığında, b değeri -4.28 ile 35.06 aralığında dağılım göstermiştir.

Çalışmada belirlenen yerel çeşitlerin meyve ağırlıkları 42.28-261.25 g olarak değişim göstermiştir (Tablo 1). Bu sonuçlar; Güleryüz ve ark. (1993) Kağızman ilçesinde yetişen elma çeşitlerinin ortalama meyve ağırlıkları 159.0-313.0 g. Miller ve ark. (2004) Amerikan Birleşik Devletleri ve Kanada'da bazı elma çeşitleri ile yürüttükleri çalışmada ortalama meyve ağırlığı 136-300 g. Farrokhi ve ark. (2011). İran (Maşhad) ilinde elmalarda meyve ağırlığı 7.1-81.67 g. Mratinić ve Akšić (2012) Gümüşhane merkez ilçede yetişen bazı standart ve yerel elma çeşitlerinin meyve ağırlığı 80.70- 195.61 g. Kırkaya ve ark. (2014) Ordu ili Perşembe ilçesinde yetiştirilen yerel elmalarda meyve ağırlığı 76.24-247.23 g. Balta ve ark. (2015) Ordu ilinin Kumru ilçesindeki yerel elma çeşitlerinde meyve ağırlığı 71.41-245.99 g. Çoşkun ve ark. (2016). Isparta (Türkiye) şehrinde 5 yerel ve 2 yabancı orijinli elma çeşidinin meyve ağırlığı 96.99 g ile 184.25 g. Öztürk ve ark. (2016) Samsun ekolojik koşullarında MM 106 elma anacı üzerine aşılı 'Cooper 7 SB2'. 'Golden Delicious'. 'Granny Smith'. 'Jersey Mac'. 'Red Chief'. 'Starkrimson Delicious' ve 'Süper Chief' elma çeşitlerinin meyve ağırlığı 112.3-173.9 g. olarak belirlenmişlerdir. Bu değerlerin bizim çalışmamızda bulunan değerlere paralel veya daha düşük olduğu tespit edildi. Bu farklılıklar genotipik farklılıklardan kaynaklanabileceği gibi ekolojik farklılıklardan da kaynaklanabilir.

Meyve boyutları bakımından yapılan değerlendirmede çeşitlerin meyve boyu değerleri 25.39-73.31 mm aralığında belirlenmiştir (Tablo 1). Bu değerler. Miller ve ark. (2004). tarafından Amerikan Birleşik Devletleri ve Kanada'da yaptığı çalışmada bulduğu değerlerle (65-80 mm). Farrokhi ve ark. (2011). İran (Maşhad) ilinde elmalarda bulduğu değerlerle (25.13-77.67 mm). Çoşkun ve Aşkın (2016) Isparta şehrinde 5 yerel ve 2 yabancı orijinli elma çeşidinde bulduğu değerlerle (53.93-65.82 mm). Özmen'in Tokat merkez ve ilçelerinde yetişen yerel çeşitlerinde bulduğu değerlerle (47.93-67.23 mm) benzerlik göstermiş ya da daha yüksek bulunmuştur.

Tablo 3 Kırgız Milli Ala Arça Parka Ait Yerli Çeşitler Araştırma İstasyonunda Yetişen Elma Çeşitlerinin Meyve Çiçek Çukuru Özellikleri
Table 3 Flower Pit Characteristics of Apple Varieties Grown in Kyrgyz National Ala Archa Park Native Varieties Research Station

№	Çiçek çukuru derinliği (mm)	Çiçek çukuru genişliği (mm)	Çiçek çukuru eni(mm)
Çeşit1	10.19	13.63	13.18
Çeşit2	8.96	13.13	14.25
Çeşit3	9.97	11.37	10.34
Çeşit4	8.18	15.31	16.74
Çeşit5	9.73	17.40	17.46
Çeşit6	10.69	12.96	12.34
Çeşit7	12.00	19.62	14.41
Çeşit8	6.63	19.86	12.80
Çeşit9	5.75	17.43	15.86
Çeşit10	17.38	16.03	8.18
Çeşit11	12.50	12.21	6.14
Çeşit12	13.75	12.94	9.27
Çeşit13	17.23	14.31	7.40
Çeşit14	9.21	8.73	4.14
Çeşit15	14.62	13.19	7.42
Çeşit16	28.69	22.61	24.00
Çeşit17	12.62	12.32	8.59
Çeşit18	18.07	14.61	11.10

Çalışmamızda meyve eni değerleri 30.58-94.19 mm. meyve kalınlığı değerleri 31.41- 91.36 mm olarak tespit edilmiştir. Bulunan değerler Farrokhi ve ark. (2011). İran (Maşhad) ilinde elmalarda yapılan araştırmada belirledikleri meyve eni (21.81-68.33 mm) ve meyve kalınlığı (25.13-77.67 mm) değerlerinden. Şenyurt ve ark. (2015) yaptıkları çalışmalarında belirledikleri meyve eni değerlerinden (57.27-80.77 mm). Kırkaya. Balta ve Kaya (2014) Ordu ili Perşembe ilçesinde yaptıkları araştırmalarda belirledikleri meyve kalınlığı değerlerinden (44.63-73.98 mm). Balta ve ark. (2015) Ordu ilinin Kumru ilçesinde elmalarda yapılan araştırmada belirledikleri meyve kalınlığı değerlerinden (61.01-95.59 mm). Coşkun ve Aşkın (2016) Isparta şehrinde yürüttükleri çalışmalarında belirledikleri meyve eni değerlerinden (64.86-76.56 mm) daha yüksek veya paralellik olarak saptanmıştır. Bu farklılığın başlıca nedeninin genotipten kaynaklandığı kaçınılmazdır. Bu sebepten dolayı incelenen genotiplerin çeşit adayı olması ve ıslah çalışmalarında kullanılabileceği düşüncesini akla getirmektedir.

Suda çözünebilir kuru madde miktarı çalışmamızda belirlediğimiz yerel çeşitlerde %3.48-16.75 değerleri arasında bulunmuştur (Tablo 4). Bu bulgular. Güleryüz ve ark. (1993). Kağızman ilçesinde yürüttüğü çalışmada SÇKM miktarını %12.35- 14.45. Miller ve ark. (2004) tarafından Amerikan Birleşik Devletleri ve Kanada'da bazı elma çeşitleri ile yürüttükleri çalışmada ortalama SÇKM oranı %12.30-15.6. Mratinić ve Akšić (2012). tarafından Güney Sırbistan'da elmaların SÇKM değerinin %12.55-19.24.

Şenyurt ve ark. (2015) yaptıkları çalışmalarında SÇKM %11.50-15.25. Kırkaya ve ark. (2014). Ordu ili Perşembe ilçesinde yetiştirilen yerel elma genotiplerinde yürüttükleri çalışmada SÇKM oranının % 9.01-13.75. Selcen (2017) Tokat merkez ve ilçelerinde yetişen yerel elma genotiplerinde yaptığı çalışmada SÇKM %9.9-16.8. Selma Boyacı (2019) Kırşehir koşullarında MM 106 yarı bodur elma anacı üzerine aşılı Mondial Gala. Red Chief. Golden Delicious. Braeburn ve Granny Smith elma çeşitlerinin performanslarının belirlenmesi amacıyla yaptığı çalışmada SÇKM %11.16- 15.41 değerleri aralığında bulmuştur. Literatürde bildirilen bu değerlerle bizim değerlerimiz benzerlik göstermektedir. Ancak bazı çeşitlerin asitlik değerlerinin düşük olduğu çeşitlerde tespit edilmiştir.

Elma çeşitlerin meyve suyu pH'larının 3.02 ile 7.40 aralığında değiştiği tespit edilmiştir. Belirlenen bu değerler. Kırkaya ve ark (2014). Ordu ili Perşembe ilçesinde yetiştirilen yerel elma genotiplerinin pomolojik. fenolojik ve morfolojik özelliklerinin belirlenmesi amacıyla 2010-2011-2012 yıllarında yaptıkları araştırmada pH değerinin 3.16-3.56. Balta ve ark. (2015). Ordu ilinin Kumru ilçesinde elma çeşitlerinde yaptığı çalışmada tespit ettiği pH miktarı 2.83-4.11. Öztürk ve ark. (2016). Samsun ekolojik koşullarında MM 106

elma anacı üzerine aşılı elma çeşitlerinin çeşitleri üzerinde yaptığı çalışmasında ölçtüğü pH miktarı 3.43-4.34. Selcen'nin (2017).

