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ICONST LST 2024

International Conferences on Science and Technology

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September 4-6, 2024 in Durres, ALBANIA

ABSTRACTS
&
PROCEEDINGS BOOK

ICONST LST 2024

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Published by

Association of Kutbilge Academicians, Isparta, Turkiye
E-Mail: info@kutbilge.org

Publication Date: 20/10/2024
ISBN: 978-625-98911-5-6

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Contents

Influence of Microbial Biosurfactants in Aquaculture Operations For Improved Production

Thavasimuthu Citarasu *[1], Ebru Yilmaz[2]

Abstract: Biosurfactants are amphiphilic compounds that have recently gained much attention because of their potential applications in aquaculture and other fields. Biosurfactants serve as immunomodulators, antibacterial, antifungal, antiviral, anti-adhesive, antioxidants, and vaccine delivery agents in the aquaculture industry. They make good substitutes for synthetic drugs and abounded antibiotics. Shrimp white spot syndrome was controlled by lipopeptide derivatives of biosurfactants derived from halophilic bacteria, and these derivatives also exhibited antibacterial activity against significant aquatic bacterial pathogens. Sixty per cent of *V. parahaemolyticus* adhesion was inhibited by biosurfactants derived from *Bacillus amyloliquefaciens* SM11. Marine bacterial species have yielded lipopeptide, glycolipid, and polymeric biosurfactants that have been shown to effectively inhibit the formation of biofilms by vibrios. Biosurfactants have been shown to have antiviral activity as well as antimicrobial properties that cause ion channels to form in lipid bilayer membranes. *Brevibacterium casei* MSI04, which produces polyhydroxybutyrate (PHB), can prevent *Vibrio* sp. from forming biofilim. Additionally, they can lessen the water's surface tension, which will enhance the nutrient and oxygen mixing and distribution in pond water. In aquatic animals, biosurfactants can improve nutrient absorption and emulsify fats to increase feed digestibility. Because biosurfactants are amphiphilic, they can interact with cell membranes and help aquatic organisms' immune cells absorb vaccine components. Certain biosurfactants have adjuvant qualities, which means they can strengthen a vaccine's immunological response in the body. In general, the application of biosurfactants in aquaculture is a viable strategy for enhancing environmental safety, health management, and sustainability in aquaculture operations.

Keywords: Antibacterial; Antiviral; Biosurfactants; Bioremediation; Immunoadjuvant; Vaccine delivery agent

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Comprehensive Characterization of Post-Extraction Products from the Biorefining of Tree Bark Biomass in the Context of Their Eco-Innovative Upcycling Potential

Aleksandra Jeżo[1], Grzegorz Kowaluk[*1]

Abstract: Wood bark contains a number of valuable components, including structural elements such as lignin and sugars, but also metabolites such as tannins, suberin, flavonoids and many others. Numerous developed methods of extracting the above-mentioned ingredients generate post-extraction waste, which in most cases has no further use and is recycled for energy purposes or thrown away. In this project, the authors plan to consider the processes of lignin extraction using the organosolv method and alcohol/alkali extraction enabling to extract the extractives and depolymerize suberin. The organosolv method allows the extraction of cleaner and more uniform lignin than conventionally known methods such as Kraft, and is also a more ecological method. The solid waste that results from extraction also contains lignin residues, extracts, suberin, hemicelluloses and others and can be further converted into a valuable source of polymeric materials, and is also a source of glucose and furfural. Moreover, after separating lignin from the organosolv filtrate, a valuable source of 5-hydroxymethylfurfural and furfural can be obtained. The target product of water-alkali extraction is often extracts used e.g. in the production of supplements or cosmetics, but often a mixture of such soluble extracts is also an element necessary to be removed before further stages of bark extraction. Soluble compounds have the potential to be used, also, among others in the pharmaceutical industry due to its anti-inflammatory and antibacterial properties. Tannins are valuable in leather tanning, the production of binders, medicines and cosmetics, and their recovery produces large-volume residues. These wastes, ignored despite their potential, are a raw material with the potential to produce cellulosic materials, as they are mainly represented by polysaccharides and lignin. Another valuable component of bark, suberin, is a natural polyester that plays a protective role in the tree against biotic factors. Suberin can be depolymerized into the form of fatty suberinic acids, which are gaining more and more recognition among researchers because they have antibacterial properties, serve UV protection, and are highly hydrophobic. The hypothesis put forward in the following project assumes that it is possible and future-proof to develop a comprehensive cascade model for the processing of tree bark, and, consequently, for the effective and sustainable valorization of this raw material. Ultimately, such a model is intended to constitute a theoretical knowledge base about the possibilities and limitations of bark biorefinery. The tree species included in the project below are: Betula, Picea and Pinus.

Keywords bark, cascade processing, extraction residues, bark extraction, biorefining, upcycling potential

Acknowledgement: The presented study was completed within the activity of the Student Furniture Scientific Group (Koło Naukowe Meblarstwa). The research presented is a part of Project BarkBuild that is funded under the ERA-NET Cofund Forest Value Program through Vinnova (Sweden), Valsts izglītības attīstības aģentūra(Latvia), Ministry of Education, Science and Sport (JIA) (Slovenia), Academy of Finland, The ResearchCouncil of Norway, and the National Science Centre, Poland (Agreement No. 2021/03/Y/NZ9/00038).The Forest Value Program received funding from the Horizon 2020 Research and Innovation Program ofthe European Union under Grant Agreement No. 773324

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Selected Aspects of the Preparation and Characterization of Multiphase Compositions of Biopolymers and Bark

Anita Wronka[1], Grzegorz Kowaluk*[2]

Abstract: Blending biopolymers, such as polylactic acid (PLA) and starch, with other biopolymers presents several challenges and opportunities. One primary issue is the inherent incompatibility between different biopolymers, which can lead to phase separation and poor mechanical properties in the resulting blend. Achieving uniform dispersion and interfacial adhesion is often difficult, requiring the use of compatibilizers or plasticizers to enhance compatibility. The thermal stability of blended biopolymers can also be problematic, as different biopolymers may have varying melting points and degradation temperatures, complicating the processing conditions. Additionally, the moisture sensitivity of certain biopolymers, like starch, can adversely affect the blend's performance and shelf life. Balancing the biodegradability and mechanical strength of the blend is another significant challenge, as improving one property can often compromise the other. The cost and availability of biopolymers can also limit the feasibility of producing blends on a large scale. Moreover, the environmental benefits of biopolymer blends must be carefully assessed to ensure that their production and disposal do not negate their intended ecological advantages. The development of innovative processing techniques and the selection of appropriate additives are crucial to overcoming these issues. Overall, while blending biopolymers holds great potential for creating sustainable materials, it requires addressing complex material science and engineering challenges to achieve optimal performance.
The aim of the presented study was to highlight and characterize the challenges encountered when preparing blends of biopolymers with residues derived from bark processing.

Keywords: biopolymer, blend, PLA, starch, bark, processing, characterization

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The Influence of Gluing Method on The Properties of Bonded Boards – On The Example of Three Layers Particleboards With Suberinic Acids Residues Dust

Gabriela Maksymiuk[1], Aleksandra Jeżo*[2]

Abstract: Suberin, a natural polyester found in tree bark, protects a tree against biotic factors, water loss and the harmful effects of UV radiation. After depolymerization, long-chain fatty suberinic acids can be accessed, after extraction of which a solid residue remains, hereinafter referred to as SAR (suberinic acid residues). SAR contains suberin monomers, lignin, cellulose, and esters, which makes it an excellent filler and hardener in wood-based composites binder. However, its ability to absorb moisture raises doubts as to its impact on the durability and stability of particleboards in various operating conditions. Finding an effective and ecological use for SAR is important from the point of view of sustainable development and industrial waste management. The use of SAR as an additive to adhesives could not only reduce waste, but also potentially reduce the cost of particleboard production, provided that its negative impact on the board's properties is minimized. Moreover, one of the additional benefits of using SAR is its ability to reduce emissions. Emissivity refers to the emissions of harmful chemicals, such as formaldehyde, often released during particleboard production. SAR, through its chemical properties, can help reduce these emissions, which is beneficial from both public health and environmental perspectives. Previous research on the addition of SAR in three-layer particleboards has shown that the addition in larger amounts reduces the positive properties of the boards. It was noticed that SAR had a high ability to absorb moisture, which could have resulted in a decrease in the properties. Therefore, this work aims to investigate how gluing methods affect the properties of particleboards. Research focuses on various bonding methods. Option 1 involves gluing particles and SAR separately. Variant 2 involves applying the glue in a conventional way. This research will provide valuable information on optimal bonding methods that will allow SAR to produce particleboards without deterioration of their properties.

Keywords: bark upcycling, suberinic acid residues, suberinic acids, particleboards bonding

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Characterizing Multiphase Biopolymer and Bark Residue Blends Using FTIR Spectroscopy

Grzegorz Kowaluk*[1], Anita Wronka[2]

Abstract: Characterizing biopolymer blends, such as those involving polylactic acid (PLA), starch, and tree bark residues, using Fourier Transform Infrared Spectroscopy (FTIR) presents several challenges. FTIR is a powerful tool for identifying functional groups and assessing chemical interactions, but the complexity of biopolymer blends can complicate spectral interpretation. Overlapping absorption bands from different components can make it difficult to distinguish individual contributions, especially when the biopolymers and bark residues have similar functional groups. Additionally, the heterogeneous nature of these blends can result in varying local compositions, leading to inconsistent spectra. The presence of moisture, often retained by hydrophilic components like starch and bark residues, can further interfere with FTIR analysis by introducing additional absorption bands. Moreover, the interaction between biopolymers and bark residues may result in new chemical bonds or altered functional groups, which can be subtle and challenging to detect. Sample preparation also plays a crucial role; improper mixing or incomplete blending can lead to non-representative spectra. The need for meticulous baseline correction and spectral deconvolution is heightened in these complex systems. Additionally, the interpretation of FTIR data requires a comprehensive understanding of the individual spectra of all components involved. Despite these challenges, FTIR remains a valuable technique for studying the chemical structure and interactions within biopolymer blends, provided that careful attention is given to sample preparation, data acquisition, and spectral analysis.
The aim of the presented study was to describe the selected properties of biopolymer blends with residues derived from bark processing, as analyzed using the FTIR technique.

Keywords: biopolymer, blend, PLA, starch, bark, processing, characterization, FTIR

Acknowledgement: The research presented is a part of Project BarkBuild that is funded under the ERA-NET Cofund Forest Value Program through Vinnova (Sweden), Valsts izglītības attīstības aģentūra(Latvia), Ministry of Education, Science and Sport (JIA) (Slovenia), Academy of Finland, The ResearchCouncil of Norway, and the National Science Centre, Poland (Agreement No. 2021/03/Y/NZ9/00038). The Forest Value Program received funding from the Horizon 2020 Research and Innovation Program ofthe European Union under Grant Agreement No. 773324
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The Effect of Using Recycled PLA Foil Bags as a Binder on the Selected Properties of MDF Boards

Igor Borysiewicz, Grzegorz Kowaluk*[2]

Abstract: MDF boards used in furniture manufacturing contain toxic formaldehyde, which can seep into the air, soil, and water due to improper storage. Nowadays, new types of binders are being sought to replace formaldehyde-based adhesives, giving the fibreboards new properties such as biodegradability and non-toxicity. This elaboration presents the research results for MDF boards bonded with polylactide (PLA) obtained from biodegradable plastic bags. Samples were made from wood fibers and 1 mm PLA particle fractions. Six types of samples with different PLA content were produced: 10, 12, 15, 20, 30, and 50 parts by weight. For each type of sample, properties such as modulus of rupture and modulus of elasticity; thickness swelling and water absorption after 2 and 24 hours, as well as density profile, were determined. Unfortunately, MDF boards made with PLA bag particles do not possess sufficient physical and mechanical properties to replace formaldehyde-based glue. However, they have potential for use in other applications, such as packaging.

Keywords: MDF, PLA, foil bags, recycling, fibreboard

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Using Dairy Industry Solid Waste As a Binder For Non-food Applications in Dry-Formed Fiberboard Technology

Julia Dasiewicz[1]

Abstract: A lot of waste is currently generated, which is very harmful to the environment. Adhesives used as binders in wood-based composites are not biodegradable and the formaldehyde they contain is toxic. Urea-formaldehyde (UF), melamine-formaldehyde (MF), phenol-formaldehyde (PF), and other combinations are the most commonly used in the current production of adhesives for wood-based panels.

The high-density fibreboard (HDF) is made of wood fibers and formaldehyde-based resin (mainly urea-formaldehyde (UF) or melamine-urea-formaldehyde (MUF)). Therefore, researchers are currently focusing on finding bio-based adhesives, as the adhesives used for wood are synthesized from non-renewable and toxic resources, making them non-recyclable.

The research investigated the possibility of using solid residues from the dairy industry as a binder in dry-formed fiberboard technology. The scope of work included the production of boards with a mass content of milk powder of 0%, 10% 12%, 15%, and 20% (concerning the arid mass of wood fibers) and the study of their selected physical and mechanical properties. Milk powder is a dairy product obtained by evaporating water from whole, standardized, or skim (skimmed) cow's milk. The water content of milk powder is approximately 2%. The results show that the properties of the produced boards are related to the mass amount of the binder. Using the right amount of binder makes it possible to obtain values that meet the requirements of the relevant European standards.

Keywords: fiberboard, MDF, HDF, binder, milk powder

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Mechanical and Physical Properties of Particleboards Made From Energetic Willow Reinforced With Nonwoven Fabric

Katarzyna Bartoszuk[1], Grzegorz Kowaluk*[2]

Abstract: This study investigates the properties and potential applications of particleboard manufactured from energetic willow (*Salix viminalis* L.) reinforced with upholstery nonwoven fabric. Energetic willow, known for its rapid growth and high biomass yield, was selected as the primary raw material due to its sustainability and cost-effectiveness. The reinforcement with upholstery nonwoven fabric is intended to enhance the structural integrity and durability of the particleboard. Nonwoven fabric is one of the many wastes generated in the production of upholstered furniture. Unfortunately, recycling of this material is not very popular and manufacturers often do not know what to do with it. Nonwoven fabric is a material used in soft furnishings, directly under the upholstery material, as a padding layer for upholstered furniture. Nonwoven fabric gives furniture the appropriate softness and fluffiness, thereby increasing the comfort of the furniture. The research involves differentiating the boards by adding nonwoven upholstery fabric in three different configurations: 1. two layers inside the board, 2. two layers outside the board, and 3. four layers, two of which are placed inside and two outside the board. Tests of selected mechanical properties (bending strength and modulus of elasticity and resistance to screw withdrawal) and physical properties (density profile, thickness swelling after immersion in water) were completed. The results indicate significant differences in physical and mechanical properties between individual configurations. This findings suggest that particleboard made from energetic willow and reinforced with upholstery nonwoven fabric could serve as an effective, sustainable alternative to conventional wood-based materials in various industrial applications.

Keywords: recycling, energetic willow, particleboard, nonwoven upholstery fabric

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Different Modifications of Hazelnut Shell Flour as a Potential Filler in Plywood Technology

Marta Wronka[1], Grzegorz Kowaluk*[2]

Abstract: The filler in the adhesive mass in plywood plays a crucial role in increasing the durability and strength of the finished product. Thanks to the filler, the adhesive adheres better to the layers of wood, preventing the plywood from delaminating under load. Additionally, the filler can improve moisture resistance, which is important for plywood used in outdoor conditions. Another advantage is the improvement of the adhesive mass's flexibility, allowing better adaptation to the natural movements of the wood. The filler can also affect the aesthetics of the plywood, ensuring a uniform surface without visible defects. Ultimately, a properly selected filler can reduce production costs by allowing the use of a smaller amount of more expensive adhesive components.

In the studies, plywood was produced with different amounts of fillers obtained from the grinding and powdering of hazelnut shells into flour. The fillers differed in their modification methods: native (unmodified) filler, chemically modified (delignified) filler, and activated carbon filler from the shells. Rye flour was used as a reference filler. The produced plywood was tested for its selected mechanical properties (bending strength, bonding quality) and physical properties (density profile, swelling, and water absorption). The studies showed differences between the tested variants of plywood and the impact of the type and amount of filler on the properties of the plywood.

Keywords: plywood, filler, bonding mass, modification, bending, density profile

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Probiotics as Biofertilizers and Biocontrol Agents: Revolutionizing Citrus Cultivation for Sustainable Agriculture

Sohaib Afzaal*[1]

Abstract: Citrus crops, including oranges, lemons, limes, and grapefruits, are economically significant but face challenges such as soil degradation, nutrient deficiencies, and susceptibility to diseases. Environmental stressors like high temperatures and drought further exacerbate these issues, leading to significant economic losses for producers. Conventional agricultural practices often rely on synthetic fertilizers and pesticides, contributing to environmental pollution. Probiotics, beneficial microorganisms known for their human/animal health benefits, have emerged as sustainable biofertilizers and biocontrol agents in agriculture. This mini-review explores the potential of probiotics to enhance citrus cultivation by improving soil health, promoting plant growth, managing diseases, and increasing stress tolerance. Probiotics such as Bacillus subtilis, Pseudomonas fluorescens, and Lactobacillus plantarum have demonstrated their efficacy in citrus cultivation through various mechanisms. These include nitrogen fixation, phosphate solubilization, production of plant growth hormones, and biocontrol of pathogens. Studies have shown that probiotics can significantly enhance the growth, yield, and quality of citrus fruits while reducing the incidence of diseases like citrus canker and root rot. Probiotics also improve the nutrient composition of citrus by-products, making them valuable for high-value food or feed materials. This review highlights the mechanisms by which probiotics benefit citrus crops, including nutrient mobilization, phytohormone production, and induction of systemic resistance. Additionally, it addresses the challenges and future perspectives of using probiotics in citrus cultivation. Despite the promising potential, issues such as variability in field performance, formulation and application methods, and regulatory considerations need to be addressed.

Keywords: Lactic acid bacteria, Citriculture, Endophytes, Beneficial Bacteria, Biofertilizers, Biopesticides.

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Why is Taxonomic Diversity not as Popular as Species Diversity? A Bibliometric Analysis

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Abstract: Biodiversity is commonly attributed to genetic diversity (GD), species diversity (SD), and ecosystem diversity (ED). However, taxonomic diversity (TD) can provide useful information for communities within ecosystems, at least as much as traditional diversity indices. When examining the literature, it is noteworthy that studies on TD are not as prevalent as those on SD. Based on this information, a bibliometric analysis was conducted by obtaining separate data for both TD and SD from the Web of Science database, allowing for comparative analysis. In terms of filtering options, articles and review articles were selected as document types, and English was chosen as the language. A total of 1,938 documents were obtained for TD, while 20,511 documents were obtained for SD. The top five countries with the highest scientific production in TD were determined as China, Usa, Brazil, France, and Russia, while for SD, they were determined as USA, China, Brazil, Germany, and India. The most common scientific fields for TD studies were found to be ecology, environmental sciences, biodiversity and conservation, plant sciences, and marine & freshwater biology. In addition, the top five keywords used were taxonomic diversity, functional diversity, diversity, biodiversity, and phylogenetic diversity. On the other hand, as for SD, the results showed that the most common scientific fields for studies were ecology, environmental sciences, biodiversity and conservation, plant sciences, and forestry, while the top five keywords used were species diversity, species richness, diversity, genetic diversity, and biodiversity. The results indicated that SD is more frequently preferred in studies, especially in the natural sciences. It is thought that this preference is influenced by the fact that species diversity calculations involve more understandable calculations and relatively more software packages available for calculations. However, the proportional increase in studies in recent years has shown that taxonomic diversity is being increasingly preferred.

Keywords: Bibliometric analysis, bibliometrix, R software, species diversity, taxonomic diversity

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Effect of Palm Date Waste as Feed Livestock on Zootechnical Performances of Small Ruminant and Broiler Chickens

Samira Meradi[1*], Miloud Aouachria[1]

Abstract: Palm date waste (PDW) is a locally accessible bioressources usually used in feed livestock in dry and semi arid regions. Valorization of palm dried leaves and seed palm date have been generated a significant enhancing in breeding productive performance. The nutritional constitutes values of seed date is reported by the average protein content from 4% to 10% dry matter, and more than 70% dry matter carbohydrate including cellulose, hemicelluloses and lignin. Seed date has a significant mineral amount of potassium 17%, magnesium 3.4% and calcium 3.5%. Furthermore the weight of seed date varies from7% to 10% of total weight date. It is mostly utilized to feed livestock: sheep, goats, camels, and poultry. Indeed, the average of seed date inclusion percentage in concentrate feed of ruminant has been limited from 20% to 70%, it provided 6 average feed conversion ratios. However, in poultry concentrate feed, the significant average seed date has been limited to less than 10% of basal diet, it provided 1.7 average feed conversion ratios. In addition, date pit has potential amount secondary metabolite of antioxidants and phenolic compounds, which is known by its oxidative stability. However incorporation of palm dried leaves was studied only in small ruminant feed, it provides a consequently results, whereas the feed conversion ratio is in average of 7 and the daily gain is in average of 130 g/D. Economically PDW is an efficiency low cost feed with an important nutrition value. It contributes to enhance the income of local farmers and breeders in arid regions of Algerians. The purpose of this paper is to investigate the effect of incorporating different levels of PDW on zootechnical performance of small ruminants and broiler chickens.

Keywords: feed, income local farmers, livestock, palm date waste; zootechnical performance.

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Using Digital Agriculture for Sustainable Food Systems in the Municipality of Pristina, Kosovo

Sokol Sallahu*[1]

Abstract: The Municipality of Pristina, Kosovo, stands at the intersection of tradition and innovation in agriculture, presenting a unique opportunity to leverage digital technologies for sustainable food systems. This study delves into the transformative potential of digital agriculture in enhancing productivity, promoting sustainability, and fostering resilience in Pristina's agricultural landscape. In recent years, the agricultural sector in Pristina has witnessed a gradual shift towards modernization, driven by the adoption of digital tools and practices. The integration of technologies such as precision farming, remote sensing, and data analytics has opened new avenues for optimizing resource use, improving crop management, and mitigating environmental impact. By harnessing the power of digital agriculture, farmers in Pristina can enhance their efficiency, reduce input costs, and increase yields while minimizing the ecological footprint of their operations. The sustainable food systems agenda in Pristina encompasses a holistic approach that addresses not only production efficiency but also environmental stewardship, social equity, and economic viability. Digital agriculture plays a pivotal role in advancing this agenda by enabling real-time monitoring of crops, soil, and weather conditions, facilitating precision irrigation and fertilization, and empowering farmers with data-driven insights for informed decision-making. Through the integration of digital technologies, the Municipality of Pristina can promote sustainable farming practices, enhance food security, and contribute to the overall well-being of its agricultural community.

Keywords: Digital Agriculture, Sustainable Food Systems, Precision Farming, Environmental Stewardship

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Some Biological Activity Studies on Endemic *Salvia potentillifolia* Boiss. & Heldr. ex Benth. (LAMIACEAE)

Rumeysa Mert[1], Batıkan Günal*[1], Gürkan Semiz[1]

Abstract: Within the Lamiaceae, *Salvia* L., commonly referred to as sage (or adaçayı in Turkish), is the largest genus. There have long been records of the usage of *Salvia* as an aromatic and medicinal herb. Salvia species are used as a herbal tea to cure rheumatic, stomach, and liver ailments as well as wounds. They are also used as a spice, sauce, and culinary addition. Furthermore, infusions and decoctions made from it are utilized as a cold remedy around the world. From natural populations in Elmalı, Antalya, *Salvia potentillifolia* was gathered (GSE 2486). Using solvents with varying polarities (*n*-hexane, chloroform, ethanol, methanol, and water), the samples were extracted. Total phenolic and total flavonoid content as well as its DPPH antioxidant capability of *S. potentillifolia* were evaluated using extracts. The Folin-Ciocalteu reagent was used to calculate the total phenolic content using the gallic acid curve. The extracts of water (157.318 ± 3.14 mg GAE/ml) and ethanol (144.303 ± 4.62 mg GAE/ml) had the maximum phenolic content. The calibration curve for quercetin and rutin hydrate was used to determine the total flavonoid concentration in the extracts. Methanol (88.009 ± 1.46 mg RU/ml, 55.157 ± 0.94 mg QE/ml) and ethanol extracts (76.043 ± 0.18 mg RU/ml, 47.427 ± 0.11 mg QE/ml) exhibited the highest flavonoid content. Using 1,1-diphenyl-2-picrylhydrazyl (DPPH), the antioxidant activity of *S. potentillifolia* extracts in various solvents was investigated. Methanol (IC50: 12.66 µg/ml) and water extracts (IC50: 13.96 µg/ml) showed the maximum antioxidant capacity, but n-hexane extract (IC50: 498.964 µg/ml) had the lowest. According to this study, *S. potentillifolia* species may be a valuable natural resource that is utilized in the production of nutraceuticals, cosmetics, and medications.

Keywords: Salvia potentillifolia, total phenolic content, total flavonoid, antioxidant, bioactivity

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Some Biological Activity Studies on Endemic *Salvia nydeggeri* Hub.-Mor (LAMIACEAE)

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Abstract: *Salvia* L., usually known as sage (or, adaçayı in Turkish), is the biggest genera within Lamiaceae containing. The use of *Salvia* as a medicinal and aromatic plant has been reported since ancient times. *Salvia* species are used in a variety of applications, such as a sauce, spice, culinary additive, and herbal tea to treat wounds and stomach, liver and rheumatic symptoms. Additionally, it is used in various regions of the world to treat colds in the form of infusions and decoctions. *Salvia nydeggeri* were collected from natural populations in Elmalı, Antalya (GSE 2485). Extraction of the samples was achieved using solvents of different polarity (n-hexane, chloroform, ethanol, methanol, water). Using extracts, total phenolic and total flavonoid content, and DPPH antioxidant capacity of Salvia nydeggeri were assessed. Using the gallic acid curve, the total phenolic content was evaluated with the Folin-Ciocalteu reagent. The highest phenolic content was found in water (110.489 ± 5.07 mg GAE/ml) and methanol extracts (109.735 ± 2.25 mg GAE/ml). The total flavonoid content in the extracts was determined using rutin hydrate and quercetin calibration curve. The highest flavonoid content was found in methanol (151.22 ± 2.64 mg RU/ml, 96.00 ± 1.71 mg QE/ml) and chloroform extracts (150.32 ± 3.15 mg RU/ml, 95.41 ± 2.03 mg QE/ml). The antioxidant activity of *S. nydeggeri* extracts in different solvents was studied using DPPH (1,1-diphenyl-2-picrylhydrazyl). The highest antioxidant capacity was observed in methanol (IC50: 12.66 µg/ml) and water extracts (IC50: 13.96 µg/ml), while the lowest was in n-hexane extract (IC50: 498.964 µg/ml). This study shows that *S. nydeggeri* species may be an important natural resource that can be used in the development of pharmaceuticals, cosmetics and nutraceuticals.

Keywords: Salvia, total phenolic content, total flavonoid, antioxidant, bioactivity

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Storm Intensity and Forest Resilience: Analyzing the Ecological and Economic Impacts of the 2017 Derecho in Poland

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Abstract: Recent research highlights an alarming increase in the frequency and intensity of storm events worldwide, a trend closely correlated with ongoing global climatic changes. This study discusses the implications of such climatic shifts on storm behavior, particularly focusing on a case study of the 2017 derecho in Poland, one of the most severe recorded in the region. The storm, characterized by hurricane-force winds, traversed over 300 km, causing unprecedented forest devastation and significant ecological and economic impacts. It destroyed nearly 10 million cubic meters of trees and affected 120,000 hectares, necessitating extensive recovery efforts and economic expenditures estimated at around 1 billion PLN. Compounding the challenges of forest renewal is the spread of an invasive Prunus serotina and various diseases, alongside heightened fire risks, which significantly impact the regeneration of previously devastated forest areas. This event underscores the growing need to integrate climate change projections into disaster preparedness and forest management strategies. By examining the 2017 derecho and subsequent ecological challenges, this study illuminates the broader implications of storm effects on the regeneration of forests once destroyed by strong winds.

Keywords: Derecho, Forest Resilience, Storm Intensity, Ecological Impact, Climate Change Adaptation.

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Investigation of Quality in the Processing of Commodities Resulting from Forest Fires in a Sawmill

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Abstract: Forests are among the natural and sustainable resources with significant economic value. However; rapidly increasing population, urbanization, increasingly diverse consumption habits, environmental pollution, climate change, desertification, large-scale forest fires, and deforestation are increasing global problems that put excessive pressure on forests. Increasing wood demand and alleviating problems caused by unpredictable factors such as fires on forests will be possible with the effective and efficient use of sustainable wood raw materials. This study addresses the problems encountered by a medium-sized sawmill processing forest materials from forest fires. The inefficiencies and quality losses that occur during the processing of burnt field materials supplied after forest fires were examined, and points for increasing the quality and efficiency of the process were determined using the p control chart for these processes. In addition, the quality status of the logs stocked in the factory log warehouse were examined and photographed. Within the scope of the study, deviations in the logs processed by the trolley band saw were measured. It was observed that 28% of the logs were cut outside the reference values (96, 97, 98 cm). Biotic pests were detected in 353 logs, color change was observed in 277 logs, and biological degradation was observed in 27 logs. As a result of the research, it was seen that there was no significant difference in the processing process of logs that emerged as fire materials compared to normal logs, but fungal and insect damage occurred more quickly in burnt materials. Therefore, processing burnt materials as soon as possible and turning them into products will prevent possible quality losses. Considering that burnt materials used as raw materials after forest fires are purchased at a lower cost compared to normal raw materials, preventing possible quality losses will provide financial gain to businesses.

Keywords: forest fire, degradation, sawmill, quality loss

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Species Distribution Modeling of the Black Pine in Aydın – Kuyucak District

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Abstract: This study aims to distribution modelling of black pine (*Pinus nigra* Arnold.), one of the ecologically and economically significant species in our country, in the Aydın – Kuyucak district. In this context, presence-only data obtained from land inventory studies and environmental and climatic variables of the target species were modeled using the Maximum Entropy (Maxent) method. Among the variables structuring the model, Bio_19 (Precipitation of Coldest Quarter) contributed the most. According to the distribution model of the target species, it preferred the rainfall of the cold season, i.e. 375 mm of rainfall in December, January and February for our country, and according to the topographic position index and valley depth, it preferred the middle and upper slopes and mountainous areas and places with metamorphic rocks with gneiss, phyllite, and metagranitoid features. The milder winter climate and precipitation in the form of rain in the Mediterranean and Aegean regions have created more suitable habitats for black pine. Even though black pine is a hardy species, the rains in such areas help the species to survive in certain regions because they help soil moisture and water retention capacity. On the other hand, the presence of metamorphic rocks, which are generally encountered in mountainous and sloping terrains, middle and upper slopes and mountainous areas point to the places above 1200 m in the region, and it has been determined that these areas have an important place in the potential distribution of black pine. In light of these results, important findings were obtained for the protection and sustainability of the distribution areas of black pine, which has a limited distribution area in the region. This study provides important information for the long-term conservation and management of the target species, especially for the development of appropriate conservation strategies against the reduction, loss and fragmentation of black pine in its distribution areas as a result of climate change.

Keywords: Aegean region, Büyük Menderes river basin, Climate change, Ecological modelling

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An Investigation on Transparentization of Poplar and Red Pine Wood

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Abstract: The research on transparent wood production possibilities, which have just started to be investigated worldwide, is increasing. In this study, it is aimed to obtain transparency on some local species. The transparentization process is basically based on the principle of removing lignin, which is one of the chemical compositions of wood. Red pine and poplar woods, which are among the abundant species, were preferred in the study and it was aimed to increase their economic values as well as their usage possibilities. The wood species used in this study were prepared according to 2.0, 3.0, and 4.0 mm thickness values and the samples were boiled for 12 hours by dipping them into the delignification solution prepared by dissolving NaOH and Na2SO3 in pure water and then placed in the bleaching solution and boiled without stirring. Then, the lignin-removed samples were placed in a container and made transparent by adding liquid resin. Some mechanical, thermal and optical properties of the produced material were investigated.

Keywords: Transparent wood, Poplar, Red Pine, chemical treatment, mechanical, thermal and optical properties.

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Impact of Climatic Conditions on the Economy of Oilseed Rape Cultivation in Conditions of Slovak Republic

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Abstract: The cultivation of oilseed rape (Brassica napus L.) is significantly influenced by climatic conditions, which have a direct impact on its economic viability. This study investigates the connection between climate factors and the Slovak Republic's oilseed rape cultivation financial results. Using data for the period 2015-2022 climatic parameters that affect production and profitability are identified by the research using a combination of meteorological data, agronomic records, and economic studies. Attention is given to brief information on the development and situation of the oilseed rape, where focus is given on the analysis of the development of selected indicators during the observed period, such as production, area sown, yields, and economic results. The results show that farmers growing oilseed rape face significant challenges due to changes in temperature and average precipitation. Based on the results, both stated hypothesis were rejected what means, that there is not any significant dependency between oilseed rape production and average temperature and precipitation in Slovakia. The study also looks at adaptive tactics and methods that help improve resilience and sustainability by reducing the negative effects of climate change. This research provides valuable insights for farmers, policymakers, and stakeholders to develop adaptive measures that ensure the economic stability of oilseed rape cultivation in the Slovak Republic.

Keywords: agriculture, oilseed rape, climate change, economics

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1. INTRODUCTION

One of the most pressing issues facing the modern world is climate change, which has drastically changed or is currently changing the planet's ecosystems. Although the earth's climate has been changing continuously over the past 100 years or more, the rate at which this variation has greater manifolds. The average temperature has increased by 0.9 °C since the 19th century as a result of human activity, mostly because of greenhouse gas (GHG) releases into the atmosphere. According to projections, this rise might be as much as 1.5 °C by 2050, given the rate of deforestation, the rise in greenhouse gas emissions, and the pollution of the air, water, and soil (Naveen Kumar, A., 2019). Sea level rise, ocean acidification, and changes in numerous other components of the climate are all associated with variations in the average temperature. According to Steffen et al. (2018), these processes combine with other global environmental changes (GECs) such as the loss of biodiversity, changes to biogeochemical cycles, and the worldwide dissemination of materials and chemicals that have a negative effect on ecosystems and interfere with services that humans depend on. Proposals have been made to refer to the current geological epoch as the Anthropocene since these changes are so significant that they will probably be seen in the geological record millions of years from now. Humans and civilizations will probably undergo radical changes as a result of GECs and our efforts to respond to them, from the local to the global (IPBES 2019).

Due to its enormous scale and susceptibility to meteorological conditions, agriculture is the industry most susceptible to climate change, which will have a significant economic impact (Mendelsohn, R., 2009). The amount of crop output is greatly impacted by variations in meteorological events like temperature and rainfall. The crop, location, and degree of parameter change all affect the effects of rising temperatures, fluctuating precipitation, and CO2 fertilization. It has been observed that rising temperatures lower yields, while rising precipitation is likely to neutralize or lessen the effects of rising temperatures. Crop productivity in Iran is contingent upon climate variables, crop type, crop response capacity, and the effect of CO2 fertilization (Karimi, V. et al., 2018). Crop yield declines have the potential to drive up food prices and have a significant impact on agricultural wellbeing worldwide, with a 0.3% yearly loss of future global GDP by 2100 (Stevanovic, M., et al., 2016). On the other hand, (Kumar, R.; Gautam, H.R.., 2014) discovered that while the impact of climate change on the global food supply is modest, poor nations will suffer greatly as a result. On the other hand, certain regions have seen a rise in agricultural productivity as a result of climate change. However, neither these regional increases nor declines would cause drastic alterations, nor they would only become more noticeable in a few low latitudes. But if the temperature rises over the point when CO2 is doubled, significant financial losses may result (Aydinalp, C.; Cresser,

M. 2008). Tropical regions of underdeveloped countries will be severely impacted by climate change, albeit this will mostly depend on the climate scenario in the area. Compared to the cooler central highland region of Sri Lanka, whose agricultural output is predicted to stay the same or maybe rise with rising temperatures, the drier north and east of the country would suffer significant losses (Seo, S.N.; 2005). Environmental policy must be dynamic and executed with adaptation and flexibility because the rate of climate change impacts society and determines the cost of adjustment (Zilberman, D.; 2004). The burden of maintaining global food and nutritional security due to population growth has placed significant strain on agriculture, a situation that is made worse by climate change. Climate change will reduce agricultural productivity in the coming years, according to a number of studies, notwithstanding the uncertainty surrounding the future climate scenario and its potential effects. The primary determinants of climate, particularly temperature, precipitation, and greenhouse gasses, greatly hindered plant metabolism, physiology, soil fertility, pest infestation, and irrigation resources. Various solutions for adaptation and mitigation have been devised to counteract the adverse effects of climate change on the sustainability of agriculture. These technologies include weather-smart activities (stress-tolerant varieties, ICT-based agrometeorological services), carbon-smart activities (zero tillage, legumes, crop residue management), and knowledge-smart activities (agricultural extensions to enhance capacity-building). Water-smart practices (laser land leveling, rainwater harvesting, micro-irrigation, crop diversification, raised-bed planting, direct-seeded rice) and nutrient-smart practices (precision nutrient application, leaf color charts, crop residue management) are among them. By lessening the negative consequences, these technologies greatly lessen the effects of climate change on crops and improve their climate suitability (Gurdeep Singh, M., 2021). Climate variations, such as variations in temperature and precipitation, can have a significant impact on food production systems. These variations can also result in pest and disease outbreaks, which can lower harvests and eventually impact the nation's food security. The exposure to and ability to adapt to and recover from global environmental change will determine the overall impact of food security. It will be necessary to manage resources like soil, water, and biodiversity carefully in order to combat the effects of climate change on agriculture. In order to address how climate change is affecting agricultural and food production (Bhattacharya, A., 2019). Hulme, M. (1996) describes four ways in which climate would have a physical effect on crops. First, changes in temperature and precipitation will alter the distribution of agro-ecological zones. Second, carbon dioxide effects are expected to have a positive impact due to, for example, greater water use efficiency and higher rate of photosynthesis. Third, water availability (or runoff) is a critical factor in determining the impact of climate change in many places, particularly in Africa. Fourth, agricultural losses can result from climatic variability and the increased frequency of extreme events such as droughts and floods or changes in precipitation and temperature variance.

Slovakia has the essence of a rural nation. It is distinct in that Slovene people are moving from the cities to the countryside in defiance of a global trend. With almost half of its inhabitants residing in rural areas, it is the second most rural state in the European Union. Slovakia's percentage of rural area is even higher. Slovakia is among the weakest nations in the intricate and methodical assessment of the Slovak Republic's agricultural performance inside the EU. Our modern agribusiness is trying to establish the land market and provide the internal market with items that are both reasonable and of high quality, but it is having difficulty dealing with organizational and legal concerns (Jankelova N. et al.2017).

Oilseed rape (Brassica napus), commonly known as canola, is a vital crop in the Slovak Republic, significantly contributing to the agricultural economy and providing raw material for various industries, including food, feed, and biofuel production. The cultivation of oilseed rape is inherently influenced by climatic conditions, which play a crucial role in determining both the yield and quality of the crop. In the context of the Slovak Republic, understanding the impact of these climatic factors is essential for optimizing production strategies and ensuring the sustainability and profitability of oilseed rape farming. It is imperative that it boost productivity, restore the land resources' production potential, and form sustainable units. In the context of subsidies, those could be suitable partners in the EU or they could compete with other organizations. The majority of the rape farmed in Slovakia is exported and used to produce methyl ester of rape oil (MERO). Rape is used less frequently for food production, the making of heating pellets, cosmetics, and animal feed (Récky, R and Horváthová, J., 2018).

2. MATERIAL AND METHOD / MATERYAL VE METOT

This methodology provides a comprehensive framework for analyzing the impact of climatic conditions on the economy of oilseed rape cultivation in the Slovak Republic. By integrating climatic, agronomic, and economic data, the study aims to offer actionable insights for enhancing the resilience and profitability of oilseed rape farming under changing climatic conditions.

This study employs a quantitative research design to analyze the impact of climatic conditions, specifically average temperature and precipitation, on the production of oilseed rape in Slovakia. The primary aim is to determine the significance of these climatic factors and their correlation with oilseed rape production. The data used in this scientific

contribution were taken from the Ministry of Agriculture and Rural Development of the Slovak Republic and from the portal Trading Economics.
The level of oilseed rape production in Slovakia during the period between 2015 and 2022 is assessed in terms of such indicators as the production of selected crops, yields, price and area harvested. Basic economic indicators of agricultural production in Slovakia were analyzed to, such as economic result, revenues and costs. To define whether there is a dependency among production of oilseed rape and avarega temperature and average percipitation in Slovakia we defined two hypotheses.

H1: We assume there is a dependency between production of oilseed rape and average temperature in Slovakia.
H2: We assume there is a dependency between production of oilseed rape and average percipitation in Slovakia.

Two separate regression models were constructed to evaluate the impact of average temperature and precipitation on oilseed rape production.

$$Yj\ ' = \beta\ 0 + \beta 1Xj$$

where:
β0 – locating constant, β1 – regression coefficient, yj´ – theoretical values depending variable, xj – values of the independent variable.

The methodology outlines a structured approach to assessing the impact of climatic conditions on oilseed rape production in Slovakia. Despite the findings of weak correlations, the study provides a foundation for further exploration of the complex factors influencing agricultural productivity in the region.

3. RESULTS

The data on agriculture production in Slovakia from 2015 to 2022 reflects positive trends in terms of economic results, revenues, and costs per hectare. Despite the rising costs, the revenues have increased at a higher rate, leading to improved economic outcomes per hectare. The substantial increase in economic result in 2021 suggests that the agricultural sector experienced particularly favorable conditions or improvements in practices and efficiencies that year.
The economic result per hectare shows significant variation over the years. It starts relatively low in 2015 (23 EUR/ha) and increases steadily, peaking in 2022 (239 EUR/ha). This indicates an overall improvement in profitability per hectare, despite some fluctuations. Revenues per hectare show a general upward trend. Starting at 1836,31 EUR/ha in 2015, revenues increase each year, reaching their highest point in 2022 (2821,63 EUR/ha). This consistent growth suggests an increase in the value of agricultural production or improved efficiency in revenue generation. Costs per hectare also increase over the years, from 1813,81 EUR/ha in 2015 to 2582,49 EUR/ha in 2022. While costs rise, the rate of increase is generally slower than that of revenues, which supports the positive trend in economic results.

Table 1. Development of economic indicators of agricultural production in Slovakia

Indicator/Year	2015	2016	2017	2018	2019	2020	2021	2022
Economic result (€ . ha^{-1} a.l.)	23	53	86	69	54	41	146	239
Revenues (€ . ha^{-1} a.l.)	1836,31	1 891,60	2 003,63	2 079,10	2 007,60	2 038,32	2 326,49	2 821,63
Costs (€ . ha^{-1} a.l.)	1813,81	1 838,40	1 917,76	2 010,00	1 955,60	1 998,87	2 192,66	2 582,49

Source: Own processing based on data from Ministry of Agriculture and Rural Development of the Slovak Republica – agriculture land

The data on oilseed rape in Slovakia from 2015 to 2022 reflects considerable variability in area harvested, yields, and production, with occasional peaks and troughs. Prices have generally remained high but saw a significant drop in 2021. The inconsistencies in yields and the large fluctuations in the area harvested suggest that the sector might be influenced by external factors such as weather, market demand, or agricultural policies.

The area harvested fluctuates significantly, with a notable spike in 2016 (124,5 thousand tonnes) and another high in 2017 (150,1 thousand tonnes). However, there is a drastic drop in 2019 (147 thousand tonnes) and 2021 (136 thousand tonnes).

Yields per hectare are inconsistent. This inconsistency could be due to varying agricultural practices, climate conditions, or other factors affecting crop yield. Production data shows peaks in 2018 (480 thousand tonnes) and 2017 (448,7 thousand tonnes. The price per tonne shows an overall high trend, peaking in 2022 (686,59 EUR/t).

Table 2. Development of oilseed rape indicators in Slovakia

Indicator/Year	2015	2016	2017	2018	2019	2020	2021	2022
Area harvested (in ths.tonnes)	119	124,5	150,1	154,2	147	146,6	136	141,4
Yields (t/ha)	2,69	3,5	3	3,1	2,8	3	3,1	3,1
Production (in ths. tonnes)	321	430,5	448,7	480	417,6	440,9	420,1	441,7
Price (Eur/t)	359,53	356,73	375,16	343,38	355,11	366,81	441,4	686,59

Source: Own processing based on data from Ministry of Agriculture and Rural Development of the Slovak Republic

Figure 1 provides annual data on average temperature and precipitation in Slovakia from 2007 to 2022. The average temperature in Slovakia shows fluctuations over the period from 2007 to 2022. The data suggest a general warming trend with occasional drops. The highest average temperature was recorded in 2014 (10.2°C). The lowest average temperature was recorded in 2010 (8.11°C). There is a noticeable increase in temperature around 2014 and again in 2018-2019, with temperatures exceeding 10°C. Despite some fluctuations, the average temperature generally remained close to 9-10°C for most of the years. Precipitation levels show significant variability over the same period. The highest level of precipitation was recorded in 2010 (1110 mm). The lowest level of precipitation was recorded in 2022 (648.74 mm). There are years with notably high precipitation such as 2010 and 2016 (902.68 mm). There are several years where precipitation levels are around the 700-800 mm range, showing a moderate level of rainfall.

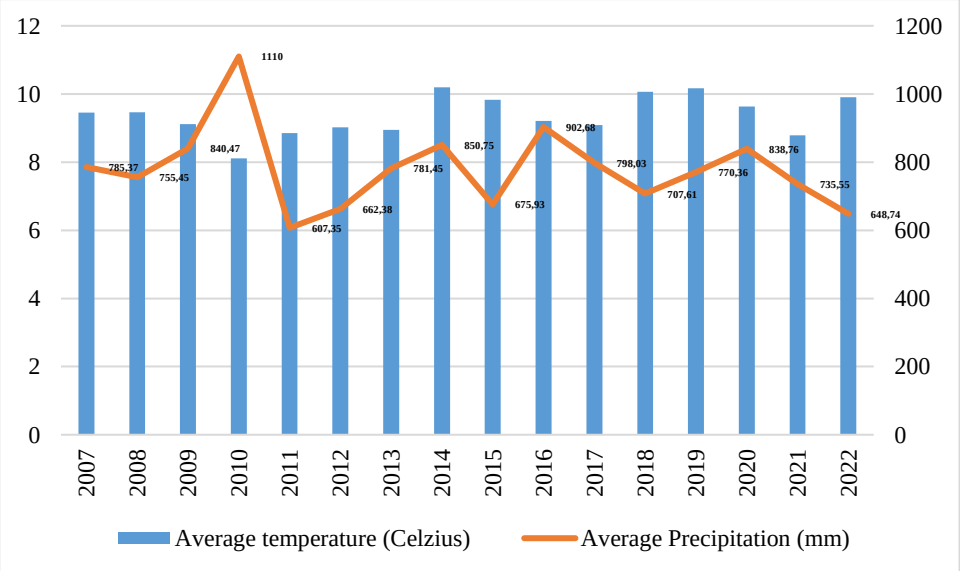

Figure 1. Development of climatic data (2007-2022)
Source: Own processing based on data from Trading Economics

To interpret the results of the regression analysis on the impact of average temperature changes on oilseed rape production in Slovakia, we will review the key statistics from the regression output.

A value of 0.2248 indicates a weak positive correlation. R Square is the coefficient of determination, showing that only 5.05% of the variation in oilseed rape production can be explained by changes in average temperature. This is very low, suggesting that average temperature alone is not a strong predictor of oilseed rape production. F-statistic tests the overall significance of the model. A low F value indicates that the model does not explain much of the variability in the outcome. The p-value associated with the F-statistic indicates the probability that the observed F-statistic would occur if the null hypothesis (no effect of temperature on production) were true. Since the p-value (0.40253) is greater than the common

alpha level of 0.05, we fail to reject the null hypothesis. This suggests that the model as a whole is not statistically significant. Other factors not considered in this analysis likely play a more critical role in determining oilseed rape production. Therefore, further research and analysis incorporating additional variables may be necessary to better understand the factors influencing oilseed rape production.

Table 3. Summary output of the regression analysis on the impact of average temperature changes on oilseed rape production in Slovakia

Regression Statistics	
Multiple R	0,2248
R Square	0,0505
Adjusted R Square	-0,017
Standard Error	75,844
Observations	16

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	4287	4286,98	0,74527	0,40253
Residual	14	80532	5752,26		
Total	15	84819			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
Intercept	103,92	317	0,32784	0,74788	-575,94	783,776	-575,94	783,776
Average temperature (X)	29,168	33,79	0,86329	0,40253	-43,298	101,633	-43,298	101,633

Source: Own processing based on available data

To interpret the results of the regression analysis on the impact of percipitation on oilseed rape production in Slovakia, we will review the key statistics from the regression output.

A value of 0.149749 indicates a weak positive correlation. R Square is the coefficient of determination, showing that only 2,24% of the variation in oilseed rape production can be explained by average percipitation. This is very low, suggesting that average temperature alone is not a strong predictor of oilseed rape production. F-statistic tests the overall significance of the model. A low F value indicates that the model does not explain much of the variability in the outcome. The p-value associated with the F-statistic indicates the probability that the observed F-statistic would occur if the null hypothesis (no effect of temperature on production) were true. Since the p-value (0,5798898) is greater than the common alpha level of 0.05, we fail to reject the null hypothesis. This suggests that the model as a whole is not statistically significant. Other factors not considered in this analysis likely play a more critical role in determining oilseed rape production. Therefore, further research and analysis incorporating additional variables may be necessary to better understand the factors influencing oilseed rape production in Slovakia.

Table 4. Summary output of the regression analysis on the impact of average percipitation on oilseed rape production in Slovakia

Regression Statistics	
Multiple R	0,149749
R Square	0,022425
Adjusted R Square	-0,0474
Standard Error	76,95853
Observations	16

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1902,05	1902,049	0,32115	0,5798898
Residual	14	82916,6	5922,615		
Total	15	84818,7			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
Intercept	303,9397	130,482	2,329364	0,03532	24,08403	583,8	24,0840	583,795
Average Precipitation	0,093833	0,16558	0,566701	0,57988	-0,2612944	0,449	-0,26129	0,44896

Source: Own processing based on available data

4. DISCUSSION AND CONCLUSIONS

The study of Racca, P. et al. (2015) demonstrates how climate change exhibits influence on the phenological development of crops. However, there are many factors of uncertainty which may further modify these predictions in a positive or negative way. Firstly, it should be noted that the ontogenetic models only use the temperature as a meteorological parameter, while rainfall is omitted although it may certainly have a great importance for the development of crop plants. Regrettably, exact estimations of the distribution of rainfall in a future period of 130 years are extremely difficult if not impossible. The results of the present study indicate not only most vulnerable areas but also those that might profit from the expected changes. Surprisingly the expected impacts (both positive and negative) of climate change in Mediterranean are in several cases smaller than those expected for northern or central Europe. This is partly explained by the possibilities for shifting some of the crop cultivation into the winter season in the Mediterranean countries in a warmer climate (Minguez et al., 2007). There is a considerable need for an increased attention on regional studies of impacts and adaptation to climate change in agriculture, since effects and responses have been shown by our study to be regionally specific depending on interactions with soils, current climate and cropping systems. These studies should include assessments of the consequences on current efforts in agricultural policy for a sustainable agriculture that also preserves environmental and social values in the rural society. The research on adaptation in agriculture has not yet provided a generalised knowledge on the adaptive capacity of agricultural systems across a range of climate and socioeconomic futures (Olesen, J.E. et al. 2011).