Tablo 4 Kırgız Milli Ala Arça Parka Ait Yerli Çeşitler Araştırma İstasyonunda Yetişen Elma Çeşitlerinin Bazı Kimyasal Özellikleri
Table 4 Some Chemical Characteristics of Apple Varieties Grown in the Kyrgyz National Ala Archa Park Domestic Varieties Research Station

№	SÇKM(%)	pH	TEA
Çeşit1	10.95	3.41	0.58
Çeşit2	12.15	3.81	0.21
Çeşit3	13.05	3.57	0.48
Çeşit4	13.75	3.73	0.66
Çeşit5	13.05	3.83	0.69
Çeşit6	14.55	3.84	0.34
Çeşit7	12.20	3.92	0.62
Çeşit8	15.50	5.21	0.79
Çeşit9	14.60	3.59	0.53
Çeşit10	16.75	3.27	1.03
Çeşit11	12.00	3.35	0.74
Çeşit12	11.00	3.69	0.23
Çeşit13	3.48	7.40	0.66
Çeşit14	11.15	3.02	1.86
Çeşit15	10.60	3.50	0.85
Çeşit16	10.87	3.26	1.35
Çeşit17	10.90	3.63	0.39
Çeşit18	13.50	3.62	0.55

Tokat merkez ve ilçelerinde yetişen yerel elma çeşitlerinde yaptığı araştırmasında saptadığı pH miktarı 2.88-5.30. Boyacı'nın (2019). Kırşehir koşullarında yetişen MM 106 yarı bodur elma anacı üzerine aşılı Mondial Gala. Red Chief. Golden Delicious. Braeburn ve Granny Smith elma çeşitlerinde yapmış olduğu çalışmaların sonucunda belirlediği pH miktarı 3.31-4.03 değerleri ile benzerlik göstermektedir. Ancak en yüksek pH olarak belirlediğimiz Çeşit 13 çeşidi (7.40) literatürde bildirilen değerlerden daha yüksek değer almıştır. Bu durumun çeşit özelliğinden kaynaklandığı söylenebilir.

Titre edilebilir asitlik miktarı bakımından Kırgız Milli Ala Arça parkta yetiştirilen yerel elma çeşitlerinin değerleri %0.21-1.86 aralığında saptanmıştır. Güleryüz ve ark. (1993). Kağızman ilçesinde yetişen Banem. Kaburgalı. Matibey. Mirizo. Şah ve Uzun elma çeşitlerinde yapılan araştırmada asit miktarları %0.29-0.44. Miller ve ark. (2004). Amerikan Birleşik Devletleri ve Kanada'da bazı elma çeşitleri üzerinde yürüttükleri çalışmada titre edilebilir asit miktarı %0.39- 0.98. Kırkaya ve ark. (2014). Ordu ili Perşembe ilçesinde yetiştirilen yerel elma genotiplerinin pomolojik. fenolojik ve morfolojik özelliklerinin belirlenmesi amacıyla 2010-2011-2012 yıllarında yürüttükleri çalışmada TEA oranının %0.40-1.64. Balta ve ark. (2015). Ordu ilinin Kumru ilçesinde elmalarda yaptıkları araştırmada titre edilebilir asit miktarı %0.22-2.01. Öztürk ve ark. (2016). Samsunda yaptıkları araştırmalarında titre edilebilir asitlik %0.39-0.90 ve Selcen'in (2017). Tokat merkez ve ilçelerinde yapmış olduğu araştırmada titre edilebilir asit miktarı %0.20-1.41 değerlerden daha yüksek değer almıştır.

Yapılan çalışmalarda tespit edilen değerlerle bizim tespit ettiğimiz değerler hemen hemen aynı değerlerdir. Elma çeşitlerinde meyve kabuk rengi tespitlerinde L değeri - 62.04 ile Çeşit 14 çeşidinde en düşük. 127.11 ile Çeşit 10 çeşidinde en yüksek olarak ölçülmüştür (Tablo 5). a değeri ölçümünde ise en yüksek değer 27.19 olarak Çeşit 10 çeşidinde. en düşük değer -37.3 olarak Çeşit 6 çeşidinde belirlenmiştir. Bir diğer renk değeri olan b'de ise renk dağılımı -16.32 ile 45.01 (Çeşit 14-Çeşit 18) arasında bulunmuştur (Tablo 5).

Belirlenen bu değerler. diğer araştırıcılardan Boyacı'nın (2019) Kırşehir koşullarında MM 106 yarı bodur elma anacı üzerine aşılı Mondial Gala. Red Chief. Golden Delicious. Braeburn ve Granny Smith elma çeşitlerinde belirlediği meyve kabuk rengi L değeri 19.50-78.86. a değeri -21.01-43.24. b değeri 10.10-51.24 değerleri ile

benzerlik göstermemiştir. Meyve kabuk rengi çeşide. yetiştirilen ekolojik faktörlere. toprak koşullarına ve uygulanan kültürel uygulamalarına göre farklılık göstermektedir.

Tablo 5 Kırgız Milli Ala Arça Parka Ait Yerli Çeşitler Araştırma İstasyonunda Yetişen Elma Çeşitlerinin Bazı Meyve Renk Özellikleri

Table 5 Some Fruit Color Characteristics of Apple Varieties Grown in the Kyrgyz National Ala Archa Park Domestic Varieties Research Station

№	Meyve Kabuk Rengi			Meyve Et Rengi		
	L	a	b	L	a	b
Çeşit 1	0.82	-5.34	0.86	-0.6	-0.54	-4.28
Çeşit 2	6.29	3.77	5.52	58.75	5.17	35.06
Çeşit 3	-2.8	6.45	-6.67	-1.42	3.51	6.37
Çeşit 4	66.37	1.07	37.83	65.85	-15.34	3.77
Çeşit 5	54.43	-0.97	18.13	33.07	-2.15	3.17
Çeşit 6	48.28	-37.3	40.89	-6.79	5.13	3.58
Çeşit 7	38.8	21.79	22.53	62.46	-10.69	8.53
Çeşit 8	34.51	13.46	13.66	0.47	1.3	0.42
Çeşit 9	28.08	12.14	8.44	76.87	-7.54	24.27
Çeşit 10	127.1	27.19	20.91	-0.51	4	1.8
Çeşit 11	29.84	26.28	11.43	68.03	-17.21	31.61
Çeşit 12	10.41	20.58	11.32	50.15	-18.06	13.06
Çeşit 13	29.13	8.48	6.92	76.2	-18.41	17.97
Çeşit 14	-62	-2.19	-16.32	21.45	-12.97	3.79
Çeşit 15	58.75	3.26	35.64	52.62	2.49	29.22
Çeşit 16	30.58	11.22	9.45	56.17	-14.75	12.8
Çeşit 17	65.57	-8.99	41.01	54.34	-0.1	26.95
Çeşit 18	67.11	1.51	45.01	17.49	-1.77	0.09

Sonuç ve Öneriler

Bu araştırma Kırgızistan'ın Çüy bölgesindeki Kırgız Milli Ala Arça Parkı'nda yer alan elma genotiplerinin pomolojik özelliklerini inceleyerek bu çeşitlerin üretime kazandırılmasının yanında ıslah çalışmaları için potansiyelini değerlendirmeyi amaçlamaktadır. Araştırma sonucunda incelenen 18 adet yerli elma çeşidinde önemli varyasyonlar tespit edilmiştir. Özellikle meyve büyüklüğü, meyve şekli ve meyve rengi bakımından büyük farklılıklar belirlenmiştir.

Çalışmada incelenen çeşitlerin meyve ağırlıkları bakımından ticari olarak yetiştirilen çeşitlerin ağırlıkları aynı zamanda Çeşit 14 dışındaki çeşitlerin tamamı ise meyve boyutları bakımından 1.sınıf meyve boyutlarına yakın veya üzerindedir. Bu çeşitler raf ömürlerinin belirlenmesi, muhafaza özelliklerinin belirlenmesi, hastalık ve zararlılara karşı dayanıklılık özelliklerinin belirlenmesi ile birlikte öncelikle Kırgızistan geneline yayılarak elma üretimine kazandırılmalı düşüncesindeyiz. Çalışmada incelenen yerel çeşitler tescil edilerek koruması ve dünya literatürüne kazandırılması gerektiği kanaatindeyiz. Yerel elma çeşitlerinin korunması ve değerlendirilmesi hem ülke ekonomisine hem de tarımsal çeşitliliğe önemli faydalar sağlayacaktır.