Temperature changes due to climate change have significant implications for agriculture in Slovakia. While there may be some potential benefits, such as longer growing seasons and increased CO2 fertilization, the negative impacts, including heat stress, increased water demand, and greater vulnerability to pests and diseases, are likely to pose substantial challenges. Effective adaptation strategies, sustainable practices, and technological innovations will be crucial in mitigating these impacts and ensuring the resilience and productivity of the agricultural sector in the face of changing temperatures.

The regression analyses indicate that neither average temperature nor average precipitation significantly impact oilseed rape production in Slovakia. The weak correlations and low R Square values suggest that other factors, which are not included in these models, play more critical roles in influencing oilseed rape production. The findings suggest that climatic conditions such as temperature and precipitation, while having some effect, are not the primary determinants of oilseed rape production in Slovakia. This aligns with the observed variability in both climate data and production levels over the years, indicating a complex interaction of multiple factors. There is a need for further research to identify and analyze additional variables that may influence oilseed rape production. These could include soil quality, farming practices, pest and disease prevalence, market conditions, and technological advancements in agriculture. Incorporating a more comprehensive set of variables into future analyses could provide better insights into the factors driving oilseed rape

production. Policymakers and agricultural planners should consider a broader range of factors beyond climate when developing strategies to enhance oilseed rape production. Investments in agricultural research, technology, and education may yield more significant improvements in production than focusing solely on climatic adaptations. In conclusion, while climate conditions such as temperature and precipitation do impact agricultural production, their effect on oilseed rape in Slovakia appears to be limited. A holistic approach that includes a variety of factors will be essential for understanding and improving the productivity and economic outcomes of oilseed rape cultivation in the region. Most European regions offer a wide range of adaptation solutions to help lessen the negative effects of climate change on crop production in Europe. But when all of the effects are taken into account, such as nutrient runoff, pesticide use, soil fertility, and crop yields, the effects of climate change are still primarily unfavorable in the majority of European regions.

Acknowledgements
The paper was created as part of the preparation and solution of the VEGA project no. 1/0344/24 “Rational Land Management with Positive Effect on Reducing the Carbon Footprint „solved at the Institute of Economics and Management.

Author Contributions
Conceptualization: only one author

Conflict of Interest
The authors have no conflicts of interest to declare.

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Konkan Karli Chronicles: Promoting Bitter gourd prominence in Konkan region of Maharashtra (India)

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Abstract: Bitter gourd (*Momordica charantia*) is one of the popular vegetable crops grown in India. The bitter gourd fruits are rich in carbohydrates, proteins, vitamins, and minerals and have the highest nutritional value of all the cucurbits. By considering the importance and demand of bitter gourd in the region, seven cultivars were evaluated for yield and quality parameters at Vegetable Improvement Scheme, Central Experiment Station, Wakawali, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. Among the evaluated types, DPL BG - 5 (Konkan Karli) recorded a maximum yield (162.55 qha-1) that was significantly superior to the other types. The growth habit of bitter gourd plants is medium viny with light green leaves. It has 8.27 number of branches per plant. It has also recorded a high-yielding variety, resistant to downy mildew and anthracnose. Besides this, a low incidence of pests and diseases was observed during the experiment. According to the research, Konkan Karli is a cultivar that is both extremely prolific and resistant to disease, which makes it a great choice for producers who want to maximise production while minimising losses from pests and diseases. The present study underscores the potential of konkan karli to improve bitter gourd yield, thereby strengthening the agricultural sustainability and financial stability of the local farmers. The long-term performance and adaptability of this cultivar to various environmental circumstances and agricultural techniques may be investigated in more detail.

Keywords: *Momordica charantia*, Carbohydrates, Proteins, Vitamins, Downy mildew, Anthracnose.

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1. INTRODUCTION

The bitter gourd (*Momordica charantia* L.) is an annual vegetable grown throughout tropical and subtropical environments (Schaefer et al., 2010). Among the Cucurbitaceae family of vegetables, bitter gourd (*Momordica charantia*) has shown some clinical research to help treat diabetes (Grover et al., 2004; Singh et al., 2004). One of the most prevalent and serious chronic illnesses in people is diabetes (Valyaie et al., 2021). It is estimated that 415 million people worldwide have diabetes and, by 2040, one in ten adults will suffer from it (642 million) (Ogurtsova et al., 2017). In this regard, bitter gourd may prove useful as a medicinal plant, as it is used in many developing countries, particularly in Asia and Africa, as a herb, medicine, and vegetable (S. P. Tan et al., 2016a). In addition to vitamin C, mineral elements, and amino acids, the fresh fruit contains many nutritional components (Behera et al., 2010) (Table 1).

Table 1. Nutrient composition of bitter gourd (*Momordica charantia* L.) fruit.

Proximate principles	Quantity/ 100 g
Moisture	83.20 g
Carbohydrates	10.60 g
Proteins	2.10 g
Fibre	1.70 g
Calcium	23.00 mg
Phosphorus	38.00 mg
Potassium	171.00 mg
Sodium	2.40 mg
Iron	2.00 mg
Copper	0.19 mg
Manganese	0.08 mg
Zinc	0.46 mg
β Carotene	126.00
Vitamin C	96.00

India seems to have a high level of genetic diversity based on morphological traits (growth stage, maturity, and various fruit attributes such as shape, size, colour, and surface texture) (Dey et al., 2006). *Momordica* species grow well in hot, humid areas but also grow abundantly in subtropical climates and are day-neutral (Kesh et al., 2020). *Momordica* is one of the largest genera in the Cucurbitaceae family, but *Momordica charantia* is the only widely cultivated crop in the genus (Kesh et al., 2020). Bitter gourd is grown for its prickly, bitter and immature fruits (Behera et al., 2010). Despite its bitter flavour, bitter gourd is widely consumed in various cultures because of its potential health advantages linked to the presence of health-promoting substances such as ascorbic acid, polyphenols, and triterpenoids (Kubola et al., 2008; M. J. Tan et al., 2008; S. P. Tan et al., 2016b). In addition to being monoecious (producing distinct male and female flowers on the same plant), the species favours cross-pollination, which encourages genotypic and phenotypic variety (Narinder P.S. Dhillon et al., 2016).

Consumers prefer bitter gourd fruit when it is naturally immature or unripe. Mature fruits contain yellow flesh with crimson seed coverings and are often split, making them inedible and unsalable. Fruit colour, shape, skin pattern, and size preferences differ between and between countries. The colours of the fruits range from white or cream to light green to dark green, and the shapes include cylindrical, elliptical, spindle, and conical types (Narinder P.S. Dhillon et al., 2016). In order to increase the market demand for bitter gourd, it is important to continuously evaluate the genetic background of bitter gourd germplasm and breed new elite varieties (Cui et al., 2022). In this connection, efforts were made to find out suitable type of bitter gourd with desirable characteristics. Also, the investigation was undertaken to identify the best combination for high yield and consumer preference.

2. MATERIALS AND METHODS

DPLBG - 5 (*Konkan Karli*) is a pedigree selection made at Central Experiment Station (CES), Wakavali, Maharashtra (India) by selecting *Konkan Tara* X *Preethi* parentage. The parentage selected shown different characteristics such as *Konkan Tara* bears medium size fruits with dark green colour (Source - Dapoli) and *Preethi* is a high-yielding variety that bears greenish-white fruits and is susceptible to fruit flies (Source- Vellanikkara). Furthermore, the experimental material for the present study comprised seven bitter gourd cultivars (viz., DPLBG-3, DPLBG-4, DPLBG-5, DPLBG-6, DPLBG—7, DPLBG-8 and *Konkan Tara*) and were tested for the growth and yield attributes from 2010 to 2015 at Vegetable Improvement Scheme (VIS), Central Experiment Station (CES), Wakawali, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth (DBSKKV), Tal. Dapoli, Dist: Ratnagiri of Maharashtra. The experiment was designed using Randomized Block Design (RBD) with three replications and treatments assigned randomly in each replication. The plants were planted on a spacing of 1.5 m x 0.50 m during the *kharif* season. Five plants from each cultivar were selected randomly and tagged to record the observations for plant characteristics and yield attributes. Vine length (cm), number of branches per vine, number of days for 50% flowering, fruit length, fruit breadth, fruit weight (g), number of fruits per vine, shelf life, yield per hectare (q), pest and disease incidence, and cost of cultivation were all recorded regularly. Data was analyzed for different parameters by a method suggested by Panse and Sukathme 1961.

3. RESULTS

3.1. Performance characteristics

3.1.1. Varietal characteristics

DPLBG - 5 (Konkan Karli) has a medium viny growth habit. The length of the vine is up to 5.51 m with light green leaves. This cultivar bears oblong-shaped fruits and the average yield is 162.55 qha^{-1}. Moreover, the shelf life of this cultivar is 4 to 5 days at an ambient temperature of 27-32° C. This cultivar is resistant to downy mildew and anthracnose and is suitable for growing in the *kharif* season in the konkan region of Maharashtra (India).

3.1.2. Plant length, number of branches per plant and 50% flowering:

Among the growth attributes, the plant length for DPLBG – 5 was observed 5.51 m, while it was minimum in DPLBG - 6 (4.31 m). Besides, the maximum number of branches per plant was recorded in DPLBG – 5 (8.27) and was minimum in DPLBG – 6 (5.93). Moreover, the maximum number of days required for 50% of flowering was in DPLBG – 3 and DPLBG – 4 (37 days) respectively, whereas it was minimum in DPLBG – 6 (35.33 days) (Table 2).

Table 2. Growth attributing characters of different types of Bitter gourd.

Sr. No.	Types	Plant Length (m)	No. of branches	50% Flowering (Days)
1.	DPLBG - 3	4.68	6.60	37.00
2.	DPLBG - 4	4.88	7.40	37.00
3.	DPLBG - 5	5.51	8.27	36.37
4.	DPLBG - 6	4.31	5.93	35.33
5.	DPLBG - 7	4.62	6.33	36.00
6.	DPLBG - 8	4.69	6.60	35.37
7.	K. Tara (C)	4.65	6.80	35.37
	SE +	0.100	0.18	0.47
	CD @ 5%	0.309	0.58	1.45

3.1.3. Fruit attributes:

Amongt the data recorded for fruit attributes, the maximum number of fruits per plant were observed in DPLBG - 5 (12.86), whereas the minimum number of fruits per plant was observed in DPLBG - 8 (8.91). Furthermore, the fruit length was maximum for DPLBG - 5 (25.52 cm) followed by DPLBG - 7 (23.38 cm), while it was minimum for Konkan Tara (13.99 cm). Fruit breadth was recorded maximum for DPLBG - 4 (4.98 cm), while it was minimum for DPLBG - 5 (3.51 cm). In addition, the weight of fruit was maximum for DPLBG - 8 (123.57 g) whereas, it was minimum for DPLBG - 3 (51.59 g) (Table 3).

Table 3. Yield attributing characters of different types of Bitter gourd.

Sr. No.	Types	Fruit weight (g)	Fruit Breadth (cm)	Fruit length (cm)	No. of Fruits/ vine
1.	DPLBG - 3	51.59	4.69	17.27	10.37
2.	DPLBG - 4	86.40	4.98	16.15	11.91
3.	DPLBG - 5	92.47	3.51	25.52	12.86
4.	DPLBG - 6	106.22	4.93	16.53	10.00
5.	DPLBG - 7	115.23	3.76	23.38	12.17
6.	DPLBG - 8	123.57	4.43	17.91	8.91
7.	K. Tara (C)	106.40	4.07	13.99	9.30
SE +		5.75	0.14	1.12	0.06
CD @ 5%		17.73	0.46	3.45	0.19

3.1.4. Yield evaluation

Among the seven cultivars mentioned, the average yield of DPLBG – 5 was high (191.48 qha^{-1}) followed by DPLBG – 3 (179.68 qha^{-1}). The average yield for DPLBG – 5 was (191.48 qha^{-1}) at the Vegetable Improvement Scheme, Central Experiment Station, Wakavali between 2010 to 2012 (Table 4). Furthermore, these cultivars were tested at six different locations viz Wakavali, Karjat, Awashi, Palghar, Rameshwar and Lanja as a part of Multi-location trials (MLT) for yield performance (Table 5-6). During this test, DPLBG – 5 was a high-yielding cultivar in the year 2014 and 2015 at all locations (Table 6-7). From 2013 to 2015, the average production of bitter gourd was determined for each location, and the DPLBG-5 cultivar of bitter gourd had the highest output (Table 8).

Table 4. Yield (qha-1) of Bitter gourd types at VIS, Wakawali (Station Trial).

Sr. No.	Types	Average yield (qha^{-1})			Pooled mean (qha^{-1})
		2010	2011	2012	
1.	DPLBG - 3	183.20	176.56	179.30	179.68
2.	DPLBG - 4	161.84	168.38	185.42	171.88
3.	DPLBG - 5	193.58	188.40	192.48	191.48
4.	DPLBG - 6	164.22	159.66	168.30	164.06
5.	DPLBG - 7	138.60	143.50	145.12	142.40
6.	DPLBG - 8	154.28	147.64	158.82	153.58
7.	K. Tara (C)	129.90	132.80	143.76	135.48
	SE +	3.181	3.352	3.467	2.89
	CD @ 5%	9.472	10.49	10.389	9.02

Table 5. Yield (qha-1) of Bitter gourd types at different locations (2013).

Sr. No.	Types	Palghar	Rameshwar	Awashi	VIS, Wakawali	Mean (qha^{-1})
1.	DPLBG - 3	188.02	179.82	183.33	169.11	180.07
2.	DPLBG - 4	171.95	135.73	168.36	155.35	157.84
3.	DPLBG - 5	168.85	189.62	167.29	159.30	171.26
4.	DPLBG - 6	159.26	131.49	146.65	147.93	146.33
5.	DPLBG - 7	154.84	126.72	160.93	145.86	147.08
6.	DPLBG - 8	152.87	120.09	124.70	141.16	134.70
7.	K. Tara (C)	148.72	132.43	139.12	130.96	137.80
	SE +	2.40	9.46	2.30	3.18	5.43
	CD @ 5%	7.48	29.16	7.79	9.91	16.27

Table 6. Yield (qha^{-1}) of Bitter gourd types at different locations (2014).

Sr. No.	Types	Palghar	Karjat	Rameshwar	Awashi	VIS Wakawali	Lanja	Mean (qha^{-1})
1.	DPLBG - 3	120.70	149.86	180.24	169.70	139.10	109.60	144.87
2.	DPLBG - 4	104.70	158.01	143.16	150.80	136.22	104.20	132.85
3.	DPLBG - 5	134.70	180.02	191.92	196.40	153.84	124.27	163.53
4.	DPLBG - 6	107.50	170.19	133.22	168.60	143.47	88.17	135.19
5.	DPLBG - 7	104.90	174.06	121.97	164.80	155.32	101.33	137.06
6.	DPLBG - 8	122.60	189.33	133.43	158.10	181.02	125.50	151.66
7.	K. Tara (C)	112.90	175.76	136.84	154.10	151.90	98.10	138.27
SE +		2.30	3.63	9.06	2.66	5.19.	0.92	5.55
CD @ 5%		6.93	10.89	27.89	7.72	16.00	2.87	16.12

Table 7. Mean Yield (qha^{-1}) of Bitter gourd types at different locations (2015)

Sr. No.	Types	Palghar	Awashi	VIS, Wakawali	Mean (qha^{-1})
1.	DPLBG - 3	94.90	121.82	125.85	114.19
2.	DPLBG - 4	120.47	119.81	136.52	125.60
3.	DPLBG - 5	147.96	152.96	157.69	152.87
4.	DPLBG - 6	130.20	124.26	141.39	131.95
5.	DPLBG - 7	190.18	183.45	186.53	186.72
6.	DPLBG - 8	130.20	142.30	146.13	139.54
7.	K. Tara (C)	119.21	137.74	131.37	129.44
	SE ±	8.74	12.37	4.46	4.38
	CD @ 5%	25.71	36.40	13.76	13.65

Table 8. Pooled mean Yield (qha^{-1}) of Bitter gourd types.

Sr. No.	Types	Average yield (qha^{-1})			Pooled mean (qha^{-1})
		2013	2014	2015	
1.	DPLBG - 3	180.07	144.87	114.19	146.37
2.	DPLBG - 4	157.84	132.85	125.60	138.76
3.	DPLBG - 5	171.26	163.53	152.87	162.55
4.	DPLBG - 6	146.33	135.19	131.95	137.82
5.	DPLBG - 7	147.08	137.06	186.72	156.95
6.	DPLBG - 8	134.70	151.66	139.54	141.96
7.	K. Tara (C)	137.80	138.27	129.44	135.70
	SE ±	5.43	5.55	4.38	6.19
	CD @ 5%	16.27	16.12	13.65	19.07

3.2. Impact of disease and pest incidence:

Among the seven genotypes studied, DPLBG - 5 reported low incidence of anthracnose (8.45%) whereas, DPLBG - 3 reported high incidence of anthracnose (12.46%). Furhtermore the downy mildew was observed maximum in DPLBG - 3 (11.34 %) and it was minimum in DPLBG – 5 (6.62 %). Moreover, the incidence of fruit fly was maximum in DPLBG - 3 (4.85 %), while the attack was low in DPLBG - 5 (2.10 %) (Table 9).

Table 9. Disease and Pest incidence of different Bitter gourd types. (Pooled mean 2013-15)

Sr.no.	Types	Anthracnose %	Downy mildew %	Fruit fly incidence %
1.	DPLBG - 3	12.46	11.34	4.85
2.	DPLBG - 4	10.14	10.20	4.34
3.	DPLBG - 5	8.45	6.62	2.10
4.	DPLBG - 6	9.88	9.73	3.16
5.	DPLBG - 7	9.17	9.55	2.67
6.	DPLBG - 8	9.90	10.01	3.15
7.	K. Tara (C)	10.67	10.34	4.11

Disease rating scale:

0 : Immune
0.1 to 10 % : Resistant
10.1 to 20 % : Moderately resistant
20.1 to 50 % : Susceptible
Above 50 % : Highly susceptible

ETL for fruit fly:- < 3% fruit infestion

3.3. Cost of economics:

From the studied seven genotypes, the cost of cultivation was maximum for DPLBG - 5 (INR. 237,616/-), whereas it was minimum for Konkan Tara (INR. 224,191/-). Besides, the gross return was higher in DPLBG - 5 (INR. 487,650/-) while it was lower in Konkan Tara (INR. 407,100/-). Moreover, the Net returns were maximum for DPLBG - 5 (INR. 2,50,034/-) while it was minimum in Konkan Tara (INR. 182,909/-). Furthermore, the B: C ratio was maximum for DPLBG - 5 (2.05) while it was minimum for Konkan Tara (1.82) (Table 10).

Table - 10. Cost of cultivation of different Bitter gourd types.

Sr. No.	Types	Cost of cultivation (INR)	Gross returns (INR.)	Net returns (INR.)	B :C ratio
1.	DPLBG - 3	229,526	439,110	209,584	1.91
2.	DPLBG - 4	225,721	416,280	190,559	1.84
3.	DPLBG - 5	237,616	487,650	250,034	2.05
4.	DPLBG - 6	225,251	413,460	188,209	1.84
5.	DPLBG - 7	234,686	470,070	235,384	2.00
6.	DPLBG - 8	227,321	425,880	198,559	1.87
7.	Konkan Tara	224,191	407,100	182,909	1.82

* Selling rate: INR. 30/kg

4. DISCUSSION AND CONCLUSIONS

The Konkan Karli Chronicles seek to illuminate initiatives to elevate bitter gourd's profile in this verdant coastal region. This project is a monument to community development, sustainable agriculture, and nutritional awareness in addition to vegetable cultivation. In order to assess the bitter gourd's performance, the seven cultivars were examined and researched in different conditions. The highest fresh fruit yield (162.55 qha^{-1}) was obtained from cultivar DPLBG - 5, while the lowest yield (135.70 qha^{-1}) was obtained from cv. Konkan Tara (Table 8). Bitter gourd fresh fruit yield is economically significant and is determined by variety and crop management practices (Ghosh et al., 2018). The average economic yield was between 8 and 10 $t.ha^{-1}$. Some cultivars can yield up to 20-40 $t.ha^{-1}$ and even more (25 to 80 tonnes) (Dey et al., 2006; Morgan et al., 2002; Raman et al., 1996). These differences can be attributed to climatic conditions, soil fertility, and cultivar. Under greenhouse conditions, grafted seedlings yielded 61-108 $t.ha^{-1}$ in Taiwan and yields ranging from 21 to 44 $t.ha^{-1}$ in India (Valyaie et al., 2021).

Our findings suggest that the various stages at which the fruit length was measured are responsible for the observed variations in fruit length. Various researchers also reported fruit lengths ranging from 2.91–38.83 cm, which is related to our findings as well (Resmi J et al., 2012; Saranyadevi et al., 2017; Singh Waikhom Jupiter et al., 2020). Fruit length is also impacted by nutrient management, as demonstrated by Dudhat and Patel (Dudhat MA et al., 2020).

According to our findings, the cv. DPLBG - 5 had the average fruit diameter of 3.51 cm, which is the lowest of all genotypes studied. Fruit diameters of 2.2 to 7.4 cm were reported by other researchers (Resmi J et al., 2012; Singh Waikhom Jupiter et al., 2020), which is in line with our findings when the fruit is unripe or semi-ripe.

5. NOTIFICATION AND SEED PRODUCTION

Konkan Karli (DPLBG - 5) a variety of bitter gourd, was released and notified by the central sub-committee on crop standards, notification, and release of varieties vide notification in the official gazette number S.O.4272 (E), dated 26th of November, 2019. The Central Experiment Station, Wakavali, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli is the maintainer of this variety and the producer of the nucleus and breeder seeds of bitter gourd.

Acknowledgements
The authors express their gratitude to J. P. Devmore, B. G. Thaware, B. R. Salvi who has worked and helped to release the cultivar.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization: P.S.; Investigation: P.S.; Material and Methodology: P.S., P.H.; Supervision: P.S.; Visualization: P.S., P.H.; Writing-Original Draft: P.S., T.C.; Writing-review & Editing: P.S., T.C.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

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Determination of *In Vitro* Antioxidant Activities, Total Phenolic and Flavonoid Contents of *Thymus vulgaris* L. (Thyme) and *Tilia L.* (Linden) Herbal Teas

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Abstract: Herbal tea is a popular drink made by steeping the leaves, petals, seeds, fruits, stems, or roots of several plant species. It has a long history of being used for promoting health and preventing diseases. As the consumption of herbal tea has risen, there has been a focus on studying their potential impact on human health and the bioactive components responsible for these beneficial benefits. Therefore, the present study aims to evaluate and compare the total phenolic, flavonoid and antioxidant capacities of thyme and linden teas prepared by the infusion method traditionally consumed in Turkey. The antioxidant capacity was assessed using three widely used methods for measuring antioxidant activity: DPPH, CUPRAC, and FIC. We also determined the total phenolic and flavonoid contents of both teas using the Folin-Ciocalteu and $AlCl_3$ colorimetric methods, respectively. TPC of thyme and linden herbal teas were calculated as 47.5 mg GAE/g-DW and 13.72 mg GAE/g-DW, respectively. We calculated the total flavonoid contents of the thyme and linden teas to be 20.48 mg CE/g-DW for the thyme tea and 5.74 mg CE/g-DW for the linden tea. Thyme and linden herbal tea infusions prepared from dried plants gave CUPRAC values of total antioxidant capacity of 0.324 and 0.147 mmol TR/g-DW, respectively. DPPH radical scavenging ability of thyme and linden herbal tea infusions was measured as 0.13 and 0.062 mmol TR/g-DW, respectively. In contrast to the CUPRAC and DPPH methods, the antioxidant capacity of linden tea was higher than that of thyme. According to the FIC method, the Fe^{2+} chelating activities of thyme and linden teas were found to be 0.004 and 0.014 mmol EDTA/g-DW.

Keywords: Antioxidant capacity, CUPRAC assay, FIC assay, DPPH, Thyme tea, Linden tea.

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1. INTRODUCTION

Oxidative stress refers to the condition of an imbalance between the activity of antioxidants and the presence of free radicals (Hussein et al., 2011). Oxidative stress arises when the natural defenses of an organism, whether they are enzymatic, non-enzymatic, or dietary in nature, are unable to cope with an excessive production of reactive oxygen and nitrogen species. Insufficient natural defense mechanisms can result in the harmful impact of excessive exposure or the formation of free radicals on cellular macromolecules (Lugemwa et al., 2013). This can contribute to the development of several degenerative illnesses, such as arthritis, cancer, cataracts, brain dysfunction, cardiovascular diseases, and aging. Nevertheless, the presence of free radicals can be effectively regulated with the use of antioxidants, which function as scavengers for free radicals (Buřičová and Reblova, 2008). The consumption of antioxidants significantly contributes to reducing the likelihood of several chronic illnesses, such as cardiovascular diseases, cancer, and diabetes (Hussein et al., 2011).

Phenolic and flavonoid compounds, which are the primary group of antioxidant compounds found in herbs that contribute to the color of flowers, fruits, and foliage, frequently demonstrate their medicinal effects by inhibiting enzymes, scavenging free radicals, and acting as cofactors for the antioxidant vitamin C. They are considered to be the primary antioxidants in the majority of plants because of their ability to function as reducing agents and hydrogen donors (Meshram & Srivastava, 2016).

Herbal teas can be prepared using a wide range of plants, utilizing various parts of the plant such as roots, flowers, seeds, berries, or bark. The choice of plant component depends on the solubility of the active elements. To make a herbal tea, place the herb parts in a glass beaker or ceramic pot and pour boiling water over them. Let them steep for the recommended amount of time. According to Campanella et al. (2003), the best way to extract tea antioxidants is by infusing the tea in hot water for 5 minutes. This method works for both ordinary and green tea. However, if the infusion time exceeds 5

minutes, the tea antioxidants will either precipitate or form micelles. This process is more likely to occur if the solution gradually cools down. As a result, both the antioxidant capacity and polyphenol content of the extract decreased. While Du Toit et al. (2001) have found that infusions made from green, black, and oolong teas exhibit notably greater ability to remove radicals compared to infusions from other herbal plants, the Turkish market offers numerous medicinal herbs that have traditionally been utilized for their health advantages for centuries. Due to the limited number of studies on the antioxidant and antibacterial activities of native Turkish herbs, it is important to investigate their antioxidant activity.

The *Tilia L.* genus, sometimes known as linden and lime, belongs to the Tiliaceae family and comprises approximately 44 species (Delnavazi et al., 2015). *Tilia cordata* is employed in traditional medicine as a sedative for the treatment of anxiety and sleep disturbances. Additionally, it possesses diuretic, demulcent, and diaphoretic characteristics (Negrie et al., 2013). It is reported that the effects of linden tea on health are due to polyphenolic compounds, including flavonoids (İnanç & Yüksel, 2018).

On the other hand, the thymus belongs to a genus that consists of approximately 350 species of fragrant perennial herbs and sub-shrubs that can reach a maximum height of 40 cm. The thymus plant is a member of the Lamiaceae family, and it can be found in arid, temperate, and cold regions of Europe, North Africa, and Asia (Qaralleh et al., 2009). *Thymus vulgaris* L., commonly known as thyme, is a popular herb that is extensively grown in various parts of the world due to its medical and culinary applications (Sonmezdag et al., 2018). The majority of herbal infusions are made from plant components belonging to the Lamiaceae family, with Thymus species being widely favored globally. Thyme is widely utilized in folk medicine for both nutritional and therapeutic purposes due to its abundance of fragrant, phenolic, antioxidative, and essential oil components (Nickavar & Esbati, 2012).

To our knowledge, there is a dearth of studies about the comprehensive phenolic, flavonoid, and antioxidant characteristics of teas prepared directly from dried thyme and linden plants without undergoing any form of processing. Therefore, the current study aimed to evaluate and compare the total phenolic, flavonoid, and antioxidant capacities of thyme and linden teas prepared by the infusion method. The antioxidant capacity was assessed using three widely used methods for measuring antioxidant activity: DPPH, CUPRAC, and FIC. We also determined the total phenolic and flavonoid contents of both teas using the Folin-Ciocalteu and $AlCl_3$ colorimetric methods, respectively.

2. MATERIAL AND METHOD

2.1. Chemicals

DPPH, ferrozine, neocuproine (2,9-dimethyl-1,10-phenanthroline), and Folin-Ciocalteu's phenol reagent were supplied by Sigma Chemical Co., Steinheim, Germany. Ammonium acetate, iron(II) chloride tetrahydrate, copper(II) chloride, 96% ethanol, Titriplex® III (ethylenedinitrilotetraacetic acid disodium salt dihydrate and the rest of the chemicals were purchased from E. Merck, Darmstadt, Germany.

2.2. Plant material

Thyme samples were collected from the experimental farm of the Agriculture Faculty, Isparta Applied Sciences University. Linden samples were collected from the campus area of Agriculture Faculty, Isparta University of Applied Sciences. We washed them in water to remove any foreign matter, then left them to dry in the shade, away from direct sunlight.

2.3. Solutions

Copper(II) chloride stock solution (10^{-2} M) was prepared by dissolving 0.4262 g dihydrate salt in distilled water, and diluting to a final volume of 250 ml. Ammonium acetate (NH4Ac) buffer at pH 7 was prepared by dissolving 19.27 g NH_4Ac in water and diluting to 250 ml. Neocuproine solution (7.5×10^{-3} M) was prepared by dissolving 0.039 g neocuproine in 96% ethanol, and diluting to 25 ml with the same solvent (should be freshly prepared). 0.2 mM DPPH solutions were prepared in methanol. Iron(II) chloride (2 mM) was prepared by dissolving 0.0397 g tetrahydrate salt in distilled water, and diluting to a final volume of 100 ml. Ferrozine solution (5 mM) was prepared by dissolving 0.0615 g ferrozine in distilled water, and diluting to 25 ml with the same solvent (should be freshly prepared). EDTA (1.0×10^{-3} M) were prepared in distilled water.

2.4. Preparation of herbal teas

The steeping method was used to obtain infusions for herbal teas. Briefly, 2 g of dry plant material mass was transferred to a glass, 200 ml of freshly boiled water was added to it and the glass was covered with a watch glass and left for 5 min. The filtrates were filtered through 0.45 µm sterile syringe filters. The prepared linden tea was illustrated in Figure 1. Similarly, thyme tea was obtained by the same method.

Figure 1. Linden tea obtained by infusion

2.5. Determination of total phenolic and flavonoid contents

Total phenolic content (TPC) of the infusions was determined according to the Folin–Ciocalteu assay (Waterhouse, 2002). Briefly, Put 40 µl sample) into a glass tube. Added 3.16 ml water, followed by 200 µl FC reagent. Mixed thoroughly by pipetting or inverting and incubated 1 to 8 min. Added 600 µL sodium carbonate solution, mix, and incubated 2 hr at room temperature (at 20°C). Measured sample absorbance at 760 nm against blank solution. (The blank solution contained distilled water instead of the sample. All other steps were the same.)

TPC = C1 x df xV/M

Where, TPC = Total phenolic content mg g^{-1} of herbal tea in GAE [Gallic acid equivalent]; C1 = The Concentration of Gallic acid established from the calibration curve µg mL^{-1};V = The volume of infusion solution [200 mL], and M = The mass of the dried herbal tea [2g]

Total flavonoid content (TFC) of the infusions was determined according to the Aluminum chloride colorimetry assay (Sakanaka *et al.*, 2005). Briefly, 0.25 ml of the infusion solutions was mixed with 1.25 ml of distilled water in a test tube, followed by addition of 75 µL of a 5% sodium nitrite solution. After 6 min, 150 µL of a 10% aluminium chloride solution was added and the mixture was allowed to stand for a further 5 min before 0.5 ml of 1 M sodium hydroxide was added. The mixture was brought to 2.5 ml with distilled water and mixed well. After 30 minutes of incubation, the absorbance was measured against blank, where $AlCl_3$ solution was substituted by water at 510 nm using a spectrophotometer.

TFC = C1 x df xV/M

Where, TFC = Total flavonoid content mg g^{-1} of herbal tea in CE [catechin equivalent]; C1 = The Concentration of catechin established from the calibration curve µg mL^{-1};V = The volume of infusion solution [200 mL], and M = The mass of the herbl teas [2g],

2.6. CUPRAC assay of total antioxidant capacity

The total antioxidant capacity of the infusions was assessed using the CUPRAC test, as described by Apak et al. (2006). To summarize, the following substances were sequentially introduced into a glass tube: 1 mL of copper(II) solution (Cu(II)), 1 mL of neocuproin solution (Nc), 1 mL of ammonium acetate buffer (NH_4Ac), 0.5 mL of a sample solution (diluted 5 times), and 0.6 mL of distilled water.

Reagent blank solution: 1 mL Cu(II) + 1 mL Nc + 1 mL NH_4Ac + 1.1 mL H_2O
Sample solution: 1 mL Cu(II) + 1 mL Nc + 1 mL NH_4Ac + 0.5 mL sample + 0.6 mL H_2O.

Incubated at 25 °C in the dark for 30 min. Absorbance values were recorded at 450 nm against reagent blank solution

$$\boldsymbol{TAC\ (mmol\ TR/g\ -DW)} = \frac{A}{\varepsilon_{TR}} x\ \frac{V_m}{V_s}\ x\ D_f\ x\ \frac{V_E}{m}$$

where ƐTR: molar absorption coefficient of Trolox compound (1.67×10^4 L $mol^{-1}.cm^{-1}$),Vs is the sample volume, Vm is the total volume of method (4.1 mL), Df is dilution factor (when needed), VE is the infusion volume (200 mL) and, m is the mass of the dried herbal tea [2g].

2.7. DPPH Free Radical Scavenging Activity

The total antioxidant potential of samples was determined by using the procedure described by Bener et al., (2022). Into a glass tube, X mL of a diluted infusion solution (diluted 5 times) was introduced, along with "2 - X" mL of 99% ethanol and 2 mL of a 0.2 mM DPPH• solution. Incubated at 25 °C in the dark for 30 min. The decrease in the absorbance at 515 nm was determined by using a UV–Vis spectrophotometer (UV-1280, Shimadzu, Japan).

$$\boldsymbol{DPPH\ (mmol\ TR/g-DW)} = \frac{\Delta_A}{\varepsilon_{TR}} x\ \frac{V_m}{V_s}\ x\ D_f\ x\ \frac{V_E}{m}$$

where ƐTR: molar absorption coefficient of TR compound in the DPPH method (2.16×10^4 L $mol^{-1}.cm^{-1}$) , Vs is the sample volume, Vm is the total volume of method (4 mL), Df is dilution factor (when needed), VE is the infusion volume (200 mL) and, m is the mass of the dried herbal tea [2g]. (ΔA was calculated by subtracting the absorbance of the sample solution from the absorbance of the control solution. The control solution contained 2 mL of 0.2mM DPPH• solution and 2 mL of ethanol (99%)).

2.8. Ferrous ion chelating (FIC) assay

The ferrous ion-chelating activities of herbal teas were assessed using the method developed by Guo et al. (2011), with minor adjustments made to the sample and reagent volumes. Briefly, an aliquot of herbal tea (1 mL) was mixed with 100 µL of $FeCl_2.4H_2O$ (2.0 mM) and 3.7 mL of distilled water. The reaction was initiated by adding 200 µL of ferrozine (5.0 mM). After 20 min of incubation at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. The decrease in the absorbance at 562 nm was determined by using a UV–Vis spectrophotometer (UV-1280, Shimadzu, Japan). EDTA was used as the positive control.

The ferrous ion-chelating ability was calculated as follows:

$$\boldsymbol{FIC\ (mmol\ EDTA/g-DW)} = \frac{\Delta_A}{\varepsilon_{EDTA}} \boldsymbol{x}\ \frac{\boldsymbol{V_m}}{\boldsymbol{V_s}}\ \boldsymbol{x\ D_f\ x}\ \frac{\boldsymbol{V_E}}{\boldsymbol{m}}$$

where $Ɛ_{EDTA}$: molar absorption coefficient of EDTA compound in the FIC method (2.67×10^4 L $mol^{-1}.cm^{-1}$), Vs is the sample volume, Vm is the total volume of method (5 mL), Df is dilution factor (here, 5), V_E is the infusion volume and, m is the mass of the dried herbal tea [2g]. (ΔA was calculated by subtracting the absorbance of the sample solution from the absorbance of the control solution. The control solution contained 4.7 ml distilled water, 100 µL of $FeCl_2.4H_2O$ (2.0 mM), 200 µL of ferrozine (5.0 mM). The blank solution contained 4.9 ml distilled water and 100 µL of $FeCl_2.4H_2O$ (2.0 Mm).

Example calculation: The linden herbal tea material, weighing 2.000 g, was measured and used to make a 200 ml infusion. From this infusion, 2 ml was taken and diluted to 10 ml before analysis, resulting in a dilution ratio of 1:5. The sample solution was analyzed using a volume of Vs=0.5 ml. The final solution used for color development in the CUPRAC method had a total volume of Vm=4.1 ml. The trolox equivalent capacity of linden, calculated using the above equation, was $(0.599/1.67 \times 10^4) \times (4.1/0.5) \times 5\ (200/2.000) = 0.147$ mmol TR/g- DW.

3. RESULTS AND DISCUSSION

3.1. Total phenolic and flavonoid contents

The content of phenolic compounds was calculated to be milligram gallic acid equivalent per gram of herbal infusion (mg GAE/g-DW). The content of flavonoid compounds was calculated to be milligram catechin equivalent per gram of herbal infusion (mg CE/g-DW). The quantity of total phenolics and flavonoid in the examined teas are presented in Figure 2.

TPC of thyme and linden herbal teas were calculated as 47.5 mg GAE/g-DW and 13.72 mg GAE/g-DW, respectively. Comparing the results found that thyme tea's total phenolic content was nearly four times higher than linden tea's. We calculated the total flavonoid contents of the thyme and linden teas to be 20.48 mg CE/g-DW for the thyme tea and 5.74 mg CE/g-DW for the linden tea. Similarly, the flavonoid content of thyme tea was 3.5 times higher than that of linden tea. The data revealed that thyme tea was richer in total phenolic and flavonoid content compared to linden tea.

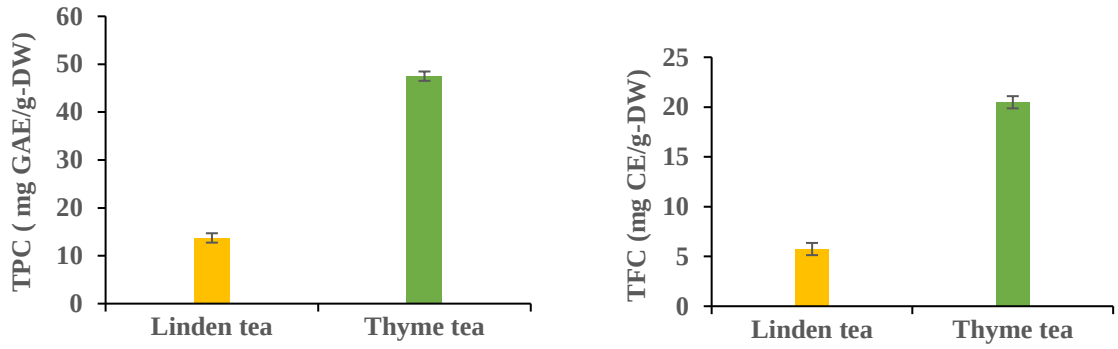

Figure 2. Total phenolic and flavonoid contents of Thyme and Linden tea

3.2. Antioxidant activity

In order to assess the overall antioxidant capacity of dried herbal teas, we conducted three commonly used and reliable procedures (CUPRAC, DPPH, and FIC) that are known for their ease of use and reproducibility. It is advisable to utilize a minimum of two techniques to ascertain the overall antioxidant capability in plants (Önder *et al*., 2023).

The Cu(II)-neocuproin (Nc) reagent, which acts as a chromogenic oxidant, is employed in the CUPRAC test to conveniently quantify the overall antioxidant capacity of plasma antioxidants, flavonoids, and dietary polyphenols (Apak et al., 2004). Thyme and linden herbal tea infusions prepared from dried plants gave CUPRAC values of total antioxidant capacity of 0.324 and 0.147 mmol TR/g-DW, respectively. In one study, the total antioxidant capacity of thyme tea was reported as 0.54 mmol TR/g-DW according to the CUPRAC method (Apak et al., 2006). In the same study, the total antioxidant capacity of linden tea was calculated as 0.18 mmol TR/g-DW as the CUPRAC value. Our findings were found to be consistent with the findings of Apak et al. (2006). As a result, according to the CUPRAC method, thyme tea exhibited stronger antioxidant activity than linden tea.

The DPPH assay was used to assess the overall antioxidant capacity of the samples by measuring their ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals in tea. In this study, free radical scavenging activity of thyme and linden tea was measured by DPPH test (Figure 3). DPPH radical scavenging ability of thyme and linden herbal tea infusions was measured as 0.13 and 0.062 mmol TR/g-DW, respectively. As in the CUPRAC method, the antioxidant capacity of thyme tea was superior to that of linden tea in the DPPH test.

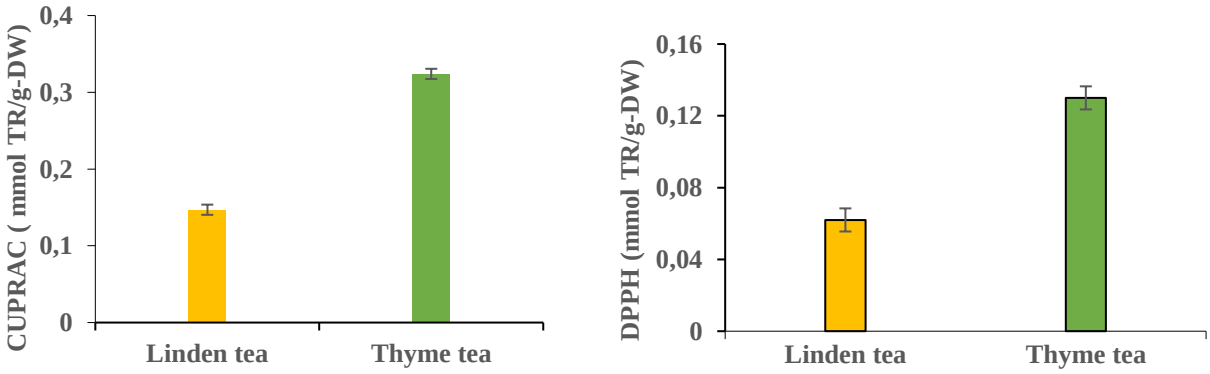

Figure 3. CUPRAC and DPPH values of thyme and linden teas

Iron can induce lipid peroxidation in a biological system through Haber-Weiss and Fenton-type processes, leading to the production of hydroxyl radicals (OH˙). In addition to its catalytic activity, the presence of the metal ion has been linked to the occurrence of arthritis and cancer (Halliwell et al., 1995). Ferrous ions are frequently present in food systems and are regarded as the most potent prooxidants. Ferrozine has the ability to form complexes with the Fe2+ ion in a way that allows for precise quantitative measurement. When chelating chemicals are present, they interfere with the development of the complex, causing a reduction in the intensity of the red color of the complex. Measuring the decrease in color allows us to estimate the metal-chelating activity of the chelator that is present.
In this assay, both the samples and the positive control disrupted the formation of the ferrous and ferrozine complex, indicating that they possess chelating activity and bind to ferrous ions before ferrozine does. As seen in Figure 4, the Fe2+ chelating activities of thyme and linden teas were found to be 0.004 and 0.014 mmol EDTA/g-DW, respectively. In contrast to the Cuprac and DPPH methods, the antioxidant capacity of linden tea was higher than that of thyme according to the FIC method. It can be said that the reason for this is related to the different mechanisms of action of each antioxidant method. Because antioxidants generally act by chain-breaking reactions, reducing the concentration of reactive oxygen species, scavenging initiating radicals, and chelating transition metal catalysts (Eroğlu et al., 2015)

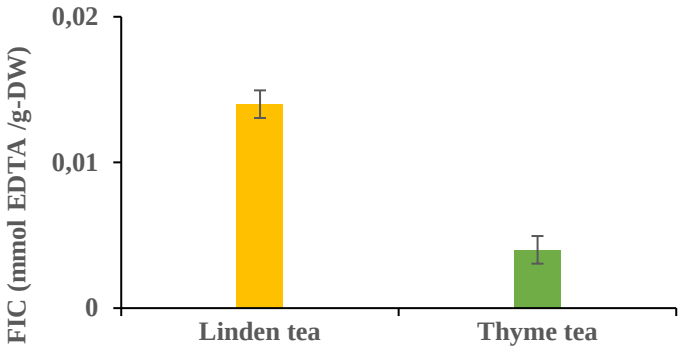

Figure 4. FIC values of thyme and linden teas

4. CONCLUSIONS

To our knowledge, there is a dearth of studies about the comprehensive phenolic, flavonoid, and antioxidant characteristics of teas prepared directly from dried thyme and linden plants without undergoing any form of processing. In the current study, Folin–Ciocalteu, Aluminum chloride colorimetry, CUPRAC, DPPH, and FIC methods were used in vitro analysis of thyme and linden herbal infusions. The determination of the antioxidant status of herbal teas will encourage further investigation into the identification and measurement of the active components in these herbs. These components have the potential to safeguard consumers from harm caused by free radicals and diseases associated with oxidative stress. Consequently, the utilization of medicinal herbs in folk medicine will be enhanced, and valuable data on finding antioxidant-rich foods and creating safe food products and additives with suitable antioxidant qualities will be collected.

Author Contributions
Conceptualization: Ü.E.; Investigation: Ü.E., M.K.; Material and Methodology: Ü.E., M.K..; Supervision: Ü.E., M.K..; Visualization: Ü.E.; Writing-Original Draft: Ü.E., M.K..; Writing-review & Editing: Ü.E., M.K.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

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Effect of Different Brewing Methods on Total Phenolic Content and Antioxidant Activity of *Logerotroimia Indica* Leaf Tea

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Abstract: Herbal tea is an important beverage consumed worldwide due to the bioactive compounds they contain. Lagerstromia is commonly used as herbal tea because of its richness in polyphenolics in some country. Lythraceae is a large family, distributed in tropical and subtropical regions. *Logerotroimia indica* is a perennial herb belonging to the Lythraceae family. Herbal tea is consumed as cold or hot tea by brewing dried flower buds, leaf stalks and branch parts. In this study, infusion and ultrasound brewing methods were used and total phenolic and antioxidant values, color and pH values were compared. Infusion brewing was carried out at temperatures of 60 ° C, 80 ° C and 100 ° C degrees for 5 minutes. Ultrasound brewing was carried out at temperatures of 60, 80 and 100 degrees for 5 min. The amplitude (A) applied for extraction was adjusted to 50 % and 100 % at each temperature. Total phenolic content was determined the Folin-Ciocalteu method by spectrophotometric and total antioxidant value was determined by (free radical scavenging) DPPH method. Total phenolic content was found to be varied in the ranges of 8.47 -31.33 mg GAE g-1. Antioxidant activities were calculated as % inhibition value and were found to vary between 77.44 % and 89.18 %. The colour values were determined as L*, a* and b* and their amounts varied between 13.55-18.92, -0.23- -0.82 and 0.80-3.17 respectively. Samples pH values were determined and pH values vary between 6.53 to 7.10, all were found to be close to neutral value. The results showed that diffrent brewing method and varied apllication change product composition.

Keywords: *Logerotroimia indica*, brewing, phenolic, antioxidant porperties

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1. INTRODUCTION

Logerotroimia indica is a perennial herb belonging to the Lythraceae family. Lythraceae is a large family that grows in tropical and subtropical regions (Labib et all., 2013). The contents of Lagerstroemia indica included phenolic glycosides (strosides A–C), alkaloids, cardiac glycosides, tannins, saponins, sterols, triterpenes, anthraquinones, reducing chemicals, and flavonoids (flavanones/dihydroflavonols and chalcones). On dry weight, the plant had 37.25 grams of carbohydrates , 22.53 grams of protein and 12.23 grams of ash (Al-Snafi, 2019). On pharmacological study demonstrated Logerotroimia indica leaf extract's have a strong biological properties, such as its analgesic, anti-inflammatory, antipyretic, antihyperglycemic, antioxidant, anticancer, antimicrobial, anti-Alzheimer's, antidiabetic, antithrombin, and hepatoprotective effects (Labib et all., 2013; Al-Snafi, 2019). İt was stated in the study that these properties may be of phenolic origin (Labib et all., 2013). Lagerstroemia indica leaves, flowers and stem used as material in many studies (Al-Snafi, 2019). In the study of Lagerstroemia indica leaves were evaluated as tea with different brewing methods was not found in the literature. In this study, tea brewing methods are infusion and ultrasound brewing methods were used. Infusion brewing was carried out at temperatures of 60 ° C, 80 ° C and 100 ° C degrees for 5 minutes. Ultrasound brewing was carried out at temperatures of 60, 80 and 100 degrees for 5 min. The amplitude (A) applied for extraction was adjusted to 50 % and 100 % at each temperature. Total phenolic and antioxidant values, colour and pH values of tea samples obtained by both methods were compared. It is thought that this study will contribute to the literature.

2. MATERIAL AND METHOD

Logerotroimia Indica leaf picked up municipality of Isparta from park gardens. It was dried in room conditions and than it was storage until the analyisis. The leaves are then cut into small pieces by hand. Distilled water was used for brewing tea.

2.1. Tea Brewing with Infusion Method

2 grams of *Logerotroimia Indica* leaves were added to 100 ml of distilled water at 60 °C, 80 °C and 100 °C and left to infuse for 5 minutes. After brewing, filtered by whatman paper and stored at +4 C for analyses.

2.2. Tea Brewing with Ultrasound Method

Ultrasound was applied 2 grams of *Logerotroimia Indica* leaves in 100 ml of distilled water at temperatures of 60 °C, 80 °C and 100 °C and at 50 and 100 % amplitude. Tea brewing with ultrasound method condition give in a Table 1.

Table 1. Tea Brewing with Ultrasound Method Condition

Sample (g)	Volume (ml)	Ultrasound (Amplitude)	Temperature (°C)
2	100	50 %	60 °C
2	100	100 %	60 °C
2	100	50 %	80°C
2	100	100 %	80°C
2	100	50 %	100 °C
2	100	100 %	100 °C

2.3. pH Analysis

The pH values of tea solutions obtained at different temperatures were measured by immersing the probe directly into the tea solution.

2.4. Colour analysis

The colour measurements of the tea samples were determined using a Minolta Colourimeter (CR-10, Konica Minolta, Osaka, Japan) and expressed in CIE (L*, a*, b*) colour system. Colour measurements were made in three zones for each sample under room conditions and the colour of the samples was determined by averaging these values (Robertson, 1977).

2.5. Determination of the Total Amount of Phenolic Substances

The total amount of phenolic compounds of tea samples were measured using the Folin-Ciocalteu spectrophotometric method at a wavelength of 765 nm, and the result was calculated as mg gallic acid equivalent (GAE)/g leaf (Singleton and Rossi, 1965).

2.6. DPPH Methods

The antiradical activity was measured by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical scavenging method according to Dorman and co-workers (2003) and the results were given as % of inhibition.