Data Availability statement
Yazar, bu çalışmayı destekleyen verilere makalede atıfta bulunulduğunu onaylamaktadır.
The author confirms that the data supporting this study are cited in the article.
Compliance with ethical standards / Etik standartlara uyum
Conflict of interest / Çıkar çatışması
Yazar herhangi bir çıkar çatışması beyan etmemektedir.

The author declare no conflict of interest.
Ethical standards / Etik standartlar
Çalışma etik standartlara uygundur.
The study is proper with ethical standards.
Authors' contributions / Yazar katkıları
Makalede adı geçen tüm yazarlar makaleye eşit oranda katkı yapmışlardır. Tüm yazarlar makaleyi incelemiş ve onaylamışlardır.
All authors mentioned in the article contributed equally to the article. All authors have reviewed and approved the article.

References

1. Bayazıt. S. D. Kılıç. and K. Gündüz. Performance of mondial gala apple cultivars grafted on M9 apple rootstock in the Mediterranean region of Turkey. MKU. Tar. Bil. Derg. 2019. 24(1) : 43-47
2. Shlyavas. A. V. et al. Genetic diversity of old and local apple (Malus × domestica Borkh.) cultivars from the collection of VIR according to AFLP analysis. Russian Journal of Genetics. 2019. 55(11). 1338–1346
3. Kırkaya. H. M. F. Balta. and T. Kaya. Perşembe İlçesinde yetişen elma genotiplerinin pomolojik. morfolojik ve fenolojik özelliklerinin belirlenmesi. Iğdır Üni. Fen Bilimleri Enst. Der. 2014. 4(3): 15-20.
4. Özrenk. K. et al. Çatak ve Tatvan yörelerinde yetiştirilen yerel elma çeşitlerinin pomolojik özellikleri. Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi. 2011. 21(1):57-63.
5. Arnold. M. and A. Gramza-Michalowska. Recent development on the chemical composition and phenolic extraction methods of apple (Malus domestica)—A Review. Food Bioprocess Technol. 2023. 10. 1–42.
6. Dzunusova. M. R. Apasov. and A. Mammadov. National report on the state of plant genetic resources for food and agriculture in Kyrgyzstan. Food and Agriculture Organization of the United Nations (FAO). Rome. 2008.
7. Uzun. A. et al. Genetic diversity in apple accessions belong to different species collected from natural populations of Tianshan Mountains. South-West Kyrgyzstan //Erwerbs-Obstbau. – 2019. – T. 61. – №. 4.
8. FAO (2023). Crops and Livestock Products. https://www.fao.org/faostat/en/#data/QCL
9. Kırgız Cumhuriyeti Ulusal İstatistik Komitesi. Tarım sektörü https://stat.kg/ru/statistics/selskoehozyajstvo/
10. Balta. M.F. et al. Ordu yöresinde yetiştirilen mahalli elma genotiplerinin fenolojik. morfolojik ve pomolojik özellikleri. Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi. 2015. 32(1): 47-56.
11. Çoşkun. S. and M.A. Aşkın. Bazı Yerli Elma Çeşitlerinin Pomolojik ve Biyokimyasal Özelliklerinin Belirlenmes. Süleyman Demirel Üniversitesi Ziraat fakültesi Dergisi. 2016. 11(1): 120-131.
12. Daler. S. M.A. Aşkın. and Y. Karakurt. Bazı birbirine benzer elma (Malus domestica L.) genotiplerinde pomolojik ve moleküler yöntemlerle genetik akrabalık derecelerinin tespiti. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi. 2016. 21(2). 444-452.
13. Farrokhi. J. et al. Evaluation of genetic diversity among iranian apple (Malus domestica Borkh.) cultivars and landraces using simple sequence repeat markers. Australian Journal of Crop Science. 2011. 5 (7): 815.
14. Güleryüz. M. and S. Ercişli. Kağızman İlçesinde Yetiştirilen Mahalli Elma Çeşitleri Üzerinde Biyolojik ve Pomolojik Araştırmalar. Atatürk Üniversitesi Ziraat Fakültesi Dergisi. 1995. 26 (2): 183-193.
15. Kuşçu. A. and Ö. Bulantekin. The effects of production methods and storage on the chemical constituents of apple pekmez. Journal of Food Science and Technology. 2016. 53(7). 3083-3092.
16. Miller. S. et al. Performance of apple cultivars in the 1995 ne183 regional project planting. Journal of the American Pomological Society. 2004. 58 (2): 65-77
17. Mratinić. E. and M. F. Akšić. Phenotypic diversity of apple (Malus sp.) germplasm in South Serbia. Brazilian archives of biology and technology. 2012. 55 (3): 349-358.
18. Özmen. Z.S. and Ç. Çekiç. Tokat yöresinde yetişen yerel elma genotiplerinde pomolojik özelliklerin tespiti. Journal of Agricultural Faculty of Gaziosmanpaşa University (JAFAG). 35(Ek Sayı). 2018. 102-107. https://doi.org/10.13002/jafag4516
19. Özmen. Z. S. and Ç. Çekiç. Tokat yöresinde yetişen yerel elma genotiplerinde pomolojik özelliklerin tespiti. Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi. 2017. 35: 102-107. http://ziraatdergi.gop.edu.tr
20. Öztürk. A. E. Aydın. and B. Ozturk. Bioactive compounds and fruit quality traits of Granny Smith Challenger apple grown under varying shade levels of green protective shade nets. Journal of Food Processing and Preservation. 2022. 46(8). e16777.
21. Öztürk. A. and B. Öztürk. Samsun Ekolojisinde Yetiştirilen Standart Bazı Elma Çeşitlerinin Pomolojik Özelliklerinin Belirlenmesi. Anadolu Tarım Bilimler Dergesi, 2016. 31(1) : 1-8.
22. Şenyurt. M. Ö. Kalkışım. and T. Karadeniz. Gümüşhane yöresinde yetiştirilen bazı standart ve mahalli elma (Malus communis L.) çeşitlerinin pomolojik özellikleri. Akademik Ziraat Dergisi. 2015. 4(2): 59-64. ISSN 2147-6403

ERRATUM

Title: "Homology Modeling of L18F Mutation on SARS-CoV-2 Spike Protein Receptor-Binding-Domain"
Published in the International Journal of Life Sciences and Biotechnology (IJLSB)
Year: 2022, Volume: 5, Issue: 3, Pages: 591–601
DOI: https://doi.org/10.38001/ijlsb.1159060

It has come to our attention that the original publication omitted a citation to a relevant conference proceeding related to the content of the study. We sincerely apologize for this oversight.

The correct reference to the related conference is as follows:

Koprululu Kucuk, G., Giritlioglu N.I. (2021). Homology Modeling of L18F Mutation on SARS-CoV-2 Spike Protein Receptor-Binding-Domain. 2nd International Sisli Science Congress, Istanbul, Turkey.

The authors regret this omission and any inconvenience it may have caused.

DÜZELTME

Makale Başlığı: "Homology Modeling of L18F Mutation on SARS-CoV-2 Spike Protein Receptor-Binding-Domain"
Yayımlandığı Dergi: *International Journal of Life Sciences and Biotechnology (IJLSB)*
Yıl: 2022, Cilt: 5, Sayı: 3, Sayfalar: 591–601
DOI: https://doi.org/10.38001/ijlsb.1159060

Yapılan incelemeler sonucunda, çalışmayla ilgili bir konferans bildirisine atıf yapılmadığı fark edilmiştir. Bu eksiklik nedeniyle özür dileriz.

İlgili konferans bildirisine ait doğru atıf aşağıdaki gibidir:

Koprululu Kucuk, G., Giritlioglu N.I. (2021). Homology Modeling of L18F Mutation on SARS-CoV-2 Spike Protein Receptor-Binding-Domain. 2. Uluslararası Şişli Bilim Kongresi, İstanbul, Türkiye.

Bu eksiklikten kaynaklanan rahatsızlıktan dolayı yazarlar üzgündür ve okuyuculardan özür diler.

Homology Modeling of L18F Mutation on SARS-CoV-2 Spike Protein Receptor-Binding Domain

Gizem Koprululu Kucuk [1]* iD **, Nazlı Irmak Giritlioglu**[2] iD

ABSTRACT

Proteins have unique properties to participate in many structural and physiological processes. Knowledge of the three-dimensional structure of proteins is important to understand their roles in the physiological processes and the functions of these processes. Any structural defect in proteins due to mutations can cause diseases, treatment unresponsiveness, and drug resistance development. The recent emergence of the new SARS-CoV-2 variants containing mutations that accelerate the spread of the virus by affecting infectiousness has been of concern. In the study, visualization of the homology model and investigation of the chemical properties of L18F mutation responsible for the formation of mutant type SARS-CoV-2 spike protein via in silico approach was intended. In this study, amino acid number, molecular weight, theoretical pI value, the percentage composition of amino acids, total negatively charged residue number, total positively charged residue number, atomic composition, formula, total atomic number, molar extinction coefficient, aliphatic index, and the average hydropathy were calculated via ProtParam. The FASTA amino acid sequence was used for visualization of the homology models via UCSF Chimera in wild-type and mutant-type spike proteins. Basic chemical calculations also were displayed on BIOVIA Discovery Studio Visualizer. ΔΔG value and the changes in the stability in L18F mutation were predicted via I-Mutant Suite software. We detected that location of the mutant residue is near a highly conserved position and the L18F mutation may not cause the damage.

Introduction

Proteins are molecules that participate in many physiological processes and are found in all organisms. Proteins participate in many physiological processes such as cell structure, cell viability, cell signals, ligand binding, and enzyme catalysis [1]. All proteins consist of amino acid sequences called polypeptide chains. In proteins in biological organisms, there are 20 types of amino acids with different characteristics, all of which have a common central carbon atom, an amino group, and a carboxyl group [2]. Each amino acid has its properties [3]. The three-dimensional structure (3D) is important for understanding protein structure [4]. The specific order of amino acids determines the 3D structure of a protein [5]. The folding of a protein into a certain conformation depends on physicochemical properties such as hydrogen bonds, ionic interactions, Van der Waals forces and hydrophobic interactions, and covalent interactions between amino acids [6]. The amino acid sequence determines how these interactions occur. The way a protein folds also depends on environmental influences such as the presence of water or lipids and the pH of the environment [7]. Knowing this structure is vital in understanding the function of the protein. Changing any amino acid can disrupt the entire structure or function of the protein by disrupting the protein and its environment, as well as the forces of interaction within the protein [8].

Knowing the three-dimensional structure of proteins is important in understanding their role in the physiological process and the functioning of this process [9]. Three-dimensional structures of proteins can be revealed by various experimental studies. However, besides these experimental studies, there are also important developments in studies on determining the three-dimensional structural models of proteins using bioinformatics methods. Any structural defect in proteins due to mutations can cause diseases, treatment unresponsiveness, and drug resistance development [10].

The emergence of new variants of SARS-CoV-2, which have emerged recently and contain mutations that affect transmissibility and accelerate the spread of the virus, has been a matter of concern [11]. Especially with the

---

[1] Department of Radiotherapy, Istanbul Sisli Vocational School Istanbul, Turkey
[2] Department of Molecular Biology and Genetics, Graduate School of Science and Engineering, Yildiz Technical University, Istanbul, Turkey
*Corresponding Author: Gizem Koprululu Kucuk, e-mail: gzm0507@gmail.com

identification of two independent strains that emerged in the United Kingdom and South Africa, studies on the detection of new mutations gained momentum. SARS-CoV-2 strains B.1.1.7 emerged in the UK (HV 69-70 deletion, Y144 deletion, N501Y, A570D, P681H, T716I, S982A, D1118H) and B.1.351 in South Africa (L18F, D80A, D215G, R246I, K417N, E484K, N501Y, and A701V) were detected in the spike protein receptor binding site (RBD) [12].

Within the scope of the study, homology modelling studies of the L18F mutation res-ponsible for the formation of mutant type coronavirus occurring in the receptor-binding domain (RBD) of spike protein by Swiss Model and amino acid number, molecular weight, theoretical pI value, the percent composition of amino acids, the total amino acid content of wild and mutant type were calculated via ProtParam.

Materials and Methods

The L18F mutation was detected in the B chain in the receptor-binding domain (RBD) of the spike protein of the Sars CoV2 virus that causes Severe Acute Respiratory Syndrome (SARS). The homology model of L18F mutation was created by bioinformatics tools. In the study, the Uniprot website and GenBank database of the National Center for Biotechnology Information (USA, NCBI) website were used detection of the amino acid sequence of the SARS CoV2 spike RBD protein [13]. Sequence information was arranged according to wild type and mutant type. Homology models of Spike protein types (wild and mutant types) were created with the Swiss Model Program which is a web-based bioinformatics tool, and the obtained three-dimensional models were examined with the UCSF Chimera program which is a visualization tool [14, 15]. Physico-chemical properties of models were obtained by ExPASy-ProtParam which is a bioinformatic tool and results were evaluated. sequence with accession numbers P0DTC2 was used for wild type in this study [16].

Swiss model analysis

The homology modelling study was carried out using Swiss-Model [17] database and the Chimera program. Wild and mutant type sequence sets were loaded into the system separately and their three-dimensional structures were obtained. The selection was made by looking at the Qualitative Model Energy Analysis (QMEAN) values of the three-dimensional structures obtained.

Homology Modelling by Chimera Program

Using UCSF Chimera program tools, all proteins were superimposed with each other, and visualization of three-dimensional structures was provided. The structural differences of the mutant protein were observed by visualizing the wild-type and mutant protein structures with a ribbon display.

Detection of Physico-chemical Properties

In the study, the Physico-chemical properties of Spike protein were calculated to understand the functional diversity. For this purpose, the ProtParam program of the ExPASy database, which is one of the bioinformatics tools, was used. FASTA formats of wild and mutant types were obtained on the GeneBank database. Physico-chemical properties of models were calculated. In both models (wild and mutant type); amino acid number, molecular weight, theoretical pI value, the percent composition of amino acids, total number of negatively charged residues, the total number of positively charged residues, atomic composition, formula, total atomic number, molar extinction coefficient, aliphatic index, and average hydropathy were calculated.

Result and Discussion

In homology modelling, protein sequence and three-dimensional structure information previously obtained by methods such as X-ray are used. Although a nature-like structure is not fully met in modelling, these studies are of great importance for clinical drug development studies. Mutations occurring in the RBD region are an important issue affecting sustained ligand binding and thus virus infectivity [18]. The changes caused by these mutations on the binding surface give us information about the degree of danger of the variation.

The amino acid sequence format that will be used as a basis in bioinformatics studies has been created based on the NCBI-P30518 accession number sequence. These arranged sequence sets were used to create the three-dimensional structure and extract the pysico-chemical properties of the models. The sequence of wild-type and mutant-type proteins is shown in Figures 1 and 2.

```
                          Wild Type

MFVFLVLLPL VSSQCVNLTT RTQLPPAYTN SFTRGVYYPD KVFRSSVLHS
TQDLFLPFFS NVTWFHAIHV SGTNGTKRFD NPVLPFNDGV YFASTEKSNI
IRGWIFGTTL DSKTQSLLIV NNATNVVIKV CEFQFCNDPF LGVYYHKNNK
SWMESEFRVY SSANNCTFEY VSQPFLMDLE GKQGNFKNLR EFVFKNIDGY
FKIYSKHTPI NLVRDLPQGF SALEPLVDLP IGINITRFQT LLALHRSYLT
PGDSSSGWTA GAAAYYVGYL QPRTFLLKYN ENGTITDAVD CALDPLSETK
CTLKSFTVEK GIYQTSNFRV QPTESIVRFP NITNLCPFGE VFNATRFASV
YAWNRKRISN CVADYSVLYN SASFSTFKCY GVSPTKLNDL CFTNVYADSF
VIRGDEVRQI APGQTGKIAD YNYKLPDDFT GCVIAWNSNN LDSKVGGNYN
YLYRLFRKSN LKPFERDIST EIYQAGSTPC NGVEGFNCYF PLQSYGFQPT
NGVGYQPYRV VVLSFELLHA PATVCGPKKS TNLVKNKCVN FNFNGLTGTG
VLTESNKKFL PFQQFGRDIA DTTDAVRDPQ TLEILDITPC SFGGVSVITP
GTNTSNQVAV LYQDVNCTEV PVAIHADQLT PTWRVYSTGS NVFQTRAGCL
IGAEHVNNSY ECDIPIGAGI CASYQTQTNS PRRARSVASQ SIIAYTMSLG
AENSVAYSNN SIAIPTNFTI SVTTEILPVS MTKTSVDCTM YICGDSTECS
NLLLQYGSFC TQLNRALTGI AVEQDKNTQE VFAQVKQIYK TPPIKDFGGF
NFSQILPDPS KPSKRSFIED LLFNKVTLAD AGFIKQYGDC LGDIAARDLI
CAQKFNGLTV LPPLLTDEMI AQYTSALLAG TITSGWTFGA GAALQIPFAM
QMAYRFNGIG VTQNVLYENQ KLIANQFNSA IGKIQDSLSS TASALGKLQD
VVNQNAQALN TLVKQLSSNF GAISSVLNDI LSRLDKVEAE VQIDRLITGR
LQSLQTYVTQ QLIRAAEIRA SANLAATKMS ECVLGQSKRV DFCGKGYHLM
SFPQSAPHGV VFLHVTYVPA QEKNFTTAPA ICHDGKAHFP REGVFVSNGT
HWFVTQRNFY EPQIITTDNT FVSGNCDVVI GIVNNTVYDP LQPELDSFKE
ELDKYFKNHT SPDVDLGDIS GINASVVNIQ KEIDRLNEVA KNLNESLIDL
QELGKYEQYI KWPWYIWLGF IAGLIAIVMV TIMLCCMTSC CSCLKGCCSC
GSCCKFDEDD SEPVLKGVKL HYT
```