3. RESULTS

The pH, colour, total phenolic content and antioxidant activity of the tea samples prepared by infusion and ultrasound methods were determined. These values of the tea samples obtained by both methods were compared.

Table 2. pH values of teas obtained at different temperatures with different brewing techniques

		Ultrasound	
Brewing Methods/Temperature	Infusion	Amplitude 50 %	Amplitude 100 %
60 °C	7.1	6.99	6.76
80 °C	7.08	6.89	6.57
100 °C	6.96	6.74	6.53

Samples pH values were determined and pH values vary between 6.53 to 7.10, all were found to be close to neutral

value. In both infusion and ultrasound methods, a decrease in pH value was observed with the increase in temperature.

Table 3. Colour values of teas obtained at different temperatures with different brewing techniques

Brewing Methods/ Temperature	Infusion			Ultrasound Amplitude 50 %			Ultrasound Amplitude 100 %		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
60 °C	13.55	-0.69	1.57	18.92	-0.82	1.20	16.22	-0.71	1.70
80 °C	14.28	-0.73	3.17	16.08	-0.23	1.54	15.99	-0.49	1.89
100 °C	14.64	-0.82	1.06	14.34	-0.51	0.80	14.55	-0.35	1.59

Colour, is one of the most important criteria for consumer acceptance. Today, objective criteria for determining the colour L*, a* and b* values are commonly is being utilised. It is sometimes possible to detect colour changes with the naked eye, but it is not meaningful. This one meaningful expression of the change is possible by measuring L*a*b* values with a spectrophotometer (Özcan, 2008). CIE L*a*b* indicates a colour, L* indicates lightness, a* indicates red/green and b* indicates yellow/blue (Bruce, 2000; Speirs, 1998). The colour values were determined as L*, a* and b* and their amounts varied between 13.55-18.92, -0.23- -0.82 and 0.80-3.17 respectively. In tea samples obtained by both infusion and ultrasound methods, a decrease in the brightness value of the tea colour was detected with increasing temperature. L* value in tea samples obtained by ultrasound method was higher than infusion method. a* and b* values showed changes at different temperatures and treatments.

Table 4. Total Amount of Phenolic Substances (mg GAE/ g leaf) of teas obtained at different temperatures with different brewing techniques

Brewing Methods/Temperature	Infusion	Ultrasound Amplitude 50 %	Amplitude 100 %
60 °C	8.47	13.57	26.58
80 °C	10.58	19.72	30.67
100 °C	12.00	22.75	31.33

The total amount of phenolic substances of the leaf teas were determined as gallic acid equivalent (GAE). The total phenolic content determined as 8.47 to 12.00 mg GAE /ml detected in teas sample obtained by infusion methods. The total phenolic content varied between 13.57 - 22.75 and 26.58 - 31.33 in samples treated with 50% and 100% amplitude ultrasound respectively. The brewing time was determined as 5 minutes in all samples and an increase in the amount of total phenolic content was observed with the increase in temperature. The total phenolic matter content of the tea samples obtained by ultrasound method was higher than the samples obtained by infusion method. In the ultrasound method, the amount of total phenolic matter in the samples with 100% amplitude was higher than the samples with 50% amplitude at all temperatures. *Logerotroimia Indica* leaf total phenolic content determined 40. 33 mu g/ml by exracted with petroleum (Ajaib and Arooj). The other work with same material, their results showed that the highest total phenolic content was detected in the leaf extract (58.80 mg GAE g-1) and the lowest amount was detected in the flower extract (43.66 mg GAE g-1) (Önder et all., 2023).

Table 5. DPPH values of teas obtained at different temperatures with different brewing techniques

Brewing Methods/Temperature	Infusion	Ultrasound Amplitude 50 %	Amplitude 100 %
60 °C	89.18	88.43	77.52
80 °C	88.93	85. 85	77.44
100 °C	87.76	84.93	72.52

DPPH radical scavenging activity (antiradical activity, % Inibition), which is an expression of the antioxidant capacity of teas, was carried out according to the method of Dorman et al. Antiradical activity of tea samples varied with respect to the temperatures of tea brewing and brewing tecniques. Antiradical activity of tea samples varied with respect to brewing of the temperature and brewing tecniques. The strongest free radical scavenger effect was determined in

ınfusion methods at 60 °C and the lowest effect was in the ultroasund method at 100 °C 100% amplitude. It is seen that the increase in temperature and applied amplitude causes a decrease in the % inhibition value. The highest DPPH inbition was determined higher than 92 % (Ajaib and Arooj). Önder and co-workers were found DPPH value 256.34 µmol Trolox^{-1} (Önder et all., 2023).

4. DISCUSSION AND CONCLUSIONS

The results showed that the methods used in the preparation of L. indica tea leaf tea have a significant effect on the tea composition. Samples pH values were determined and pH values were found to be close to neutral value. In both infusion and ultrasound methods, a decrease in pH value was observed with the increase in temperature. In tea samples obtained by both infusion and ultrasound methods, a decrease in the brightness value of the tea colour was detected with increasing temperature. L* value in tea samples obtained by ultrasound method was higher than infusion method. a* and b* values showed changes at different temperatures and treatments. The total phenolic matter content of the tea samples obtained by ultrasound method was higher than the samples obtained by infusion method. In the ultrasound method, the amount of total phenolic matter in the samples with 100% amplitude was higher than the samples with 50% amplitude at all temperatures.The strongest free radical scavenger effect was determined in ınfusion methods at 60 °C and the lowest effect was in the ultroasund method at 100 °C 100% amplitude.

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Preventing Phase Separation Problem with Different Gelators in Poppyseed Paste

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Abstract: The poppy is an annual plant belonging to the Papaver somniferum L. species of the Papaveraceae family. Poppy seed paste is a product obtained by crushing poppy seeds and is traditionally consumed in our country. Poppy seed is considered an important source in human nutrition with its rich oil content, unsaturated fatty acid composition, especially rich in oleic acid and omega fatty acids, and vitamin and mineral content. Poppy seeds and their paste are widely used in pastries such as bread, bagels, pies, buns, cakes, and desserts such as halva, semolina, and molasses. In this study, it was aimed to prevent phase separation and increase the spreadability of poppy seed paste and to produce it in spreadable form for breakfast. Different gelling agents were used to make poppyseed paste stable spreadable form. Then, the color, texture and sensory properties of the poppyseed paste samples were determined. In order to prevent oil leakage and improve spreadability in poppyseed paste samples, certain concentrations (1% and 3%) of mono-di glyceride, stearin, beeswax and carnauba wax were added to poppy paste. The control sample was compared with the other gelling agent added samples in terms of colour texture and sensory evaluationThe control sample hardness and stickiness values were determined 408.51 - -95.49 g force respectively. Hardness and stickness values of 1% gelling agent added samples vary between 423.83-757.65 g force and -95.51- -48.56 g force. In the samples to which 3% gelling agent was added varies between hardness values 759.87-1839.41 g force and stickiness values -108.05- -45.75 g force. As the gelling agent concentration increased, the hardness value increased and the stickiness value decreased. In terms of colour, L*, a* and b* values of the control sample were determined as 40.38, 6.69, 21.77, respectively. Although there was no significant difference between the control sample and the other samples in terms of L*, a* and b*, the closest values were found in the samples to which monoglyceride was added. The colour, taste and odour characteristics of the poppy paste samples were sensory evaluated and the poppy paste samples containing 1% gelling agent were the most appreciated. In addition, the addition of low concentration improved the spreadability and effect on oil stability in paste.

Keywords: Poppyseed paste, gelators, texture and sensory properties

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1. INTRODUCTION

Poppy is a plant of the poppy family and in our country, there are 36 species, 10 of which are endemic (Arslan, 2009). The poppy plant likes places with hot summers and moderate rainfall. Its homeland is the Eastern Mediterranean and It has been cultivated in India and Anatolia for a long time. In our country, it is cultivated mainly in Afyon, Denizli, Uşak, Konya, Burdur, Isparta and Kütahya (Göksoy, 1995). Poppy seeds contain 40-55% oil, 18-27% protein, 5-9% ash (Arslan, 2009). Poppy seed oil contains 62-74.5 % linoleic acid, 15-20% oleic acid, 4.8-9.5% palmitic acid, 2-2.9% stearic acid from saturated fatty acids. It's s a rich oil in omega fatty acids and contain vitamins and mineral substances useful for human health (Arslan, 2009, Bozan and Temelli, 2008). Poppy seeds are recognised as important seed crops in oilseeds (Bozan and Temelli, 2008). Poppy seeds and poppy paste are widely used in poppy seed bread, bagels, buns, rolls, pies, pastries, cakes, kete, cakes, cookies, rusks, katmer, pastries, halva, semolina, revani, sugar pudding, molasses, etc. desserts. Haşhaş tohumlarının yağı alındıktan sonra arta kalan küspe protein, yağ ve azotsuz öz maddelerce zengin olup, hayvan yemi olarak çok değerlidir (Arslan, 2009).

Previous studies have reported the chemical composition and oxidative stability of poppy seed oils (Bozan and Temelli, 2008), rheological properties of poppy seed paste (Davulcu, 2012) and rheological properties of poppy seed paste and grape molasses blends (Süren, 2010), investigation of bioactive properties, phenolic compounds and fatty acid profiles of wheat breads enriched with poppy seed paste (Juhaimi et all., 2024).In this study, it was aimed to prevent phase separation and increase the spreadability of poppy seed paste and to produce it in spreadable form for breakfast.

2. MATERIAL AND METHOD

Poppy seed paste (Gümüş brand poppy seed paste produced in Afyon), stearin and mono-diglyceride (gelling agent) supplied from Toposmanoğlu company in Isparta. The refined beeswax and carnauba wax obtained from the Kahlwax brand from Ejder Kimya A.S.

2.1. Spreadable Poppyseed Paste Production

In the study, poppyseed paste served as the base material while beeswax, carnauba wax, stearin and mono-diglycerides were utilized as gelling agents for producing spreadable poppyseed paste. The spreadable poppyseed paste was obtained by adding beeswax, carnauba wax, stearin and mono- diglyceride to poppy seed paste in accordance with the specified ratios provided in Table 1. In spreadable poppyseed paste production, the mixing temperature was adjusted according to the wax melting temperature (80-90 ◦C) and after the wax completely melted in the mixture, it was kept at this temperature for a certain time (5 minutes).

Table 1. Gelling Agents Ratio Used in Spreadable Poppyseed Paste Production

Beeswax (%)	Mono-di glyceride (%)	Stearin (%)	Carnauba Wax (%)
1	1	1	1
3	3	3	3

2.2. Determination of Textural Properties of Spreadable Poppy Seed Paste

To determine the textural properties of the poppyseed paste, the poppyseed paste spread samples were refrigerated overnight at A temperature of +4 °C after production. Following this period, the hardness and stickiness values of the samples were determined using a Texture Analyzer equipped with stainless ball probe (P/0.75S). The probe's speed was set at 1 m/s before the test and 2 m/s after the test. As soon as the probe reached a force of 0.5 g during the test, it penetrated the sample by 15 mm and then the hardness and stickiness values of the samples were recorded (Moskowitz, 1987).

2.3. Color Analysis

Spreadable poppyseed paste samples were storage overnight at refrigerator temperature (+4 °C) after production. The color values of these samples were determined using a Minolta Color Measurement device (CR-10, Konica Minolta, Osaka, Japan). Color measurements are expressed with the CIE (L*, a*, b*) color system. Three different measurments were taken for each sample under room conditions and the samples's color was determined by averaging these values (Robertson, 1977).

2.4. Sensory Analysis

To determine the appropriate gelling agent and its proportion in the production of spreadable poppyseed paste, gelling agents were added to the poppyseed paste in specified proportions and left at room temperature overnight to achieve a spreadable consistency. Subsequently, sensory analysis of the samples that had attained a spreadable form was conducted using a hedonic scale. On this scale, evaluation of spreadable poppyseed paste samples was based on criteria such as surface appearance (color : dull white-light yellow, matte-shiny, homogeneous color distribution, oily appearance, rough appearance), structure (hardness, elasticity, chewability, rough structure, wetness in the mouth), odor (bad smell, unique pleasant smell) and taste (bland pleasant, rancid taste, unpleasant foreign taste). This analysis helped determine which gelling agent would be suitable for use in the production of spreadable poppyseed paste.

3. RESULTS

In order to prevent oil leakage and improve spreadability in poppyseed paste samples, certain concentrations (1% and 3%) of mono-di glyceride, stearin, beeswax and carnauba wax were added to poppy paste. Different gelling agents (mono-di gliserit, stearin, carnauba wax and beeswax) were used to make spreadable poppyseed paste form and were evaluated for its textural properties, color values and sensory properties. The production of poppyseed paste spread at various concentrations with mono-di glyceride, stearin, beeswax and carnauba wax as well as the hardness and stickiness values of the resulting resulting spreadable poppyseed paste were determined based on the textural values provided in Table 2.

Table 2. The Spreadable Poppyseed Paste Samples Textural Values (Hardness and Stickiness values)

Samples	Concentration(%)	Hardness (g force)	Stickness (g force)
Control	-	408.51	-95.49
Mono-di gliserit	1	484.29	-45.75
Mono-di gliserit	3	759.87	-89.54
Stearin	1	757.65	-48.56
Stearin	3	1839.41	-100.89
Beeswax	1	492.15	-95.51
Beeswax	3	1138.57	-100.83
Carnauba Wax	1	423.83	-79.43
Carnauba Wax	3	1247.92	-108.05

The control sample was compared with the other gelling agent added samples in terms of colour texture and sensory evaluationThe control sample hardness and stickiness values were determined 408.51 - -95.49 g force respectively. Hardness and stickness values of 1% gelling agent added samples vary between 423.83-757.65 g force and -95.51- -48.56 g force. In the samples to which 3% gelling agent was added varies between hardness values 759.87-1839.41 g force and stickiness values -108.05- -45.75 g force. As the gelling agent concentration increased, the hardness value increased and the stickiness value decreased. There is no study on the oil stability and spreadability of poppy seed paste in the 32iteratüre. However, Öğütçü and co-workers have studied the oil stability and spreadability of sesame seed paste. As the amount of wax added increased, the hardness and stickness value of the product increased. Literature findings are similar effect to our findings (Öğütçü et all., 2018).

Table 3. Color Values of Spreadable Poppyseed Paste Produced by Different Gelling Agents

Samples	L*	a*	b*
Control	40.38	6.69	21.77
Mono-di gliserit	40.91	5.43	17.41
Mono-di gliserit	41.16	6.40	21.99
Stearin	39.31	5.94	21.01
Stearin	37.49	5.49	19.01
Beeswax	38.92	5.72	19.74
Beeswax	41.41	6.20	20.25
Carnauba Wax	37.35	5.19	18.11
Carnauba Wax	37.69	5.42	19.45

In terms of colour, L*, a* and b* values of the control sample were determined as 40.38, 6.69, 21.77, respectively. Although there was no significant difference between the control sample and the other samples in terms of L*, a* and b*, the closest values were found in the samples to which monoglyceride was added. In our study colour values changes wax type nad concentration. The other work of showed that, sesame seed paste physico-chemical features of colour value, to change wax type and concentration (Öğütçü et all., 2018).

4. DISCUSSION AND CONCLUSIONS

In conclusion, natural waxes addition was effective to restrict the phase separation, hence, the problem of oil leakage, which negatively affects consumer preferences in poppy seed paste samples, will be eliminated and product stability and quality will be maintained throughout the shelf life. The colour, taste and odour characteristics of the poppy paste samples were sensory evaluated and the poppy paste samples containing 1% gelling agent were the most appreciated. In addition, the addition of low concentration improved the spreadability and effect on oil stability in paste. Poppy seed paste can also be made into a spreadable product by adding natural beeswax and used for breakfast.

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Determination of Soil Compaction and Loss Relationships Caused by the Extraction of Industrial Wood Raw Material by Agricultural Tractors in Forestry

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Abstract: This study investigates the soil compaction and loss resulting from the extraction of wood raw materials using agricultural tractors on skid trails. The production areas with slopes ranging from 0% to 33% in Balıklı Forest Management Directorate (Düzce) were selected as the study area. In the study, each identified skid trail was sampled at 10-meter intervals. Soil compaction and soil loss measurements on the skid trails were modelled using a hand penetrometer at a soil depth of 0-5 cm. As a result of the study, it was determined that an area of 703 m² per hectare was exposed to soil compaction on the skid trails. Due to continuous skidding on the skid trails, soil erosion occurred at different depths, with a total soil loss of 53.39 m³ (134,009 tons) per hectare identified. According to the results of the PCA (Principal Component Analysis) conducted on soil compaction and loss, the total variance was found to be 73.63%. It was determined that soil loss is positively correlated with skid trail width, soil compaction, and skid trail length, thereby influencing soil compaction and loss. Additionally, a low negative correlation was identified between the slope parameter and soil compaction and loss.

Keywords: PCA, soil compaction, soil loss, forestry.

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1. INTRODUCTION

The production of industrial wood raw materials (logs, poles, industrial wood, etc.) in forestry generally consists of the stages of cutting, extraction from the compartment, and transportation. The location and method of these stages can vary depending on the organization (Özçamur 1981; FAO 1982; Dykstra and Heinrich 1996). In Türkiye, the production stages of wood raw materials are managed by the General Directorate of Forestry (OGM). Forest enterprises extract the wood raw materials produced in the forest in a way that does not harm the ecosystem and deliver them to the market, obtaining a significant portion of their income from this process. Within this process, extraction from the compartment constitutes the most critical stage.

The extraction phase from the compartment is the most challenging, costly, and environmentally damaging stage of wood raw material production. Additionally, according to the SPO (2007), approximately half of our country's forests are located in mountainous areas with slopes exceeding 44%, which negatively affects this stage.

Different tools and methods are applied in extraction operations, one of the stages of industrial wood raw material production, based on technical, economic, and ecological factors. One of these methods is the extraction of industrial wood raw materials from skid trails using tractors to bring them to temporary stacking sites (ramps) (Figure 1).

In our country, the use of tractors in extraction operations is carried out irregularly, depending on the experiences of forest workers. Consequently, along with the emergence of negative environmental impacts in many areas of forest land, there can also be temporal and economic losses.

Figure 1. Extraction from the compartment by skidding on a skid trail using an agricultural tractor.

The approach to forest utilization and forest conservation complicates the decision-making process related to forestry operations. Wood raw material production causes varying degrees of damage to the soil, the remaining stand, and the produced product (Bayoğlu 1972; Gürtan 1975; Aykut 1984; Acar 1994). The environmental damages resulting from the skidding of industrial wood raw materials on the ground can be categorized as follows: degradation of the physical properties of the soil (compaction, reduction in pore volume, decreased water and air capacity due to compaction, and increased bulk density, soil loss through surface runoff and erosion, soil transport and mixing), decline in plant growth and changes in species diversity (reduction in root development due to impaired soil properties, hindrance of nutrient uptake), deterioration in the conditions and activities of soil organisms and humification and mineralization in soil organic matter and litter, and nitrogen losses in the soil through denitrification (Erdaş 1993; Messina et al. 1997; Wang 1997; Bengtsson et al. 1998; Arocena 2000; Marshall 2000; Gilliam 2002; Buckley et al. 2003; Williamson and Neilsen 2003; Godefroid and Koedam 2004; Johnston and Johnston 2004; Makineci et al. 2007).

This study investigates the soil compaction and loss occurring during extraction from the compartment by skidding on skid trails using agricultural tractors. The study identified parameters affecting soil compaction and loss using skid trail width, soil compaction, skid trail length, loss of soil volume, and slope parameters. PCA analysis was utilized to determine the parameters influencing soil compaction and loss. As a result of the analysis, the directions of positive or negative impacts of environmental parameters on soil compaction and loss were identified.

2. MATERIAL AND METHOD

2.1. Study Area

This study was conducted in compartments numbered 48, 50, 51, 52, 53, 61, 63, 64, 66, 68, 69, 74, 75, and 76 of the Balıklı Forest Management Directorate in the Gölyaka district of Düzce Province. The Balıklı Forest Management Directorate is located between 40º 38' 40" - 40º 42' 40" North latitude and 30º 57' 35" - 31º 06' 45" East longitude. The location map of the study area was presented in Figure 2.

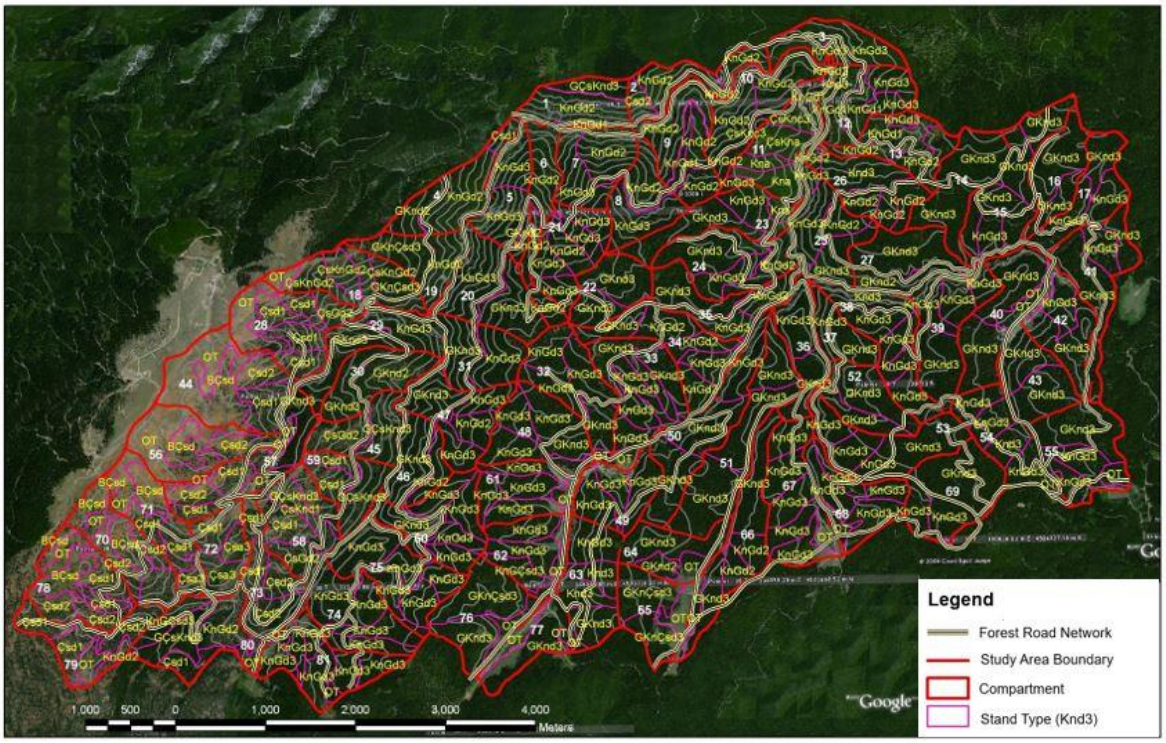

Figure 2. Location map of the study area boundary

The entire area of the Balıklı Forest Management Directorate is covered with forested land and has a total size of 5,822 hectares, with not bare areas present. Of the forested area, 5,469 hectares consist of tree-covered land, while 353 hectares are non-forested (herbaceous) land. The climate is generally cool in summer and rainy and cold in winter. The average annual temperature of the region is 13.3 ºC, with a maximum temperature of 42.0 ºC and a minimum temperature of -20.5 ºC. The average annual precipitation is 884.9 mm, and the relative humidity is 76% (Anonymous 2001). The study area consists of a mixed stand of Nordmann fir (*Abies bornmulleriana Mattfeld*) and oriental beech (*Fagus orientalis Lipsky*). The stands are in three age classes: closed, medium, and dense tree cover. The average slope of the study area is 17%, and it faces Southeast. Industrial wood raw materials are extracted from the compartment by skidding directly along skid trails using chains attached to agricultural tractors or with a cable from a mounted winch on the tractor.

2.2. Method

The primary cause of environmental damage resulting from the skidding of industrial wood raw materials on the ground is the compaction occurring in forest soil. Measurements were conducted to model the conditions of soil compaction and loss in the skid trails within the research area. Each skid trail was sampled at 10-meter intervals. In the samples taken from the skid trails, soil compaction was measured using a hand penetrometer (soil compaction meter) at a soil depth of 0-5 cm (Karaöz 1989a; Karaöz 1989b; Karaöz 1992; Makineci et al. 2007). To determine the soil loss occurring in the skid trails, the geometric shapes resulting from soil erosion were measured with a meter to find the cross-sectional areas, which were then multiplied by the length of the skid trail to calculate the volume of soil loss (Türk 2006; Menemencioğlu 2006). The soil loss value was provided in cubic meters and tons, with the average bulk density of forest soil taken as 2.510 tons/m³ for calculating the ton unit (Kara and Bolat 2008).

The parameters related to soil compaction and loss have been modelled using principal component analysis (PCA). PCA is one of the oldest and most commonly used techniques in research (Jolliffe, 2002). Through principal component analysis, fewer new variables or principal components, which can be described as combinations of the original variables, are created. The principal components are independent of one another, thus eliminating the dependency structure among the variables (Jolliffe, 2002). Additionally, PCA is one of the solutions suggested in the literature for the problem of multicollinearity (Abdi and Williams, 2010; Paul et al., 2013; Karamizadeh et al., 2013;Kurita, 2019; Salem, 2021; Özdemir and Çınar, 2023). In multivariate statistical analysis, *p* variables (features) related to *n* individuals (objects) are examined. The interrelation of many of these variables and the large number of variables (*p*) complicate various assessments. In such cases, principal component analysis is the most important technique employed. It is a multivariate statistical method that explains the variance-covariance structure of a set of variables through linear combinations of these

variables, facilitating data reduction and interpretation. Generally, this technique, used for eliminating the dependency structure among variables and/or for dimensionality reduction, serves as a standalone analysis as well as a data preparation technique for other analyses. In this method, p variables exhibiting mutual dependency, with n measurements, are transformed into k ($k \leq p$) new variables that are linear, orthogonal, and independent of one another (Jolliffe, 2002).