Fig 1 Wild-type sequence for the formation of the three-dimensional structure

```
                         L18F Mutation

MFVFLVLLPL VSSQCVNFTT RTQLPPAYTN SFTRGVYYPD KVFRSSVLHS
TQDLFLPFFS NVTWFHAIHV SGTNGTKRFD NPVLPFNDGV YFASTEKSNI
IRGWIFGTTL DSKTQSLLIV NNATNVVIKV CEFQFCNDPF LGVYYHKNNK
SWMESEFRVY SSANNCTFEY VSQPFLMDLE GKQGNFKNLR EFVFKNIDGY
FKIYSKHTPI NLVRDLPQGF SALEPLVDLP IGINITRFQT LLALHRSYLT
PGDSSSGWTA GAAAYYVGYL QPRTFLLKYN ENGTITDAVD CALDPLSETK
CTLKSFTVEK GIYQTSNFRV QPTESIVRFP NITNLCPFGE VFNATRFASV
YAWNRKRISN CVADYSVLYN SASFSTFKCY GVSPTKLNDL CFTNVYADSF
VIRGDEVRQI APGQTGKIAD YNYKLPDDFT GCVIAWNSNN LDSKVGGNYN
YLYRLFRKSN LKPFERDIST EIYQAGSTPC NGVEGFNCYF PLQSYGFQPT
NGVGYQPYRV VVLSFELLHA PATVCGPKKS TNLVKNKCVN FNFNGLTGTG
VLTESNKKFL PFQQFGRDIA DTTDAVRDPQ TLEILDITPC SFGGVSVITP
GTNTSNQVAV LYQDVNCTEV PVAIHADQLT PTWRVYSTGS NVFQTRAGCL
IGAEHVNNSY ECDIPIGAGI CASYQTQTNS PRRARSVASQ SIIAYTMSLG
AENSVAYSNN SIAIPTNFTI SVTTEILPVS MTKTSVDCTM YICGDSTECS
NLLLQYGSFC TQLNRALTGI AVEQDKNTQE VFAQVKQIYK TPPIKDFGGF
NFSQILPDPS KPSKRSFIED LLFNKVTLAD AGFIKQYGDC LGDIAARDLI
CAQKFNGLTV LPPLLTDEMI AQYTSALLAG TITSGWTFGA GAALQIPFAM
QMAYRFNGIG VTQNVLYENQ KLIANQFNSA IGKIQDSLSS TASALGKLQD
VVNQNAQALN TLVKQLSSNF GAISSVLNDI LSRLDKVEAE VQIDRLITGR
LQSLQTYVTQ QLIRAAEIRA SANLAATKMS ECVLGQSKRV DFCGKGYHLM
SFPQSAPHGV VFLHVTYVPA QEKNFTTAPA ICHDGKAHFP REGVFVSNGT
HWFVTQRNFY EPQIITTDNT FVSGNCDVVI GIVNNTVYDP LQPELDSFKE
ELDKYFKNHT SPDVDLGDIS GINASVVNIQ KEIDRLNEVA KNLNESLIDL
QELGKYEQYI KWPWYIWLGF IAGLIAIVMV TIMLCCMTSC CSCLKGCCSC
GSCCKFDEDD SEPVLKGVKL HYT
```

Fig 2 Mutant-type sequence for the formation of the three-dimensional structure

3D structures of the spike protein were obtained by using wild and mutant-type sequence sets. 3D structure of Spike protein homology modelling was performed using the Swiss-Model (www.swissmodel.expasy.org) database and the Chimera program,

By examining the QMEAN values of the three-dimensional structures obtained, high models were selected and used as models (Table 1). We used the P0DTC2 accession number sequence for the wild type as the basis. The selection was made by evaluating the QMEAN values of the 3D structures obtained as a result of Swiss Homology modelling. The QMEAN for the wild type was detected at -1.59 and the mutant type was detected at -1.58.

Table 1 QMEAN values of the models that homology modeling was built by Swiss-Model

	QMEAN
Wild-type	-1,59
L18F	-1,58

Basic chemical calculations were displayed on BIOVIA Discovery Studio Visualizer (Table 2). Leucine is a more hydrophobic amino acid than phenylalanine (Hydrophobicity values are 3.8 and 2.8, respectively). Protein-ligand binding needs hydrophobic interactions. L18F mutation at the RBD site of the spike protein gains more hydrophilic character to the protein. Changing the stability and ΔΔG value in L18F mutation was predicted via I-Mutant software. The conditions were selected as 25°C, pH: 7.0 (default settings), and the software showed that the stability of the mutant spike protein decreased. Also, the graph of temperature–ΔΔG was created in Microsoft Excel. According to the data, ΔΔG is increased when the temperature is decreased. The increased temperature causes a more unstable mutation at the B: 18F site.

Table 2 Calculation of hydrophobicity by BIOVIA Discovery Studio Visualizer

	Full name	Hydrophobicity	Secondary structure
Wild-type	B:Leu18	3.8	Coil
Mutant-type	B:Phe18	2.8	Coil

Also HOPE web service was utilized for determining the structural effects of the mutant protein in this study. The location of the mutant residue is near a highly conserved position and L18F mutation may not cause damage. Receptor-ligand interactions could be changed negatively in the structural base because the mutant residue is bigger than the wild-type residue. The amino acid change was detected for the mutant type (Figure 3).
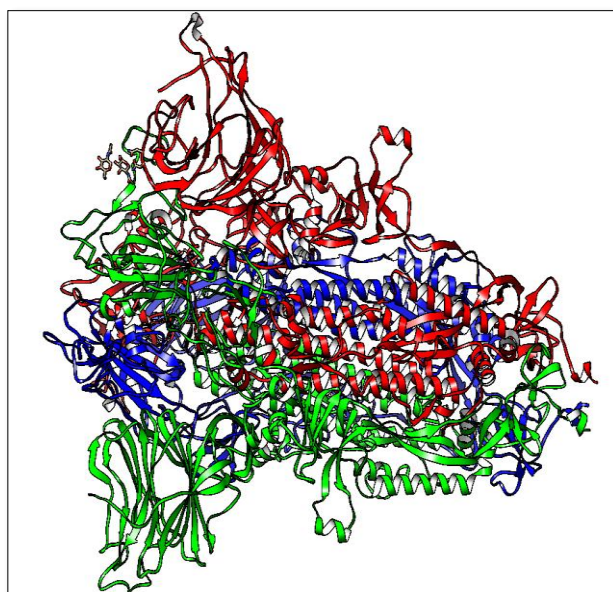
Fig 3 Wild-type ribbon structure

The change in the receptor-binding domain of the mutant type Spike protein is shown in Figure 4. The amino acid Leucine at position 18 in the wild-type receptor-binding domain of the spike protein was converted to mutant-type as phenylalanine. When the ribbon structure of both models was examined, conformational change was not observed.
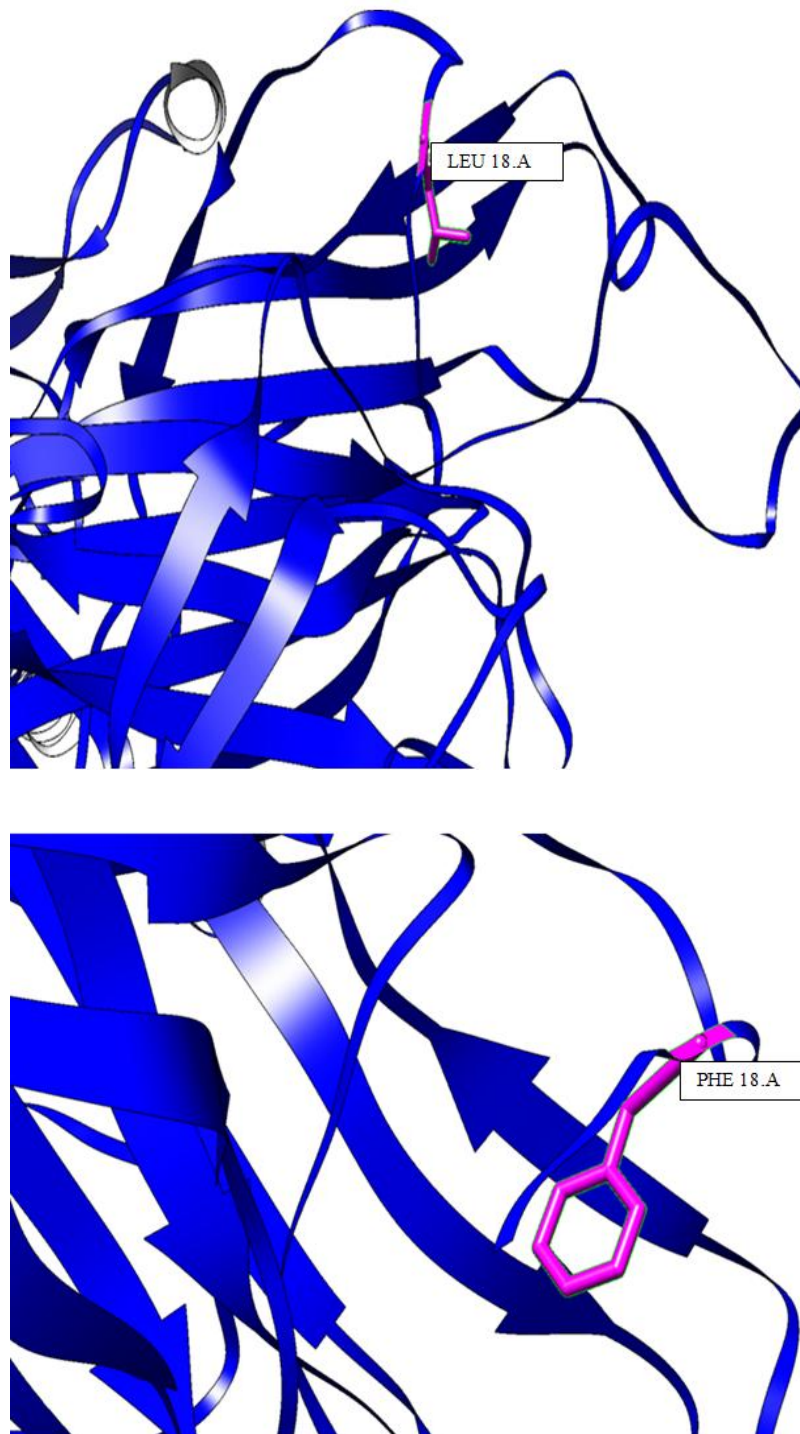
Fig 4 Amino acid changes

Leucine is a more hydrophobic amino acid than phenylalanine (Hydrophobicity values are 3.8 and 2.8, respectively). Protein-ligand binding needs hydrophobic interactions. L18F mutation at the RBD of the spike protein gains more hydrophilic character to the protein. Also HOPE web service was utilized for determining the structural effects of the mutant protein in this study. The location of the mutant residue is near a highly conserved position and L18F mutation may not cause damage. Receptor-ligand interactions could change negatively in the structural base because the mutant residue is bigger than the wild-type residue.

Travel restrictions have been imposed in most countries since the pandemic was declared. However, the SARS-CoV-2 virus continued to mutate. New SARS-CoV-2 variants occurred and continued to spread across continents.

Substitution of phenylalanine with aromatic ring instead of leucine with aliphatic chain impairs steric elasticity in the protein chain. It may cause the ligand not to cleave. It breaks conformational elasticity

sterically. Aromatic rings increase stability [19]. As the increased stability also strengthens the ligand-protein bonds, continuous binding takes place. This increases the damage of the mutation. It is a negative situation in methods such as xray in the study of protein ligand interactions. Because the relaxed structure of the protein cannot be visualized. There is no such disadvantage in homology modelling [20].

In a study, Kuzmina A. et al. detected that the L18F mutation (B. 1. 351 variant) is more resistant to greater infectivity and antibody neutralization [21]. Moreover, they showed that when K417T/E484K mutants were compared with L18F, it was detected that these mutants resisted the post-vaccination serum neutralization similarly to L18F.

On the contrary, Thomson EC. et al. found that N439K facilitated resistance to antibody neutralization [22]. Boon S. et al. speculated that N439S, T478S, and N501K mutations gave the virus a chance to infect host cells more efficiently and with low antigenicity. They detected that these mutations allow SARS-Cov2 to enter the host cell easily and make it easier to infect the cell [23].

Shahhosseini N. et al. used the SWISS-MODEL database to create homology models for SARS-CoV-2 Spike protein mutants [24]. We also used the SWISS-MODEL database for homology modelling. However, we cannot obtain hydrophobic interactions, hydrophobic structure, or atom-bond visualizing for protein by SWISS-MODEL. Because of this situation, we used the Chimera program and we backed up our models with Chimera.

Conclusion

Detection of the effects of changes in the protein sequences of the SARS-CoV-2 virus is an important element in determining the spread and severity of COVID-19. In particular, since spike protein is closely related to receptor proteins such as ACE2, FURIN, and TMPRSS2 in humans, it is extremely necessary to determine the rate and direction of change in this protein. The change from leucine to phenylalanine at amino acid 18 is found in the VOC strain that has a replicative advantage, and it is clear that such mutations result in subspecies with novel properties. When we consider the drug development process from a technological point of view, vaccine studies against COVID-19 are at the beginning and it is a matter of debate whether they are sufficient against variants. We think that it is important to model all newly formed variants just like in our study and to examine them from all chemical aspects. In addition to our in silico study, performing in vitro studies will be important in terms of understanding the beta coronavirus species and subspecies that are likely to occur in the future.

References

1. Lee, M. J., and M.B. Yaffe, Protein regulation in signal transduction. Cold Spring Harbor Perspectives in Biology, 2016. 8(6).
2. Alberts, B., A. Johnson, and J. Lewis, The Shape and Structure of Proteins. 2002, United States: Molecular Biology of the Cell.
3. Lopez, M J. and S.S. Mohiuddin, Biochemistry; Essential Amino Acids. StatPearls. Retrieved, 2020.
4. Schmidt, T., A. Bergner, and T. Schwede, Modelling three-dimensional protein structures for applications in drug design. Drug Discovery Today Elsevier Ltd., 2014.
5. Suryanto, C. H., H. Saigo, and K. Fukui, Structural Class Classification of 3D Protein Structure Based on Multi-View 2D Images. IEEE/ACM Transactions on Computational Biology and Bioinformatics, 2018. 15(1): p. 286–299.
6. Englander, S. W., and L. Mayne, The nature of protein folding pathways. Proceedings of the National Academy of Sciences of the United States of America. National Academy of Sciences, 2014.
7. Sun, P. D., C.E. Foster, and J.C. Boyington, Overview of protein structural and functional folds. Current Protocols in Protein Science, 2004.
8. Fuentes-Prior, P., and G.S. Salvesen, The protein structures that shape caspase activity, specificity, activation, and inhibition. Biochemical Journal, 2004.
9. Torrisi, M., G. Pollastri, and Q. Le, Deep learning methods in protein structure prediction. Computational and Structural Biotechnology Journal, 2020.
10. Longley, D. B., and P. G. Johnston, Molecular mechanisms of drug resistance. Journal of Pathology, 2005.
11. Khandia, R., et al., Emergence of SARS-CoV-2 Omicron (B.1.1.529) variant, salient features, high global health concerns and strategies to counter it amid ongoing COVID-19 pandemic. Environmental Research, 2022.