3. RESULTS

The arithmetic means, standard deviations, maximums, and minimums of the data related to the sample areas where measurements are taken on the dragging strips and the control points are provided in Table 1. The average length of the existing dragging strips per hectare is found to be 281 m, and the average area is 703 m².

Table 1. Data related to variables measured in drag strips

Parameters	**Unit**	**Minimum**	**Maximum**	**Average**	**Standard Deviation**
skid trail width	m	1,62	3,03	2,43	0.25
skid trail length	m	81	1418	389	281.8
soil compaction	psi	179	780	386.04	129.2
loss of soil volume	m^3/ton	2/5020	530/1330300	76/190760	80.0
loss of soil volume (100 m)	m^3/ton	2/5020	61/153110	19/47690	11.4

In the field of research, it has been determined that the extraction of industrial wood raw materials by dragging them along skid trails results in varying degrees of erosion on the skid trails. In the study area, the average skid trail density is found to be 281 m/ha. Accordingly, in Table 1, the average soil loss amount in a skid trail that is 100 m long, when compared to the skid trail density, indicates that there is a soil loss of 53.39 m³ (134,009 tons) per hectare.

In the evaluation of PCA analysis, variance explanation ratios and eigenvalues are taken into account. It is recommended to consider the axes where the eigenvalues are greater than 1 and the variance explanation ratio is more than 10%. In this way, the total variance of the PCA analysis is determined (Özdemir and Çınar, 2023; Çınar et al. 2023). The eigenvalues and percentage of variance explanations for the PCA analysis are provided in Table 2. In the performed analysis, Axis 1 and Axis 2 meet these criteria.

Table 2. Eigenvalues and variance explanation rates of PCA

Component	**Eigenvalue**	**Percentage of variance**
1	1.92	48.06
2	1.02	25.57
3	0.89	22.29
4	0.16	4.07

According to the results of the PCA analysis, it has been determined that axis 1 and axis 2 meet these criteria. The results for component 1 and component 2 of the PCA analysis was provided in Figure 3.

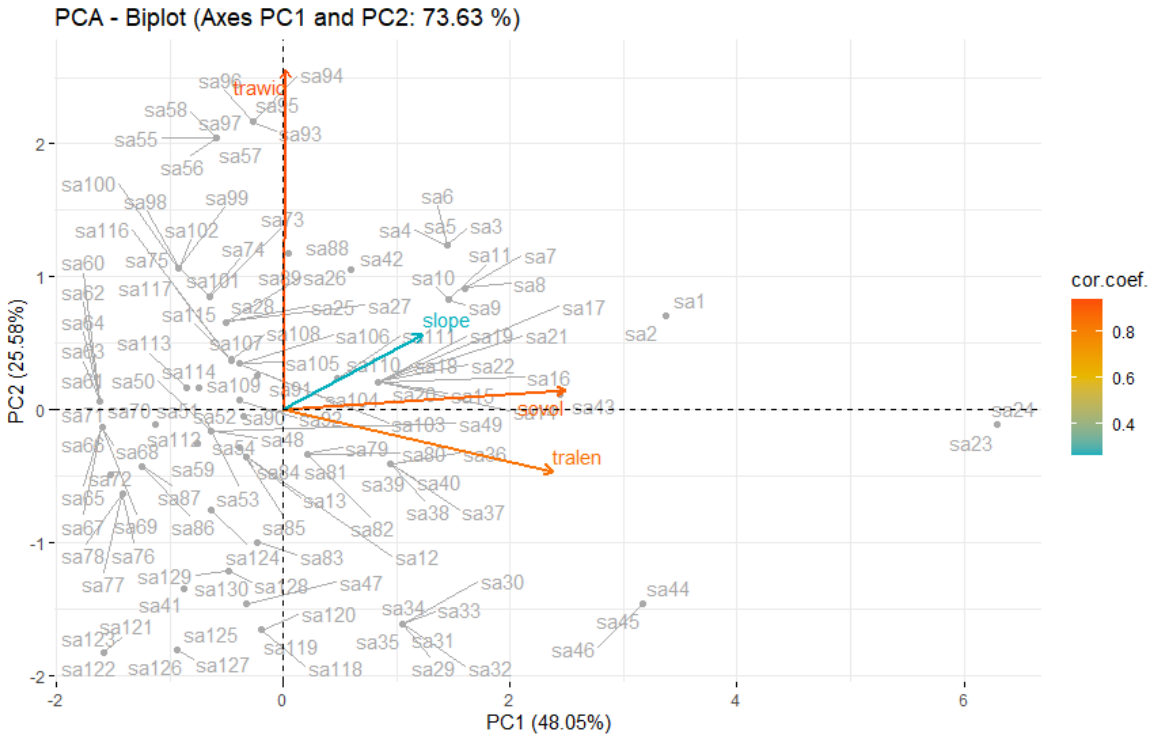

Figure 3. PCA result of component 1 component 2

As can be seen from Table 2, Axis 1 is the component with the highest variance explanation ratio. Accordingly, the variables soil compaction (socom) and skid trail width (trawid) show the highest correlation with Axis 1, and a positive relationship has been identified between these parameters. When Figure 3 is examined, it is observed that these variables are positioned together on Axis 1. On the other hand, the variable slope (slop) is positioned inversely on Axis 1, indicating a weak negative relationship with the other parameters. Overall, Axis 1 and Axis 2 explain 73.63% of the total variance.

4. DISCUSSION

In areas where soil loss occurs, erosion first accelerates, followed by the formation of rill channels that lead to soil losses, which in turn triggers further erosion and exposes bedrock in places. In the study conducted by Sidle et al. (2004), they estimated the amount of surface erosion occurring on skid trails as a result of extraction operations to be 272 ± 20 tonnes ha^{-1} $year^{-1}$. They also noted that the erosion rate on skid trails in steeper areas (%20<) is higher (320 ± 24 tonnes ha^{-1} $year^{-1}$) compared to that in less steep areas (245-264 tonnes ha^{-1} $year^{-1}$). The soil loss data obtained in this study are annual assessment results. However, the soil loss value obtained in this study is derived from skid trails where extraction has been conducted for a long time. Therefore, the soil loss value found in the field is higher compared to other studies. Gürtan (1975), Yıldırım (1989), and Tomasic (1996) indicated that forest soil can be damaged during the extraction of harvested products from the forest, particularly in sloped areas. These damages typically occur in the form of erosion-prone rills. Soil erosion in forest areas develops in direct proportion to factors such as vegetation cover, slope, transportation, and rainfall amounts. Görcelioğlu (2004) argued that one of the determining factors in the planning of skid trails is their effects on erosion and that machines that create wheel ruts in the forest soil lead to compaction and erosion, and therefore, these machines are not recommended. If light-skidding equipment is to be used, he suggested that skid trails should be routed through gently sloping (broad) terrain, and if agricultural tractors are utilized for skidding, the road slope should not exceed 25%.

As a result of the study, it was determined that the compaction on the skid trail was greater than at the control point, with 703 m^2 of area per hectare being exposed to soil compaction on the skid trails. Makineci et al. (2007) noted that there is a significant difference in soil compaction between the protected area and the skid trail (tractor path), indicating that compaction on the skid trail is greater than in the protected area, and that the amount of dead and living ground cover on the skid trails has significantly decreased compared to the protected area.

5. CONCLUSIONS

Instead of skidding industrial wood raw materials to loading sites without planning skid trails, skid trails have been planned to minimize adverse environmental impacts. The model coefficients of the factors affecting skidding damages have been determined. In the study, it was found that 703 m² per hectare of the skid trails area was subjected to soil compaction, and skid trails exhibited more compaction compared to natural areas. Due to continuous skidding on the skid trails, soil erosion occurred at various depths, with a soil loss of 53.39 m³ (134,009 tonnes) per hectare being identified. Currently, the average skid trail density has been found to be 281 m/ha. In this study, certain environmental damages (soil compaction, soil loss damages) were identified during the extraction of wood from the compartment using agricultural tractors. More detailed studies should be conducted to identify other environmental damages. To minimize the degradation of forest soil, the use of chippers and slash (cutting residues such as branches and leaves) covering the skid trails should be evaluated. Micro transport planning should be implemented, and the skid trail network should be included in the micro transport plan. In the extraction of industrial wood raw materials from the compartment, the negative impacts arising after production can be reduced, and work efficiency can be increased by providing job training to the skidder operator.

Acknowledgements

This study was supported by the Research Projects Management Unit of Karadeniz Technical University under Project No. 2008.113.001.3. We would like to express our gratitude to the unit staff.

Ethics Committee Approval
N/A

Author Contributions / Yazar Katkıları
Conceptualization: Y.T. T.Ç.S.G.; Investigation: Y.T. T.Ç.; Material and Methodology: Y.T. T.Ç.S.G.; Supervision: Y.T. T.Ç.S.G.; Visualization: Y.T. T.Ç.; Writing-Original Draft: Y.T. T.Ç.; Writing-review & Editing: Y.T. T.Ç.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

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Driving Digital Innovation for Sustainable Growth: A Case Study from Slovakia

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Abstract: Innovation is necessary for economic growth and sustainable development, which includes social, economic, and environmental prosperity. The European Union emphasizes innovation and aims for R&D investments to reach 3 % of GDP. Slovakia has experienced significant transformation driven by digital innovation, as evidenced by data on digital readiness and business skills across industries. The main aim of this paper is to assess innovation activities in Slovakia, focusing on the gross domestic expenditures on R&D in the selected period. Research and development (R&D) spending, a key metric for technological advancement and economic growth, is predominantly concentrated in the Bratislava region because its strategic role as Slovakia´s political, economic, and cultural center attracts substantial investments. Innovation expenditures in Slovakia vary by business size, with large companies leading due to their financial resources and sizable workforces. Small companies face resource constraints that limit their R&D investments. Dynamic trends in Slovakia show an increasing percentage of companies of business engaged in innovation activities which is a significant fact, because innovation is essential for the competitiveness, prosperity, and sustainability of European member states, including Slovakia. Slovakia´s commitment to R&D, particularly in the Bratislava region, along with the positive trend in business participation in innovation, highlights the potential for a more innovative and competitive environment for companies.

Keywords: Slovakia, research and development, investment, innovation, region

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1. INTRODUCTION

Digitalization is an important part of innovation that includes the wider social and institutional use of these technologies, whereas digitization refers to the process of transferring analog information to digital form, even if the terms are commonly used synonymously (Bican & Brem, 2020). Another definition of digitization characterizes it as a transformation caused by the widespread adoption of digital technologies for the generation, processing, sharing and transmission of information (Nagy & Somosi, 2022). Three pillars are necessary for sustainable development: social, economic, and environmental well-being. Protecting vulnerable populations, promoting healthy social contact, and cultural enrichment are the main goals of a sustainable society. Yet there are connections among these pillars. A society's long-term well-being depends on both a robust economy and a clean environment (R. Khan, 2016). In a world where science fiction comes to life by constant innovation, the last few decades have seen an explosion in technological and digital breakthroughs (Tolić et al., 2022). Innovation plays a crucial role in the economic development and growth of European countries. Research has shown that higher innovation performance is directly linked to economic growth (Akçomak & Weel, 2009). Even while digital technologies have the potential to significantly improve company, their strategic deployment is necessary to realize their full potential and create new business models or operational excellence (Moschko & Blažević, 2023). Countries like Germany, Italy, and Spain have demonstrated the ability to generate significant commercial output from innovation-related activities, highlighting the importance of fostering innovation (Makkonen & Inkinen, 2013). Additionally, the European Union has recognized the significance of innovation in achieving lasting economic growth and improving economic and social conditions (Mazur-Wierzbicka, 2019). Efforts to promote innovation in Europe involve multi-level policies at the European, national, and regional levels, taking into account each country's unique characteristics such as infrastructure, investment levels, and education standards (Arbussà & Llach, 2018). The European Union has set ambitious goals for innovation policies and research and development (R&D) investments, aiming for R&D to represent 3% of GDP, although challenges persist in achieving this target (Ješić et al., 2021). Innovation is not only crucial for economic growth but also for addressing societal challenges. The manufacturing sector, for instance, is seen as a key player in providing innovative solutions to significant societal

challenges, further underlining the importance of innovation in Europe (Sautter, 2016). Innovation is a crucial driver of economic development and competitiveness for countries worldwide. Research has shown that innovation is closely linked to productivity and economic progress (Crespi & Zúñiga, 2012). Developed countries, in particular, benefit significantly from innovation as it contributes to sustainable development and overall economic advancement (Malik, 2020). Technological innovation, especially in sectors like renewable energy, is highlighted as a key element for countries with strong innovative bases and substantial investments in research and development (Khan & Su, 2022). National innovative capacity is influenced by factors such as investment in research and development, innovation-oriented policies, and the level of education within a country (Hegerty & Weresa, 2022). Innovation is not only vital for economic growth but also plays a significant role in enhancing a country's competitiveness and gaining a competitive advantage (Domazet et al., 2022). Moreover, innovation is essential for firms' competitiveness, leading to economic growth at the national level (Mariev et al., 2020). It is also considered a critical element for surviving in global competition, maintaining competitiveness, improving economic performance, and contributing to national economic development (Puzović et al., 2019). Businesses alter their perspectives not only in response to consumer demand or national and international regulations and standards, but also because implementing environmental management techniques opens up new business prospects (Loučanová et al., 2022)

Early in the new millennium, Slovakia was among the EU 25 region's worst innovators. Low innovation performance was a result of multiple causes. Some of them were objective, such the long transformation of the economic and social system. Subjective factors, such as a weak national innovation system and unambitious innovation strategies, also hindered the development of technologies. These were also reflected in low expenses for research and development (Baláž, 2005). The adoption of sustainable development initiatives could indicate the beginning of a fast, broad, and worldwide transition to an ecological economy that supports growth by allocating resources to the protection of natural resources (Loučanová et al., 2023). Slovakia's development has been greatly impacted by EU regional policy since its 2004 entry. This effect was reinforced by the National Strategic Reference Framework, which was developed for the 2007–2013 period and which directs Slovakia's use of EU funding for social and economic development (Rentková, 2018).
The main aim of this paper is to assess innovation activities in Slovakia, focusing on the gross domestic expenditures on R&D in the selected period. In this paper, the following hypotheses were established:

Hypotheses 1: The companies do invest the same average amount of investments from 2001 to 2020 to innovations regardless of the company size.
Hypotheses 2: The companies do invest in the same areas of investments from 2001 to 2020 regardless of the company size.

2. MATERIAL AND METHOD

Based on the necessity to assess trends and developments of the level of innovation within Slovak enterprises, a research approach was chosen. A variety of broad scientific methods, particularly statistical analysis and graphical data presentation were employed to analyse the degree of innovation. To illustrate the overview of the innovation level in Slovakia, the study uses statistical data from 1996 for the Gross domestic expenditures on R&D by region and from 2001 for other parts of the research. Data from the statistical office were supplemented with results from a questionnaire survey, where we determined the degree of innovation and the level of investments in companies.
Data were prepared and processed by Microsoft Office 365 and statistically analysed by Jamovi 2.5.6. Reliability Analysis with Correlation Heatmap was used to identify the reliability of the data and potential correlations between the debt ratio variables. Then the various dependencies were quantitively identified by Non-parametric One-way ANOVA (Kruskal-Wallis test) which, together with the Paired Samples Contingency Tables identified dependencies and correlations between variables and evaluated hypotheses.

3. RESULTS

The Slovak Republic, a country in central Europe, has undergone a dramatic transformation in recent years, mostly because of the pervasive influence of digital technology. A unique opportunity to gauge the scope of this transformation is provided by publicly available data, which also reveals the digital readiness and skills of businesses across numerous industries. The following conclusions serve as a basis for both the empirical investigation and a more thorough evaluation of the opportunities and difficulties that lie ahead.

The switch from planned to market economies in the post-communist countries resulted in a significant increase in regional economic variation, and the early-transition regional disparities in unemployment rates remained remarkably stable (Terrell, 2009). The geographical distribution of research and development (R&D) spending in the Slovak Republic

is shown in Figure 1. Research and development spending is a key metric for assessing a region's commitment to technological advancement, innovation, and economic growth. During the duration of the study period, the Bratislava region has consistently dominated in terms of R&D spending. This continued leadership role has been attributed to a number of factors, chief among them being Bratislava's strategic location as the nation's capital. As the political, economic, and cultural centre of the country, Bratislava has understandably attracted a disproportionate number of investments. Following Bratislava, Trnava region holds the second position in terms of R&D spending. Influencing factors are industrial areas and the location, which is located near the Bratislava region. As we gradually move from the western regions, through the center to the east, R&D investments gradually decrease. On the contrary, the unemployment rate increases towards the east (Koisova et al., 2018). The spending in the remaining regions (Trenčín, Nitra, Žilina, Banská Bystrica, Prešov, Košice) is according to Figure 1 lower.

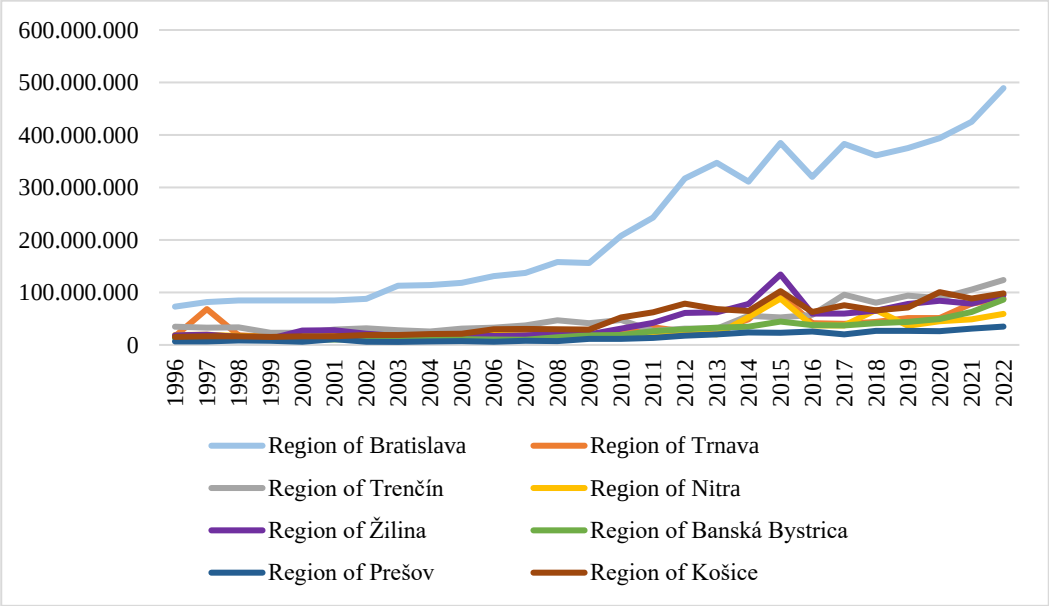

Figure 1. Gross domestic expenditures on R&D by regions
Source: Own elaboration based on data from the Slovak Statistical Office

Figure 2 displays a summary of innovation spending divided into business size classes. The data shows a pattern where a company's size and the amount it spends on innovation are positively associated. The three distinct size groups—small, with 0–49 employees —medium, comprising 50–249 workers and large, comprising 250+ workers—display varying levels of financing assistance for innovation initiatives. Small businesses exhibit the smallest innovation expenditures, which is understandable given the resource constraints that these organizations typically face. Even though small businesses contribute significantly to innovation, their limited resources often limit the amount of investments into R&D. Medium-sized enterprises are outspending small organizations when it comes to innovation spending. These businesses are moderately financially capable, which enables them to allocate more financial resources to innovation and carry out a wider range of research and development. Big companies lead the way in innovation investment because they have a sizable workforce and substantial funding. The substantial investments made by these companies show their commitment to the latest research, technological development, and industry-leading innovation, all of which significantly improve their competitiveness. The framework for the analysis that follows, which encourages a closer examination of the variables impacting these detected trends, is innovation spending by size classes. By diving further into the factors influencing innovation and digitization investment across different firm size classes, we expect to find important insights that will contribute to a more comprehensive understanding of the innovation and digitization environment within the business environment.

Figure 2. Innovation expenditure by size classes
Source: Own elaboration based on data from the Slovak Statistical Office

Slovakia's spending on innovation is shown in Figure 3. The diagram gives a quick summary of Slovakia's financial commitment to innovation and displays dynamic trends in expenditure on innovation over time. The graph displays a progressive trend with peaks and troughs that represent times when innovation investment was higher.

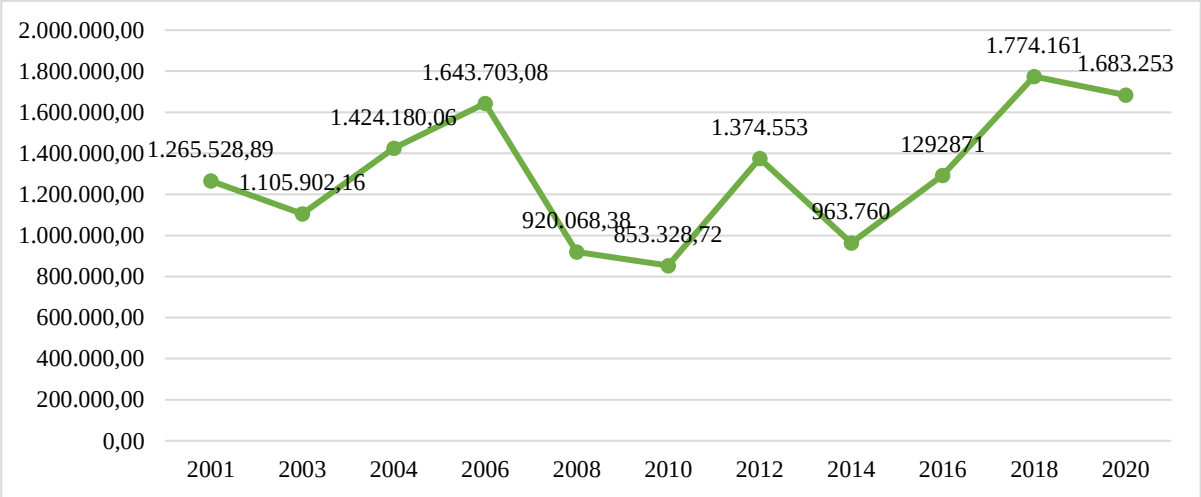

Figure 3. Innovation expenditure in the Slovak Republic
Source: Own elaboration based on data from the Slovak Statistical Office

An engaging depiction of the innovation environment in Slovak companies can be seen in Figure 4, which displays the percentage of businesses engaged in innovation activities relative to all businesses. Over the course of the observation period, a pattern emerges that implies the majority of organizations do not consistently engage in innovative projects. While still important, this majority exhibits a downward tendency, suggesting a gradual shift in the innovation landscape and a gradual rise in the percentage of companies actively pursuing innovation. The declining proportion of non-innovating corporations underscores a significant shift as businesses increasingly recognize the need for innovation to stay competitive. In the meantime, a progressive and dynamic corporate environment is being created as evidenced by the steady rise in the percentage of companies participating in innovative initiatives.

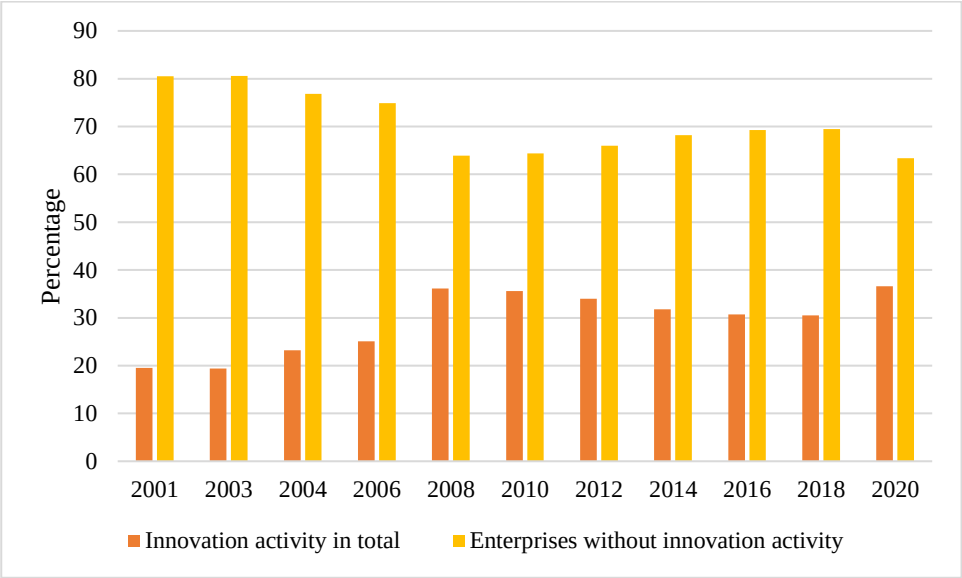

Figure 4. Share of enterprises with innovation activity on the total number of enterprises in %
Source: Own elaboration based on data from the Slovak Statistical Office

Table 1. Descriptive statistics

	Size	Area of investment	Avg. amt. of investments (2001-2020)
N	250	250	250
Mean	2.38	2.57	3.34
Std. Error mean	0.0521	0.0758	0.120
Median	3.00	3.00	4.00
Standard deviation	0.823	1.20	1.90
Minimum	1	1	1
Maximum	3	4	6
Shapiro-Wilk W	0.692	0.827	0.830
Shapiro-Wilk p	< .001	< .001	< .001

Source: Own elaboration

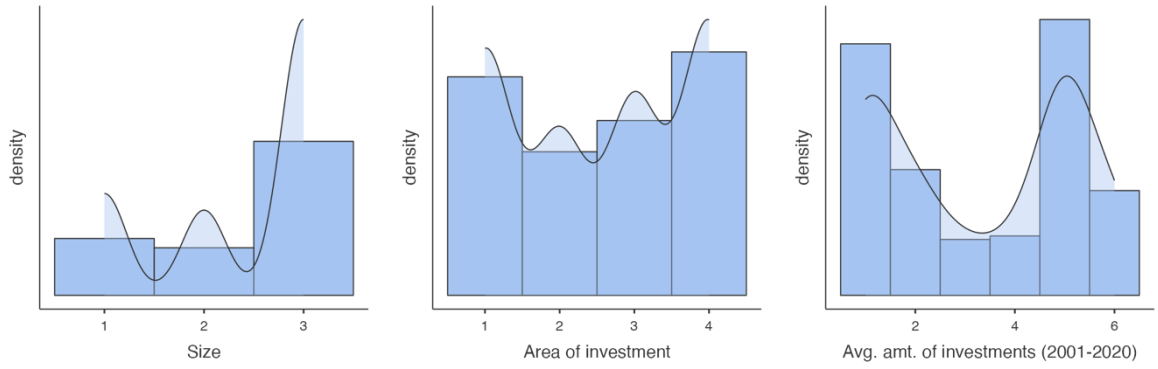

Figure 5. Plots with densities
Source: Own elaboration

From the descriptive and plots with densities (which were calculated from coded data), we can identify these data as abnormal; therefore, it is appropriate to use only non-parametric testing.