12. Naveca, F., et al., Phylogenetic relationship of SARS-CoV-2 sequences from Amazonas with emerging Brazilian variants harboring mutations E484K and N501Y in the Spike protein. Virological, 2021. p. 1–12.
13. https://www.ncbi.nlm.nih.gov/ Access date: 15.06.2022.
14. Waterhouse, A., et al., SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Research, 2018. p.296-303
15. Pettersen, EF, et al., UCSF Chimera—A Visualization System for Exploratory Research and Analysis. Journal of Computational Chemistry, 2004. 25(13): p.1605-12.
16. Gasteiger, E., et al., Protein Identification and Analysis Tools on the ExPASy Server, J.M. Walker, Editor. 2005, Humana Press. U.S.: U.S. p. 571-607.
17. www.swissmodel.expasy.org. Access date: 15.06.2022.
18. https://bmcmolcellbiol.biomedcentral.com/articles/10.1186/s12860-021-00403-4, Accessed: 18.10.2022 .
19. Biancalana, M., et al., Aromatic cluster mutations produce focal modulations of β-sheet structure. Protein Science, 2015. 24(5): p. 841–849.
20. Celej, MS., G.G. Montich, and G.D. Fidelio, Protein stability induced by ligand binding correlates with changes in protein flexibility. Protein Science, 2003. 12(7): p. 1496–1506.
21. Kuzmina, A., et al., SARS-CoV-2 spike variants exhibit differential infectivity and neutralization resistance to convalescent or post-vaccination sera. Cell Host Microbe, 2021. 29:p. 522–528.
22. Thomson, EC., et al., COVID-19 Genomics UK (COG-UK) Consortium, et al. Circulating SARS-CoV-2 spike N439K variants maintain fitness while evading antibody-mediated immunity. Cell, 2021. 184: p. 1171–1187.
23. Boon, S. S., et al., Temporal-Geographical Dispersion of SARS-CoV-2 Spike Glycoprotein Variant Lineages and Their Functional Prediction Using in Silico Approach. American Society for Microbiology, 2021. 12(5).
24. Shahhosseini, N., et al., Mutation signatures and in silico docking of novel sars-cov-2 variants of concern. Microorganisms, 2021. 9(5).

Bir sonraki sayıda görüşmek ümidiyle..
Hope to see you in the next issue..

GENERAL PRINCIPLES

1. The article should be submitted by the responsible author responsible as Microsoft Word (doc, docx).
2. The responsible author is responsible for monitoring all the processes of the article.
3. The main section headings should be bold and the first letter in capital letters, the first letter of the first word in the 2nd degree headings should be large. If possible, articles with the third degree titles should not be used in our journal.
4. The main headings and sub-headings should not be numbered.
5. Turkish and English titles of the articles should be short, descriptive and not more than fourteen words (except prepositions).
6. **In the Pre-Control and Evaluation processes, the authors must submit the proposed corrections within 30 days at the latest. Otherwise the article will be rejected.**
7. **Please click here to see the article written according to the rules of our journal**.
8. Except for the Turkish and English titles of the work, all remaining parts should be written on the right side.
9. The text should be written on an A4-size page, in 12-font size Times New Roman, and double-spaced.
10. There should be 3 cm margin on the left, right and top of the page.
11. Text should be written in a single column, all pages should be given a page number.

About the Ethics Committee

Are ethics committee permissions required for each article?

Ethics committee permissions are not requested for every article. It is requested for all articles in Turkish or foreign addresses that "require the permission of the ethics committee".

Research that requires the permission of the ethics committee;

• All kinds of research conducted using qualitative or quantitative approaches that require collecting data from participants using survey, interview, focus group study, observation, experiment, interview techniques,

• The use of humans and animals (including materials/data) for experimental or other scientific purposes,

• Clinical trials conducted on elderly people,

• Previous research on animals,

• Retrospective studies in accordance with the law on the protection of personal data.

Also;

• Stating that the ”Illuminated Consent Form" was taken in the case reports (case analysis),

• Obtaining permission from the owners for the use of scales, questionnaires, photos belonging to others and specifying,

• It should be stated that the copyright regulations are complied with for the intellectual and artistic works used.

• You can take advantage of the diagrams on the link at the point of whether the studies require the permission of the ethics committee. Research that requires the Permission of the Ethics Committee.pdf (link will be added)

What permissions should be obtained other than Ethics Committee permissions?

• In case reports, information about the signing of the informed consent / consent form should be included in the article.

• It should be stated that the permission document was obtained from the institution where the research was conducted (Ministry of National Education, Ministries, etc.).

• It should be stated that permission has been obtained from the owners for the use of scales,

• questionnaires, photos belonging to others.

• ABSTRACT It should be stated that the copyright regulations are complied with for the ideas and works of art used.

Note: The information given in the section About Ethics Committee authorization was taken from the TRDizin web page. Attention is paid to this issue in our journal.]

About the Plagiarism Report

It is necessary to conduct a plagiarism scan for the article with the plagiarism program e in the Dergipark article upload section. There should be a maximum similarity rate of 15%, excluding the bibliography.

Note: Similarity reports are reviewed by editors. Not meeting every similarity requirement does not mean that the publication has committed plagiarism, and meeting every similarity requirement does not guarantee that plagiarism has not occurred. Plagiarism refers to unauthorized copying, while similarity may be mandatory in certain cases. For detailed information, you can obtain information from the links below.

- **Scientific Research and Publication Ethics Directive**

- Plagiarism" Based on Administrative Judgments

- Plagiarism Control

Files to be Uploaded during Article Submission

1- **Article (Template) file** (Scientific text of the article)

2- **Author and Article information** (Special information belonging to the Author and the Article)

3- Plagiarism Report *(it will be made by "dergipark" in the article upload section)*

4- **Declaration of Ethical Responsibility and Copyright Agreement Form**

5- Ethics committee permission (if ethics committee permission is required in your work)

Some Reminders

- It is not possible to submit articles to the journal without your membership in the journal. Click here to register.

- Make sure that you have sent all the requested information correctly before submitting your article.

- Don't forget to check your articles after the issue is published. After the article is published in the journal, an editing period of 5 days is given. When this period

ends, the article is not edited. a correction article should be published in the next issue. In the case of our journal, this means that the correction will be published after six months. Therefore, please contact us as soon as possible for corrections that will not be subject to peer review after your articles are published.

The issues that are recommended to check after the article is published
-The title of the article in the first and second languages,
-Year, Volume, Number information, -Accuracy of charts and figures, -Possible layout errors,
-Spelling mistakes
-Erroneous statements, etc.

Note: The errors mentioned above should not be subject to the referee's opinion. In other words, requests for changes that involve altering the data in an article that has undergone referee review and has been accepted for publication in its current form should not be made and will not be taken into consideration.

WRITING RULES
Download the Spelling Rules file from this link: **Writing rules**

NAMES SECTION
A double-blind peer reviewer system is applied in our journal. Therefore, the names and addresses of authors should not be given in the main text when loading the Manuscript to the system. Author names and contact information should be written on a separate cover page. The cover page is available in the article submission section of our journal.

ABSTRACT
The article can be written in Turkish and English. English and Turkish abstracts should be written at the beginning of the Turkish manuscripts.

The abstract should contain brief and clear information about the purpose, method and results of the article. 10 font size, single line spacing and maximum 250 words should be written. No reference should be made to the "Abstract".

Key words: Key words should be 10 font sizes, minimum 3 and maximum 5 words. Keywords must be separated with a comma (,) sign and should be in lowercase.

INTRODUCTION

The sections of the main part of the study should be written in 14 font sizes; Bold and the first letter should be capitalized; Article should have Introduction, Methods, Results, Discussion and Conclusion sections. The second level titles should be written in the left, in 12 font sizes, the first letter of the first word should be capitalized, bold and numbered. A line in the previous paragraph must be separated by a space.

FIGURES AND TABLES

In the Turkish article for Figures and Tables;" Şekil", "Tablo"; whereas in English article "Fig", "Table" should be used (Table 1, Fig 1).

Figure and Table words should be written in bold, and at the end of the description of figures and tables should not have a dot (.)

Figures, graphics, photographs and the like should be written under Figures and written with 11 fonts. Figure and Table should be given in the main document in the relevant places, should not be uploaded as separate files or should not be added to the end of the text.

Examples

Table 1 Possible effects of genetically modified organisms

Table 2 Methods of tissue culture in tomato plants

Fig 1 Amount of GMO production in the world

Fig 2 Countries with the highest number of GM cultivation in the world

CITATION INSIDE THE TEXT

In the text, the author should be numbered. Name and year should not be specified.