Table 2. Scale Reliability Statistics

	Cronbach's α	McDonald's ω
Scale	0.795	0.795

Source: Own elaboration

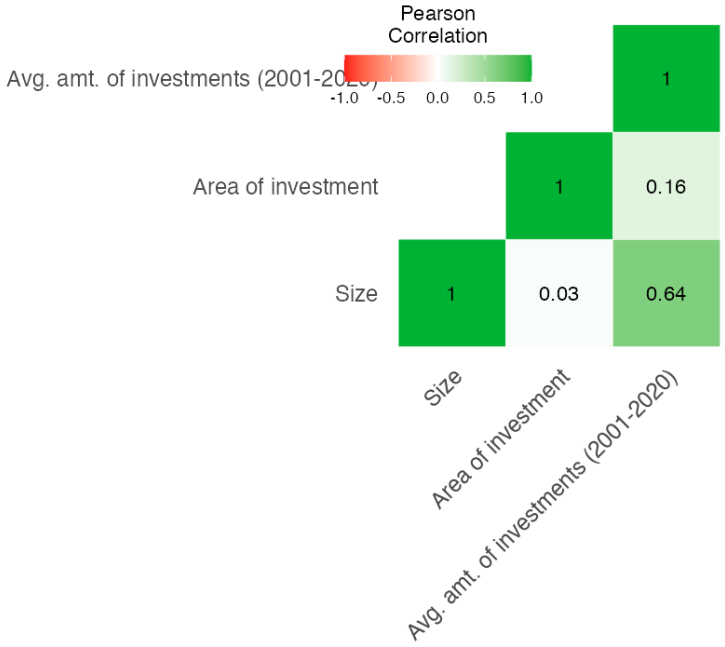

Figure 6. Correlation heatmap
Source: Own elaboration

Based on the reliability test, it is possible to confirm that these data are suitable for further statistical verification since the value of the measure of internal consistency of the data reached 0.795 according to Cronbach's alpha and 0.962 according to McDonald's omega.

Based on the results of the correlation heatmap, it is possible to prove the correlation between the size of the companies and the average amount of investments from 2001 to 2020.

Table 3. Kruskal-Wallis

	χ^2	df	p
Area of investment	1.92	2	0.382
Average amount of investments from 2001 to 2020	113.66	2	< .001

Source: Own elaboration

Based on the results from the Kruskal-Wallis test it is possible to reject the null hypotheses and accept the alternative hypothesis of the H1 because the value of p is <.001. It is possible to identify that at least one of the sizes of the companies does not invest the same average amount of investments from 2001 to 2020 to innovations as the others. However, based on the results above, it is not possible to reject the null hypotheses of the H2 because the value of p is 0.382 which means that the companies do invest in the same areas of investments from 2001 to 2020 regardless of the company size.

Table 4. Paired Sample Contingency Table

Avg. amt. of investments (2001-2020)								
Size		**more than 250 th. €**	**200-249 th. €**	**150-199 th. €**	**100-149 th. €**	**50-99 th. €**	**To 49 th. €**	**Total**
10-49	**count**	2	2	4	1	4	42	**55**
	% within row	3.6 %	3.6 %	7.3 %	1.8 %	7.3 %	76.4 %	
	% within column	6.7 %	2.5 %	23.5 %	6.3 %	11.1 %	58.3 %	
50-249	**count**	2	1	3	4	13	23	**46**
	% within row	4.3 %	2.2 %	6.5 %	8.7 %	28.3 %	50.0 %	
	% within column	6.7 %	1.3 %	17.6 %	25.0 %	36.1 %	31.9 %	
250 and more	**count**	26	76	10	11	19	7	**149**
	% within row	17.4 %	51.0 %	6.7 %	7.4 %	12.8 %	4.7 %	
	% within column	86.7 %	96.2 %	58.8 %	68.8 %	52.8 %	9.7 %	
Total	**count**	**30**	**79**	**17**	**16**	**36**	**72**	**250**
	% within row	**12.0 %**	**31.6 %**	**6.8 %**	**6.4 %**	**14.4 %**	**28.8 %**	
	% within column	**100.0 %**	**100.0 %**	**100.0 %**	**100.0 %**	**100.0 %**	**100.0 %**	

Source: Own elaboration

Based on the Kruskal-Wallis test results and the derivated paired sample contingency table, it is possible to identify that companies with over 250 employees emphasize investments, as up to 51% of companies invested 200-249 thousand € in innovations, which represents 96.2% of the total investments in this range. A similar trend can also be identified in investments over 250 thousand €, where the share of companies with more than 250 employees in all investments in innovations reached 86.7%. On the contrary, large enterprises with more than 250 employees are not strongly interested in smaller investments in innovations.

4. CONCLUSIONS

Innovation plays a critical role in the competitiveness, prosperity, and sustainability of European nations. It is the engine of social advancement, economic growth, and the resolution of urgent issues. European nations can secure long-term prosperity and improve their global competitiveness by promoting innovation through focused policies and investments. Innovation is essential to nations because it promotes sustainable development, increases competitiveness, and accelerates economic progress. Nations that allocate resources towards innovation, technology, and research and development are more likely to achieve sustained prosperity and prosper in the global economy. Spending on research and development (R&D) has been consistently committed by Slovakia, especially the Bratislava region, where investments are largely drawn by Bratislava's key location as the capital of the country. Furthermore, a company's size and innovation spending are clearly correlated, with larger businesses investing more in innovation since they have stronger financial standing. Despite this, a positive trend shows that the percentage of companies participating in innovation activities has been rising over time, demonstrating a greater understanding of the significance of innovation for preserving competitiveness. Although the existing data offers insightful information about some aspects of Slovakia's innovation scene—most notably, the increasing involvement of larger companies in innovation—a more thorough analysis of investment trends across various business sizes and industries would provide a more nuanced picture. However, the data shows how crucial it is to create an atmosphere that is conducive to innovation in order to propel Slovakia's economic expansion and competitiveness. Despite the constraints associated with the accessible data, the results indicate a hopeful path for Slovakia's innovation ecosystem. More investigation and data gathering are necessary to provide a more accurate picture of investment trends and their effect on innovation outcomes. All things considered, these results demonstrate how dynamic Slovakia's innovation scene is and point to the potential for a more creative and competitive corporate environment.

Acknowledgements

Author Contributions

Conceptualization: A.J., M.Č., D.Č.; Investigation: A.J., M.Č., D.Č.; Material and Methodology: A.J., M.Č., D.Č.; Supervision: A.J., M.Č., D.Č.; Visualization: A.J., M.Č., D.Č.; Writing-Original Draft: A.J., M.Č., D.Č.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

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Comparison of WorldClim and CHELSA Climate Data

Ahmet ACARER*[1]

Abstract: In this study, the bioclimatic concordance (i.e., the degree of concordance in temperature and precipitation values) between the recently updated WorldClim and CHELSA global databases was investigated. In order to reveal the effect of choosing which bioclimatic database on species response curves, the worldwide distributed Eurasian magpie (*Pica pica*) was examined in this study. Maximum Entopy (MaxEnt) method, which is frequently preferred in wildlife habitat suitability models, was preferred. According to the results of the WorldClim global climate model, the Eurasian magpie habitat suitability model with a training data set AUC value of 0.877 and a test data set AUC value of 0.875 was selected as the best model, and according to the results of the CHELSA global climate model, the model with a training data set AUC value of 0.899 and a test data set AUC value of 0.892 was selected as the best model. When the results of both models were evaluated according to Baldwin's Auc classification, it was determined that they were in the "very good" model category. In addition, when the average AUC value jackknife graphs were examined with all variables contributing to the two different habitat suitability models, it was determined that worldclim was 0.87 and Chelsa was 0.89. As a result, it was determined in this study that the CHELSA climate data preferred on bird species distribution produced slightly higher results.

Keywords: AUC, Climate comparison, Eurasian magpie, Maximum Entopy, WorldClim

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1. INTRODUCTION

The damage inflicted by humans on the long-protected natural balance of forest areas has been substantial over the past century (Watson et al., 2018). Since the late 19th century, following the onset of the Industrial Revolution, the losses experienced through forest depletion, fragmentation, and even destruction have continued to escalate (Lewis et al., 2015). Methods employed by humans to meet their needs from forests have led to disruptions in natural balance, resulting in environmental pollution and various ecological issues (Bengtsson et al., 2000). This ongoing situation has contributed to the degradation of natural ecosystems and placed their future at risk (Balmford and Bond, 2005). The disruption of ecological balance has resulted in a decline in biodiversity, desertification, and the emergence of climate change (Hailu, 2023).

Globally changing climate conditions have emerged as one of the fundamental issues faced by humanity in the early 21st century, generating significant concern among scientists (Lubchenco, 1998; Clark et al., 2005). Consequently, a variety of scientific research on climate change has been conducted by different researchers (Acarer, 2024a; Acarer, 2024b; Acarer, 2024c). In other words, with the recent impacts of climate change becoming more pronounced over a shorter time frame, studies focusing on this issue have gained prominence (Acarer, 2024d; Acarer, 2024e; Acarer and Mert, 2024). These investigations examine not only the effects of climate change on ecosystems but also its implications for human health, agriculture, water resources, and economic stability (Viviroli et al., 2011; Chang and Bonnette, 2016). Furthermore, there is a strong scientific consensus on the necessity of reducing greenhouse gas emissions and developing climate adaptation strategies (Klein et al., 2005). To effectively address these challenges in the future, adopting an interdisciplinary approach is critical for both policymakers and scientists. In conclusion, climate change is highlighted as a complex and multifaceted issue that encompasses not only environmental but also social, economic, and political dimensions.

Global climate change has been characterized as a range of adverse processes that significantly impact ecosystems by increasing the frequency and intensity of extreme weather events, including rising temperatures, droughts, excessive rainfall, and severe winds (Ummenhofer and Meehl, 2017; Walsh et al., 2020; Seneviratne et al., 2021; Clarke et al., 2022). When these processes are combined with factors that alter the dynamics of the climate system, such as rising sea levels and glacial melting, they create profound effects on the balance of ecosystems (Higgins et al., 2002; Harley et al.,

2006; Upadhyay, 2020). Consequently, climate changes arising from rising temperatures coupled with low precipitation or high rainfall combined with low temperatures are among the primary factors influencing the distribution of certain wildlife and bird species within forest ecosystems (Leech and Crick, 2007).

Eurasian magpie (*Pica pica*), belonging to the Corvidae family, is a social and defensive species of birds (Linsdale, 1937; Vernelli, 2013). This bird, characterized by its long tail, has white feathers on the inner sides of its shoulder, belly, and wing flight feathers (Linsdale, 1937). Its body feathers are a glossy metallic black, while its tail consists of metallic green plumage (Linsdale, 1937). Additionally, its legs and beak are black (Linsdale, 1937). The species has a wide distribution, ranging from Asia to Europe and North America, with the subspecies found in Turkey known as the "Eurasian Magpie". Global climate change is impacting the Eurasian magpie across its extensive geographical range. To mitigate the effects of global climate change or to adapt with minimal damage, various global climate models are being highlighted. Among these, WorldClim and CHELSA are notable for providing up-to-date and detailed information (Karger et al., 2017; IPCC, 2024).

WorldClim and CHELSA global climate models are critical for climate change and ecosystem research, as they help inform necessary measures and provide forecasts for future years (Randall et al., 2007; Leichenko and O'Brien, 2024; Power et al., 2024). These models play a crucial role in conducting statistical evaluations related to changing climates (Desmet et al., 2024; Piccolroaz et al., 2024; Rawat et al., 2024). By analysing various parameters in oceans, terrestrial surfaces, and the atmosphere, they assist in elucidating how the climate responds to current greenhouse gas emissions. Therefore, global climate models are among the most advanced systems available. However, the analysis of these climate models and the interpretation of their results is a complex process. Nevertheless, it is worth considering the global climate modelling processes that have the potential to produce predictions for both the near and distant future, as well as to implement protective measures (Evcin and Kalleci, 2020; Evcin, 2023; Evcin, 2024a; Evcin, 2024b).

Throughout history Anatolia has been affected by global climate change, having hosted various civilizations (Öztürk, 2002; Demir, 2009; Haldon et al., 2014). In addition to climate change, years of unauthorized and irregular poaching activities have placed certain mammal and bird species at risk of extinction. To carry out conservation activities for mammal and bird species distributed across different ecosystems, and to prevent habitat fragmentation or slow its progression, species distribution models are emphasized. Species distribution modelling can be categorized into two classes: connection and mechanistic approaches. Connection methods are preferred over mechanistic methods, as the latter require eco physiological characteristics specific to the target species. In contrast, connection methods do not have such requirements (Özkan and Şentürk, 2012). One of the connection methods is the Maximum Entropy approach (Elith et al., 2011).

The MaxEnt (Maximum Entropy) method is a widely used statistical approach, particularly in ecological and geographic information systems. This method is notable for its effectiveness even in situations with incomplete data on wildlife and bird species, as well as its ability to optimally utilize existing data. Furthermore, it allows for the integration of different types of datasets, including continuous and categorical data, thereby facilitating the modelling process. With the capability to produce high-quality predictions using minimal presence data, MaxEnt supports decision-making processes. Additionally, its ability to model interactions among multiple variables enables more comprehensive analyses and aids in the understanding of global climate change. Finally, the integration of techniques such as cross-validation enhances the model's accuracy and reliability. Due to these attributes, the MaxEnt method is frequently preferred in habitat suitability modelling for wildlife (Phillips and Dudik, 2004; Phillips et al., 2006; Evcin et al., 2019; Kaky et al., 2020; Zhang et al., 2021; Zhao et al., 2021; Sarı, 2022; Evcin, 2023; He et al., 2023; Li, et al., 2023).

This study aims to develop a habitat suitability model for the Eurasian Magpie species in the Mediterranean region of Türkiye. To achieve this goal, the MaxEnt method, widely used in wildlife ecology and management, has been employed. Two different global climate models, WorldClim and CHELSA, have been used as independent variables. As a result, a habitat suitability model has been established that corresponds to the global climate variable with the highest explanatory power for the distribution of the Eurasian Magpie in the lake region.

2. MATERIAL AND METHOD

2.1. Study area and Eurasian Magpie presence data

This study was carried out in the Mediterranean region, one of the 7 regions of Turkey. The Mediterranean region has a climate dominated by hot and dry summers and mild and rainy winters (Lionello et al., 2006; Lionello et al., 2012; Perry, 2014). This climate includes a rich vegetation and various topographic features (Cowling et al., 1996; Rundel et al., 2016). This area, which is generally covered with mountainous terrain, hosts important mountain ranges such as the

Taurus Mountains and there are plains suitable for agriculture between the mountains. There are many bays and beaches on the coasts of the Mediterranean region. Therefore, the elevation of the study area varies between the lowest 0 m and the highest 3561 m (Figure 1). While the maquis vegetation specific to the climate of the study area is enriched with resistant plants such as olive, thyme and lavender, agricultural products such as olive, citrus and grape increase the economic importance of the region.

In terms of wildlife diversity, the Mediterranean region stands out with its rich wildlife diversity and is home to many species in terms of wildlife thanks to its inclusion of both terrestrial and marine ecosystems. Some of these include mammals such as wolves, jackals, hyenas, foxes, and numerous migratory bird species, especially albatrosses, pelicans, and flamingos, which are frequently seen in wetlands. In addition, various reptile species such as turtles, snakes, and lizards are also common. Eurasian Magpies are generally distributed in the highlands of northwest America, Asia, Europe, Tibet, India, Ladakh (Leh and Kargil) and Pakistan (Tatner, 1982; Bokotej, 1997; Dos Santos et al., 2024; Plateau et al., 2024). Due to its wide geographical distribution, presence data of Eurasian Magpie were obtained from GBIF (Global Biodiversity Information Facility) network.

GBIF (Global Biodiversity Information Facility) is an international network that collects, shares and distributes biodiversity data worldwide. GBIF collects biodiversity data from various sources (museum collections, observation records, research projects) and makes these data openly accessible to researchers by sharing them. GBIF enables monitoring and analysing the status of global biodiversity by including different types of data such as species records, observation data, distribution maps and ecological data. With the open data principle, all data are freely accessible, and this helps support scientific research and environmental protection efforts. In addition, it provides users with various analysis tools and visualization options to better understand biodiversity data. Therefore, 5331 presence data belonging to the Eurasian Magpie target species (GBIF, 2024), which is also distributed in the Mediterranean region, was obtained from the GBIF infrastructure and is shown in green on the map (Figure 1).

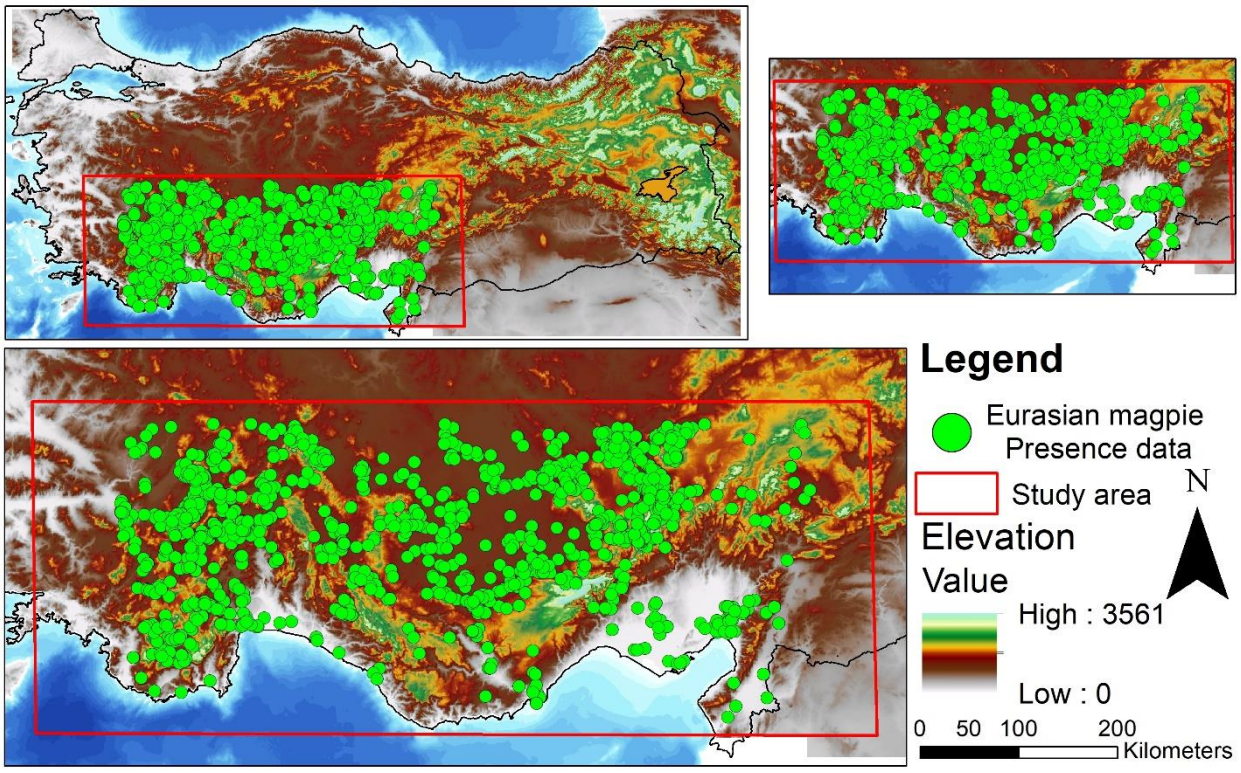


Figure 1. Study area location and Eurasian Magpie presence data (5331) mapping

2.2. Preparation of Environmental, CHELSA and WorldClim digital base maps

It is stated in the literature that numerical and model-based base maps are needed before starting species distribution modelling (Acarer, 2024f). Based on this, the digital elevation model (30 arc second resolution ~1 km) of the study area was first obtained from the internet address https://www.usgs.gov/. Then, the production process of environmental base maps that could be effective in Eurasian Magpie habitat suitability models was started. Based on the digital elevation

model obtained according to the study boundaries, the base maps of the Slope Degree, Slope Percentage, Aspect, Aspect Classes, Elevation, Elevation Class, Landform Classification (Jenness), Hillshade, Heat Load Index (McCune 2002), Area Solar Radiation, Roughness, Terrain Ruggedness and Topographic Moisture index of the study area were produced with the help of ArcGIS Pro+ software. In addition, considering that the illumination at different hours within the study area may influence Eurasian Magpie distribution, solar illumination index ((Solar Illumination Index)/20:00, 18:00, 16:00, 14:00, 12:00, 10:00, 08:00, 06:00, total illumination) base maps of the study area were produced with ArcGIS Pro+ software (Tekeş, 2017; Tekeş and Cürebal, 2019).

Environmental variables such as stand type, stand area (ha), age classes and closure that may affect the distribution of Eurasian Magpie, which are likely to be seen in many ecosystem environments, were obtained from the management plans of the General Directorate of Forestry. The base maps obtained in vector format were converted to raster format with the help of the "Polygon to Raster" add-on of ArcGIS Pro+ software. Aspect suitability index, temperature index and radiation index base maps were produced based on different formulas and previously produced digital base maps with the "raster calculator" add-on of ArcGIS Pro+ software. After the production period of the environmental base maps was completed, the production phase of CHELSA and WorldClim global climate models was started.

Global climate models provide information about the potential distribution of the target species with the help of statistical analyses, considering current climate conditions. One of these models, the CHELSA V2.1 climate scenarios, has high resolution (30 arc second resolution ~1 km) (Karger et al., 2017). Therefore, to contribute to Eurasian Magpie habitat suitability modeling, current CHELSA V2.1 climate scenarios were obtained from www.chelsa-climate.org (Karger et al., 2017). In this study, which aims to reveal the effects of Chelsa and WorldClim climate variables on species distribution, WorldClim global climate models (30 arc second resolution ~1 km) were obtained from https://www.worldclim.org/. These global climate maps downloaded at world scale were resized based on the study area and the "GCS_WGS_1984" projection system was applied. Thus, 19 CHELSA and 19 WorldClim climate variables were obtained according to the study boundary. As a result, the modeling phase started with the environmental variable, CHELSA and WorldClim climate variable base maps in raster format produced according to the study boundary.

2.3. Eurasian Magpie habitat suitability modelling

In this study, which was conducted to reveal the effects of CHELSA and WorldClim global climate models on Eurasian Magpie distribution, Maximum Entropy (MaxEnt) version 3.4.4, one of the species distribution methods, was used. MaxEnt method is an approach that has an important place in the fields of statistical modelling and machine learning. It is widely used especially in species distribution modelling, ecosystem management and environmental analysis. The basic principle of the Maxent method is to determine the distribution with the highest entropy under uncertainty based on the available data. In this way, it means that a certain distribution for the target species is created in the most probable form based on the observed data. In other words, MaxEnt considers some restrictions while trying to find the distribution with the highest entropy based on the observed data. These restrictions usually express the effects of some features (e.g. environmental and climatic variables) related to the observations on the distribution. To establish the working principle of the MaxEnt method, the data set must first be prepared. At this stage, environmental and climatic data (such as temperature, humidity, altitude) related to the regions where the target species is observed are collected. During the creation of the model, the observed data set is associated with certain features (feature functions). These features reveal the relationship between the observed distribution and certain environmental and climatic conditions. In summary, the MaxEnt method uses these feature functions to ensure that the model output has the highest entropy under certain restrictions (Phillips et al., 2006; Phillips and Dudík, 2008; Elith et al., 2011; Phillips et al., 2017). The Maxent formula is generally expressed as follows.

$$P(x) = \frac{1}{Z(\lambda)} exp\left(\sum_i \lambda_i f_i(x)\right)$$

According to the formula, $P(x)$ is the probability distribution of the event (x), λi are the parameters representing the restrictions, $fi(x)$ are the feature functions and the relationship of x with these features, $Z(\lambda)$ is the normalization factor (partition function), which makes the sum of all probabilities have a value of 1. The MaxEnt method is one of the most used methods for species distribution modeling. This method is used to understand which environmental conditions show the best performance for determining the distribution of wildlife target species. In addition, the MaxEnt model is an important tool for estimating the potential distribution of species under climate change scenarios. While traditional statistical models usually require more data, MaxEnt provides meaningful results even with limited data. The accuracy of the model results produced with the MaxEnt method should be checked. The accuracy of the MaxEnt model is generally evaluated with performance criteria such as AUC (Area Under the Curve). According to the AUC values, it is necessary to ensure that the training data set is higher than the test data set AUC value. In addition, it is necessary to

ensure that the test data set value is not higher than the training data set value. Baldwin (2009) classified the models developed on wildlife species according to the AUC values of the training and test data sets as 0.9< AUC "very good", 0.7< AUC <0.89 "good" and AUC <0.69 "uninformative". In addition, the Jackknife graph obtained should be examined. According to the Jackknife graph, it should be ensured that the contribution level of the variables contributing to the model alone does not exceed the contribution to the entire model. In short, the MaxEnt method has become an indispensable method for researchers and practitioners as it stands out as a flexible and powerful modelling technique that can make the best estimates in cases where wild animal species distribution is uncertain (Acarer, 2024g; Acarer, 2024h). As a result, the workflow diagram of this study, which aims to reveal the relationship between the Maxent method and global climate models of the Eurasian Magpie, is given below (Figure 2).

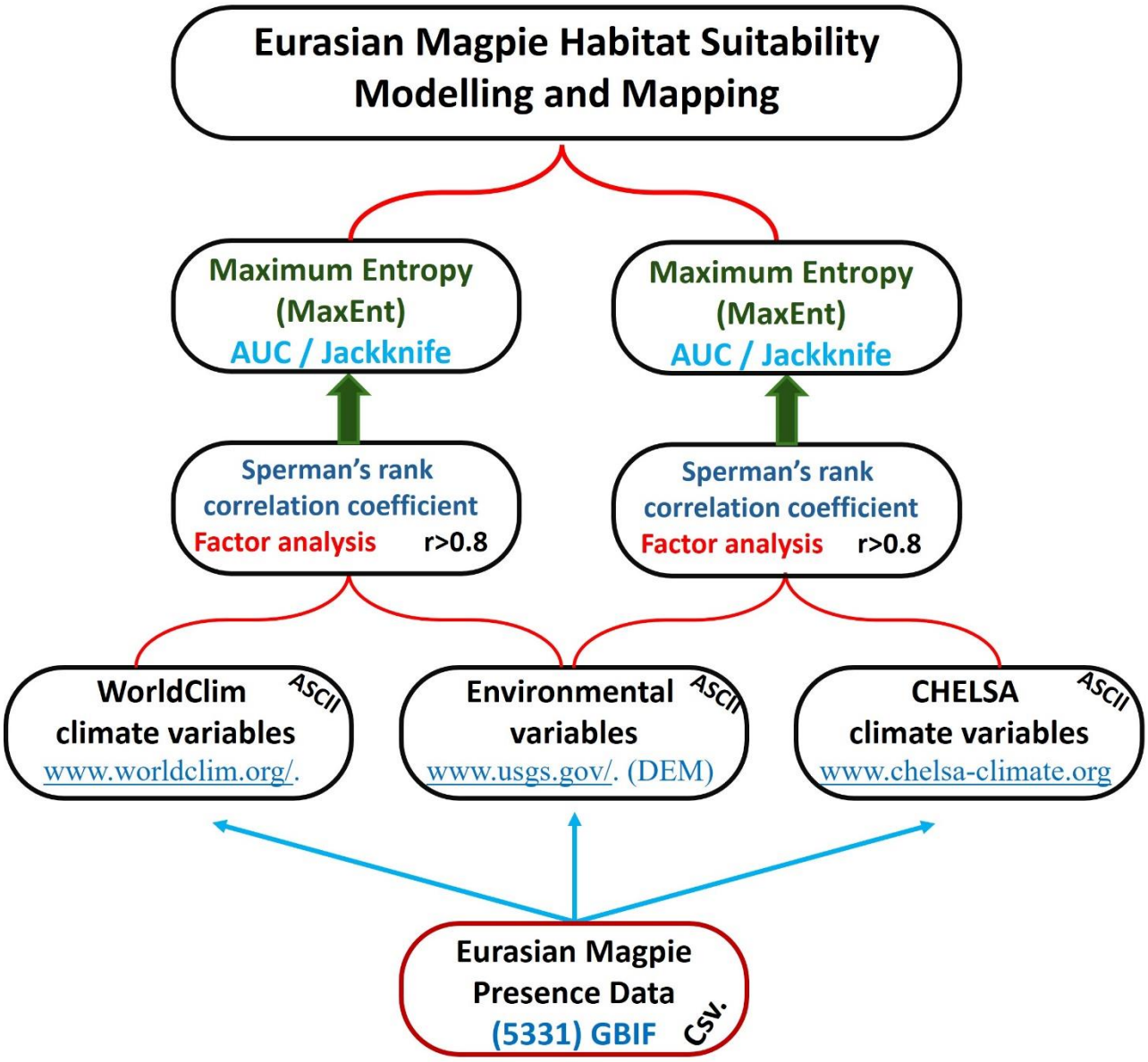


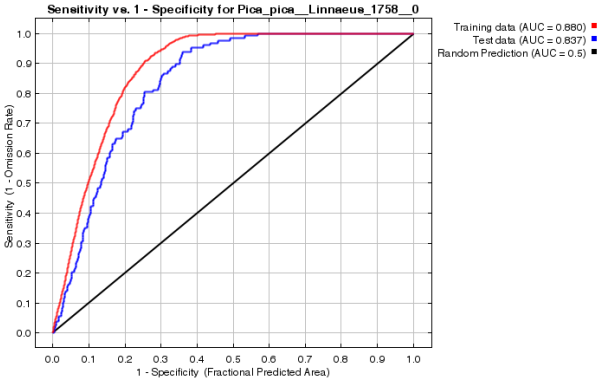
Figure 2. Flowchart of the study

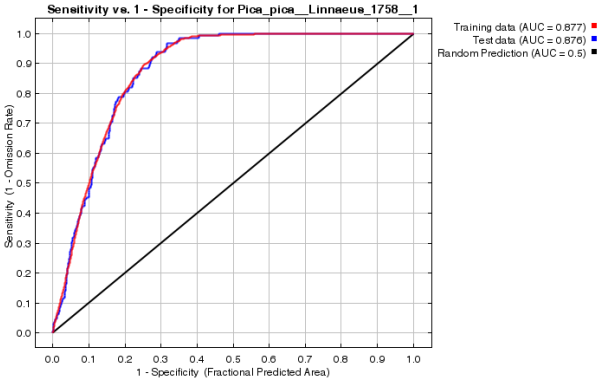
3. RESULTS AND DISCUSSION

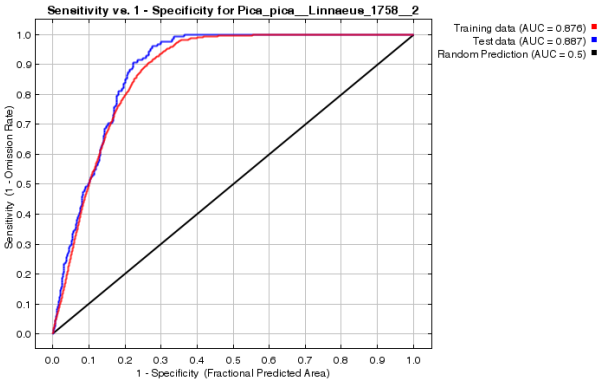
The modelling process of habitat suitability modelling of Eurasian Magpie was started with environmental variables and both CHELSA climate variables and WorldClim climate variables. The cross-validation option of the MaxEnt method, which is one of the species distribution models, and 10% of the available data belonging to the target species were preferred as test and 10 repetitions. While the models presented with both CHELSA and WorldClim climate variables were trained using 4798 presence records belonging to the target species, 533 records were used for testing. In this

process, two different models with an average of 5000 repetitions were presented based on the results of 10 repetitions. A total of 26 environmental and climate variables (such as annual mean temperature and annual precipitation) belonging to different global climate models (such as CHELSA and WorldClim) within the Mediterranean borders were processed. Digital base maps produced according to the study area borders were converted to ASCII format to be used in the MaxEnt method. The modelling phase was started with the digital base maps prepared for the MaxEnt method and the presence data belonging to the Eurasian Magpie. Among the variables contributing to the models, the variables with the lowest permutation importance and the lowest percentage value were removed from the model and the modelling process was continued. The process was continued until at least two variables remained contributing to the model and the best model was selected according to the AUC values and accuracy levels without deviation in the neglect graph between the models. The AUC values of the habitat suitability model of the magpie species presented with WorldClim climate data are given in Figure 3a. At the same time, the AUC values of the habitat suitability model of the magpie species presented with CHELSA climate data are given in Figure 3b.

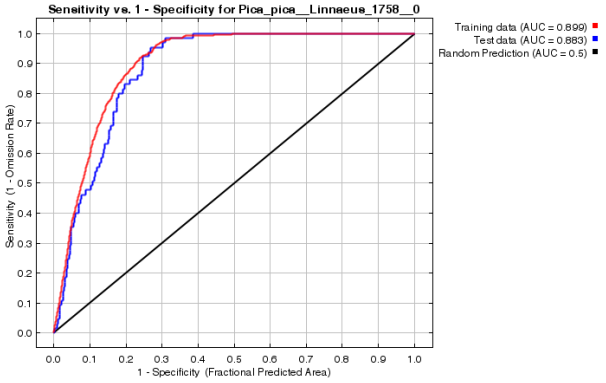
WorldClim climate variable modelling

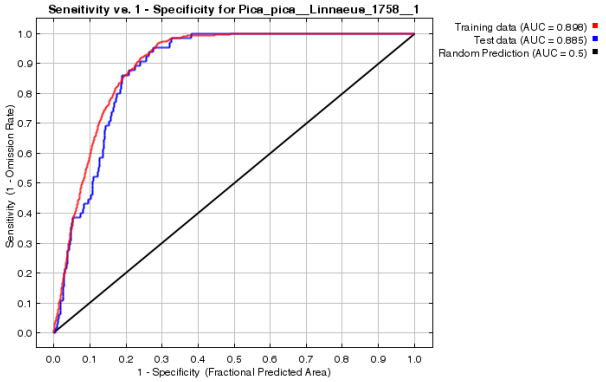


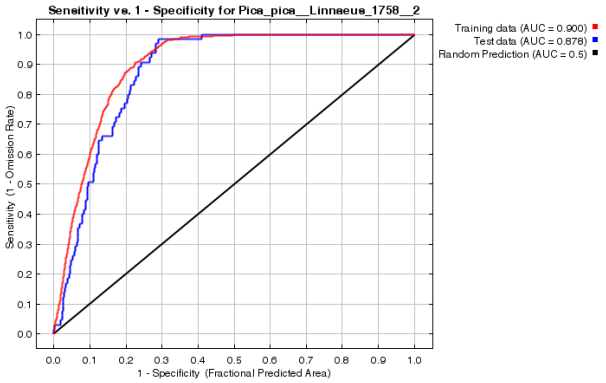


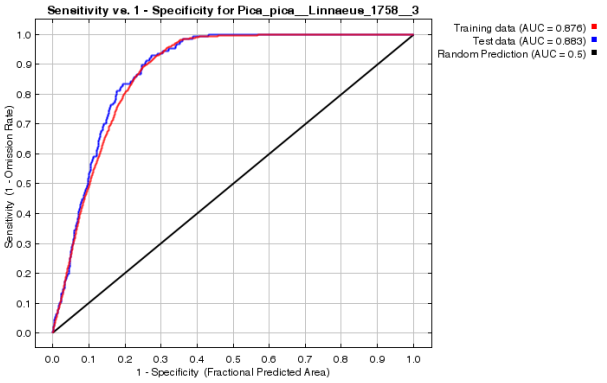


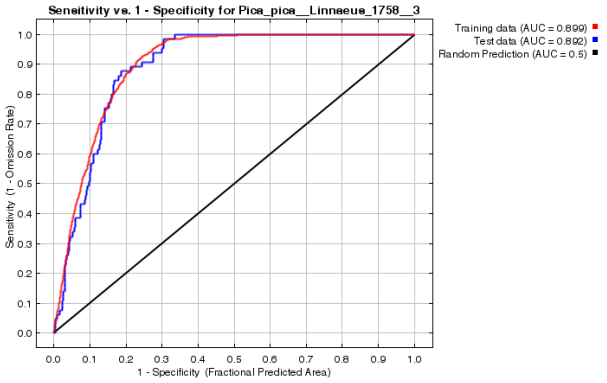
CHELSA climate variable modelling

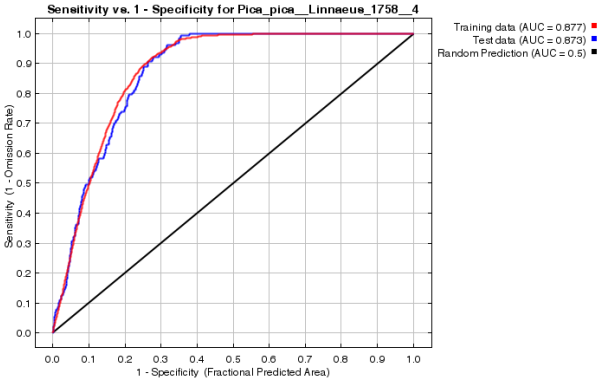


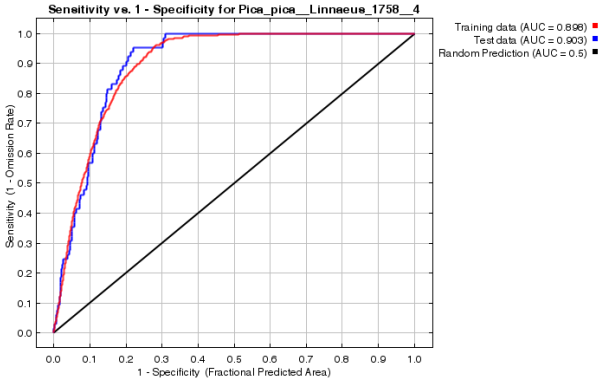


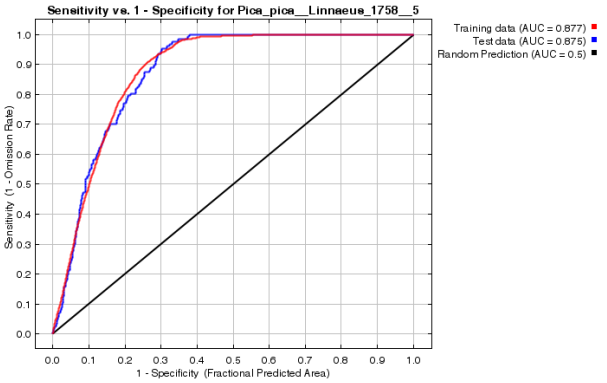


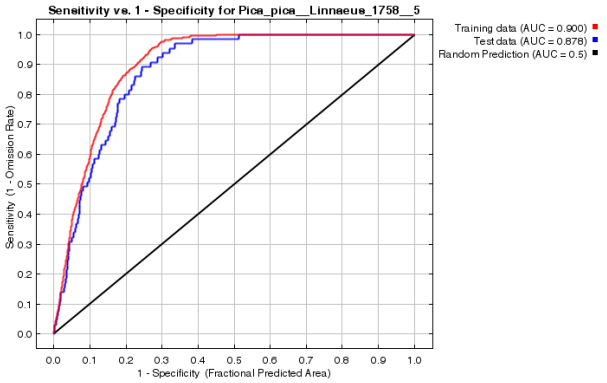

Sensitivity vs. 1 - Specificity for Pica_pica__Linnaeus_1758__3
Training data (AUC = 0.876)
Test data (AUC = 0.883)
Random Prediction (AUC = 0.5)
Sensitivity (1 - Omission Rate)
1 - Specificity (Fractional Predicted Area)

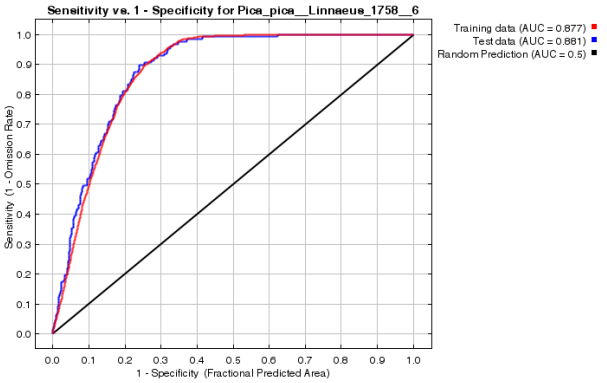

Sensitivity vs. 1 - Specificity for Pica_pica__Linnaeus_1758__3
Training data (AUC = 0.899)
Test data (AUC = 0.892)
Random Prediction (AUC = 0.5)
Sensitivity (1 - Omission Rate)
1 - Specificity (Fractional Predicted Area)

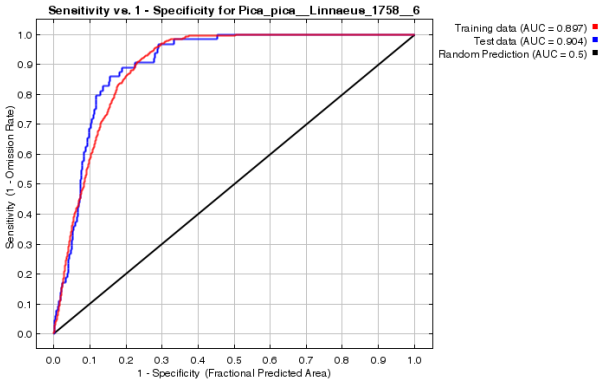

Sensitivity vs. 1 - Specificity for Pica_pica__Linnaeus_1758__4
Training data (AUC = 0.877)
Test data (AUC = 0.873)
Random Prediction (AUC = 0.5)
Sensitivity (1 - Omission Rate)
1 - Specificity (Fractional Predicted Area)


Sensitivity vs. 1 - Specificity for Pica_pica__Linnaeus_1758__4
Training data (AUC = 0.898)
Test data (AUC = 0.903)
Random Prediction (AUC = 0.5)
Sensitivity (1 - Omission Rate)
1 - Specificity (Fractional Predicted Area)


Sensitivity vs. 1 - Specificity for Pica_pica__Linnaeus_1758__5
Training data (AUC = 0.877)
Test data (AUC = 0.875)
Random Prediction (AUC = 0.5)
Sensitivity (1 - Omission Rate)
1 - Specificity (Fractional Predicted Area)


Sensitivity vs. 1 - Specificity for Pica_pica__Linnaeus_1758__5
Training data (AUC = 0.900)
Test data (AUC = 0.878)
Random Prediction (AUC = 0.5)
Sensitivity (1 - Omission Rate)
1 - Specificity (Fractional Predicted Area)


Sensitivity vs. 1 - Specificity for Pica_pica__Linnaeus_1758__6
Training data (AUC = 0.877)
Test data (AUC = 0.881)
Random Prediction (AUC = 0.5)
Sensitivity (1 - Omission Rate)
1 - Specificity (Fractional Predicted Area)


Sensitivity vs. 1 - Specificity for Pica_pica__Linnaeus_1758__6
Training data (AUC = 0.897)
Test data (AUC = 0.904)
Random Prediction (AUC = 0.5)
Sensitivity (1 - Omission Rate)
1 - Specificity (Fractional Predicted Area)

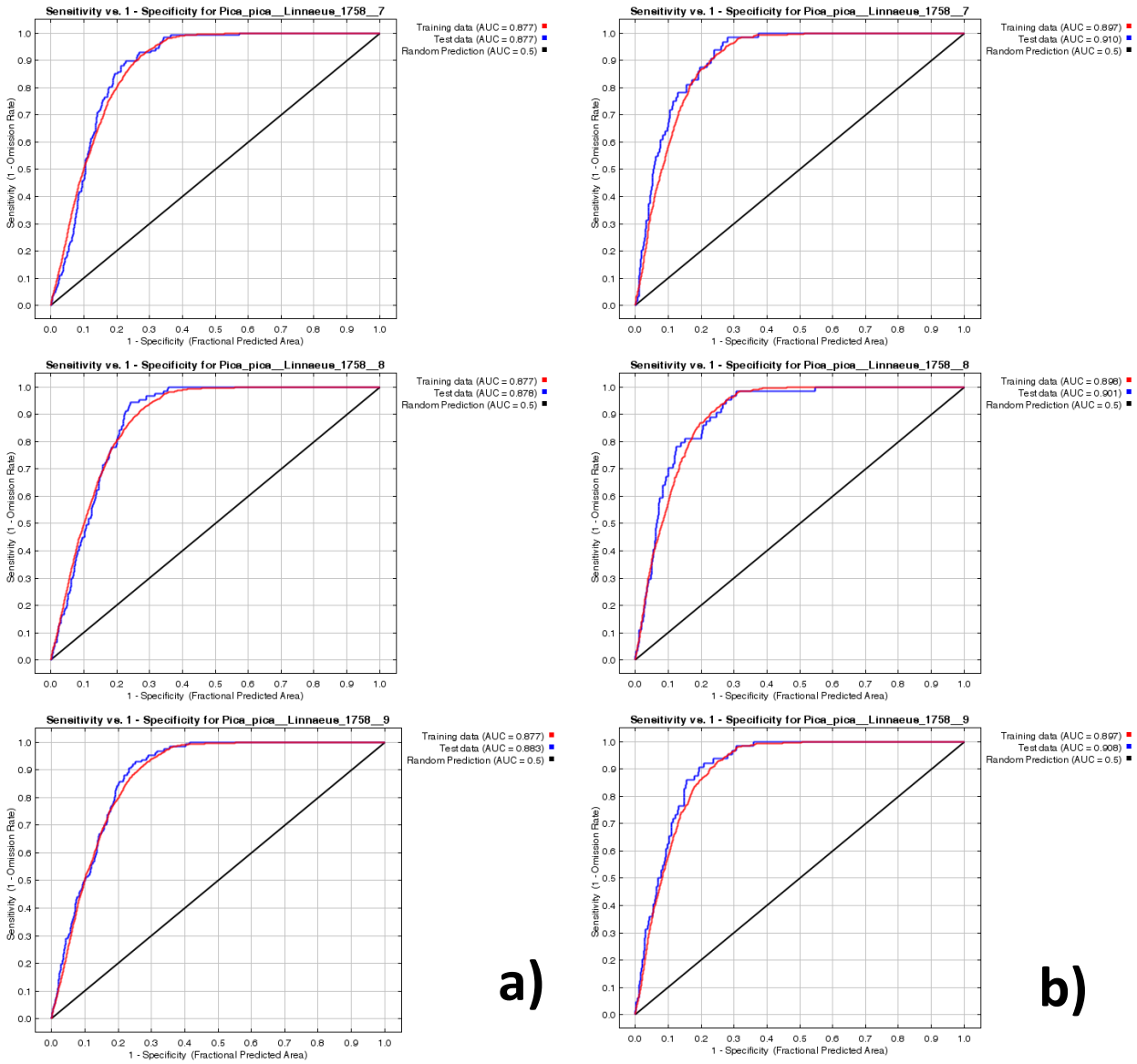


Figure 3. Eurasian Magpie **a)** habitat suitability modelling using WorldClim climate variables and, **b)** habitat suitability modelling using CHELSA climate variables

The AUC values of the habitat suitability model of the Eurasian Magpie, presented according to different climate scenarios, are examined in more detail in the table below (Table 1).

Table 1. Training and test dataset AUC values of the model developed with CHELSA and WorldClim climate data

			0	1	2	3	4	5	6	7	8	9
Eurasian Magpie habitat suitability modelling	**WorldClim climate variable**	Training data (AUC)	**0.880**	**0.876**	**0.876**	**0.876**	**0.877**	**0.877**	**0.877**	**0.877**	**0.877**	**0.877**
		Test data (AUC)	**0.837**	**0.876**	**0.887**	**0.883**	**0.873**	**0.875**	**0.881**	**0.877**	**0.878**	**0.883**
	CHELSA climate variable	Training data (AUC)	**0.899**	**0.898**	**0.900**	**0.899**	**0.896**	**0.900**	**0.897**	**0.897**	**0.898**	**0.897**
		Test data (AUC)	**0.883**	**0.885**	**0.878**	**0.892**	**0.903**	**0.878**	**0.904**	**0.910**	**0.901**	**0.908**

When Table 1 is examined, the Eurasian magpie habitat suitability model was selected according to the results of the WorldClim global climate model with an AUC value of 0.877 for the training data set and an AUC value of 0.875 for the test data set. At the same time, according to the results of the CHELSA global climate model, the model with the training dataset AUC value of 0.899 and the test dataset AUC value of 0.892 was selected. When both model results were evaluated according to the Baldwin (2009) AUC classification, it was determined that they were in the "very

good" model category. However, according to these results, when the CHELSA climate model results were compared to the WorldClim climate model results, it was determined that both the training data set AUC and the test data set AUC values were higher. In addition, when the average AUC value Jackknife graphs were examined with all variables contributing to the two different habitat suitability models presented, it was determined that the WorldClim was 0.87 (Figure 4a) and CHELSA was 0.89 (Figure 4b). As a result, it was determined in this study that the CHELSA climate data preferred on bird species distribution produced slightly higher results. Morales-Barbero and Vega-Álvarezn (2019) used the Random Forest method to construct species distribution models (SDMs) in their study titled "*Input matters matter: Bioclimatic consistency to map more reliable species distribution models*" and aimed to determine the degree of overlap between spatial projections resulting in different bins. To address the impact of selecting a single global climate database, sixteen wild mammal species worldwide were modelled. Thirteen of the preferred wild mammal species were terrestrial wild mammal species. In the comparison between the terrestrial wild mammal habitat suitability models, it was revealed that the mapping produced by WorldClim and CHELSA climate models showed the highest overlap values for 11/13 terrestrial mammals. In addition, although the average AUC values of the model results presented with CHELSA climate models were high for five wild mammal species, the average AUC values of the model results presented with WorldClim climate models showed that accurate and reliable results were obtained for two wild mammal species. As a result, it was determined that CHELSA climate models have a higher explanatory power than WorldClim climate models for habitat suitability modelling of wild mammal species. When these results are compared with this study, they are in the same direction as the literature.