Example: Potatoes produced on a global scale are used in many basic areas [1, 2, 3]. Fifty percent of primarily produced potatoes are used for fresh consumption, such as baking, frying, boiling [4, 5, 6, 7].

REFERENCES

The references section should be written in 10 font sizes and without hanging indentations. In the references section, italics should not be written except in italic words such as "in vivo, in vitro, ex-situ" and species names. References should be written according to "Chicago style". **Besides, there is an endnote style preparing for "International Journal of Life Sciences and Biotechnology". You can prepare your references using that style. For Endnote style, you can reach it by clicking on the endnote at the bottom right part of the main page of the journal.**

Example of an article with one author;

Marakli, S., A Brief Review of Molecular Markers to Analyze Medicinally Important Plants. International Journal of Life Sciences and Biotechnology, 2018. 1 (1): p. 29-36. Doi:xxxxxxxxxxxxxxxxxxxxxxxxx

Example of an article with two authors;

Kocacaliskan, I. and I. Tailor, Allelopathic effects of walnut leaf extracts and juglone on seed germination and seedling growth. The Journal of Horticultural Science and Biotechnology, 2001. 76 (4): p. 436-440. Doi:xxxxxxxxxxxxxxxxxxxxxxxxx

Example of an article with three authors;

Aksoy, H.M., Y. Kaya, and T.H. Tengku Abdul Hamid, Expression of the dspA/E gene of Erwinia amylovora in non-host plant Arabidopsis thaliana. Biotechnology & Biotechnological Equipment, 2017. 31(1): p. 85-90.Doi:xxxxxxxxxxxxxxxxxxxxxxxxxxx

Examples of articles with four or more authors;

Kaya, Y., et al., Evaluation of transgenic Nicotiana tabacum with dehE gene using transposon based IRAP markers. American Journal of Plant Sciences, 2013. 4(08): p. 41. Doi:xxxxxxxxxxxxxxxxxxxxxxxxx

Example for the book;

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:xxxxxxxxxxxxxxxxxxxxxxxxxx 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.

Ethical standards for publication exist to ensure high-quality scientific publications, public trust in scientific findings, and that people receive credit for their ideas.

Plagiarism

All journals published by IJLSB are committed to publishing only original material, i.e., material that has neither been published elsewhere nor is under review elsewhere. Manuscripts that are found to have been plagiarized from a manuscript by other authors, whether published or unpublished, will incur plagiarism sanctions.

<u>Manuscripts are checked by Ithenticate Plagiarism System.</u>

Duplicate Submission

Manuscripts that are found to have been published elsewhere, or to be under review elsewhere, will incur duplicate submission/publication sanctions. If authors have used their own previously published work, or work that is currently under review, as the basis for a submitted manuscript, they are required to cite the previous work and indicate how their submitted manuscript offers novel contributions beyond those of the previous work.

Citation Manipulation

Submitted manuscripts that are found to include citations whose primary purpose is to increase the number of citations to a given author's work, or to articles published in a particular journal, will incur citation manipulation sanctions.

Data Fabrication and Falsification

Submitted manuscripts that are found to have either fabricated or falsified experimental results, including the manipulation of images, will incur data fabrication and falsification sanctions.

Improper Author Contribution or Attribution

All listed authors must have made a significant scientific contribution to the research in the manuscript and approved all its claims. It is important to list everyone who made a significant scientific contribution, including students and laboratory technicians.

Redundant Publications

Redundant publications involve the inappropriate division of study outcomes into several articles.

Sanctions

In the event that there are documented violations of any of the above mentioned policies in any journal, regardless of whether or not the violations occurred in a journal published by IJLSB, the following sanctions will be applied:

Immediate rejection of the infringing manuscript.

Immediate rejection of every other manuscript submitted to any journal published by IJLSB by any of the authors of the infringing manuscript.

The prohibition against all of the authors for any new submissions to any journal published by IJLSB, either individually or in combination with other authors of the infringing manuscript, as

well as in combination with any other authors. This prohibition will be imposed for a minimum of 36 months.

Prohibition against all of the authors from serving on the Editorial Board of any journal published by IJLSB.

In cases where the violations of the above policies are found to be particularly egregious, the publisher reserves the right to impose additional sanctions beyond those described above.

Aim & Scope

International Journal of Life Sciences and Biotechnology (Int J. Life Sci. Biotechnol.) is an international peer-reviewed journal that publishes original articles from all biology and molecular biology studies, particularly in life sciences and biotechnology. The publication is in Turkish and English—also the main objective of Int J. Life Sci. Biotechnol. is to provide quality publications to scientists, researchers, and engineers from both academia and industry who want to communicate the latest developments and practices in their field.

Int J. Life Sci. Biotechnol. publishes original papers in various fields of Life Sciences and Biotechnology that cover, but are not limited to, the following areas:

- Agricultural Biotechnology

- Animal (livestock and fish production, physiology, breeding and genetics, biotechnology, etc),

- Animal Biotechnology

- Biochemical Genetics,

- Biochemistry

- Biodiversity and biodiscovery

- Bioinformatics and system biology

- Biology and Molecular Biology,

- Bioremediation and biodegradation

- Biotechnology

-Bioethics (Life Sciences and Biotechnology)

- Botany,

- Evolution and Population Genetics,

- Food Biotechnology

- Genetic engineering and cloning,

- Lichens,

- Genetics,

- Biotechnological product and Halal Food,

- Industrial Biotechnology,

- Medical Biotechnology

- Molecular Genetics

- Plant (Plant production, physiology, breeding and genetics, biotechnology, agronomy, horticulture, plant protection, etc.),

- Plant Biotechnology

- Soil (soil ecology, physics, chemistry, etc.)

-Polar Science (Life Sciences and Biotechnology)

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

Gulfidan Kuyumcu[1*], Muhammed Majed Abed[2]

Author Addresses: [1] Samsun Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, Samsun / Turkey
[2] Samsun Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, Samsun / Turkey

*Corresponding Autor: Gulfidan Kuyumcu, e-mail: ijlsb@intsa.org

Please write the e-mail addresses and Orcid ID numbers of all authors **(required fields)**

Author 1 mail and Orcid ID:

Author 2 mail and Orcid ID:

Author 3 mail and Orcid ID:

INTERNATIONAL JOURNAL OF LIFE SCIENCES AND BIOTECHNOLOGY
ETHICAL RESPONSIBILITY STATEMENT AND COPYRIGHT FORM*

I agree that *the International Journal of Life Sciences and Biotechnology* bears no responsibility for the content, conclusions and interpretations of the article titled:

written by the author(s) whose names appear here:

I hereby guarantee that the article I have submitted is a work in accordance with ethical rules, that it has not been submitted for publication in any other journal, that it has not been published before, and if it has been published in whole or in part, that all necessary permissions have been obtained for its publication in the above-mentioned journal and that it has been sent to the International Journal of Life Sciences and Biotechnology together with the original copyright form.

The copyrights of all articles published in the journal belong to the relevant author or authors and are protected by the relevant laws, the editorial board is authorized to be published in International Journal of Life Sciences and Biotechnology, the article published by me can be provided free open access in the International Journal of Life Sciences and Biotechnology, the users of my article in the journal can download my article provided that (1) they give reference to the copyright holder and (2) do not use the articles for commercial purposes, and share it on computer or electronic media. I give my consent for my article published in the International Journal of Life Sciences and Biotechnology to be included in paid or free academic/scientific databases within the knowledge and approval of the Journal or to be republished by the Journal, that in case I publish the article published here in other media later, I will give full reference that the article or work was published by the International Journal of Life Sciences and Biotechnology;
I hereby declare and approve.

Author's Name Surname:

Institution:

Date:

Signature / Confirmation:

Ethics Committee Report

Article If an Ethics Committee Report is required to conduct a research on animals and humans and to conduct this research, it is mandatory that the **Ethics Committee Report be scanned and uploaded as a PDF file**. Otherwise, the article is returned to the author at the preliminary examination and other stages.

1.REVIEWER SUGGESTİON

DEGREE:

FIRST NAME:

LAST NAME:

MAİL ADDRESS:

INSTITUTION:

DEPARTMENT:

SUBJECTS:

2.REVIEWER SUGGESTİON

DEGREE:

FIRST NAME:

LAST NAME:

MAİL ADDRESS:

INSTITUTION:

DEPARTMENT:

SUBJECTS:

3.REVIEWER SUGGESTİON

DEGREE:

FIRST NAME:

LAST NAME:

MAİL ADDRESS:

INSTITUTION:

DEPARTMENT:

SUBJECTS: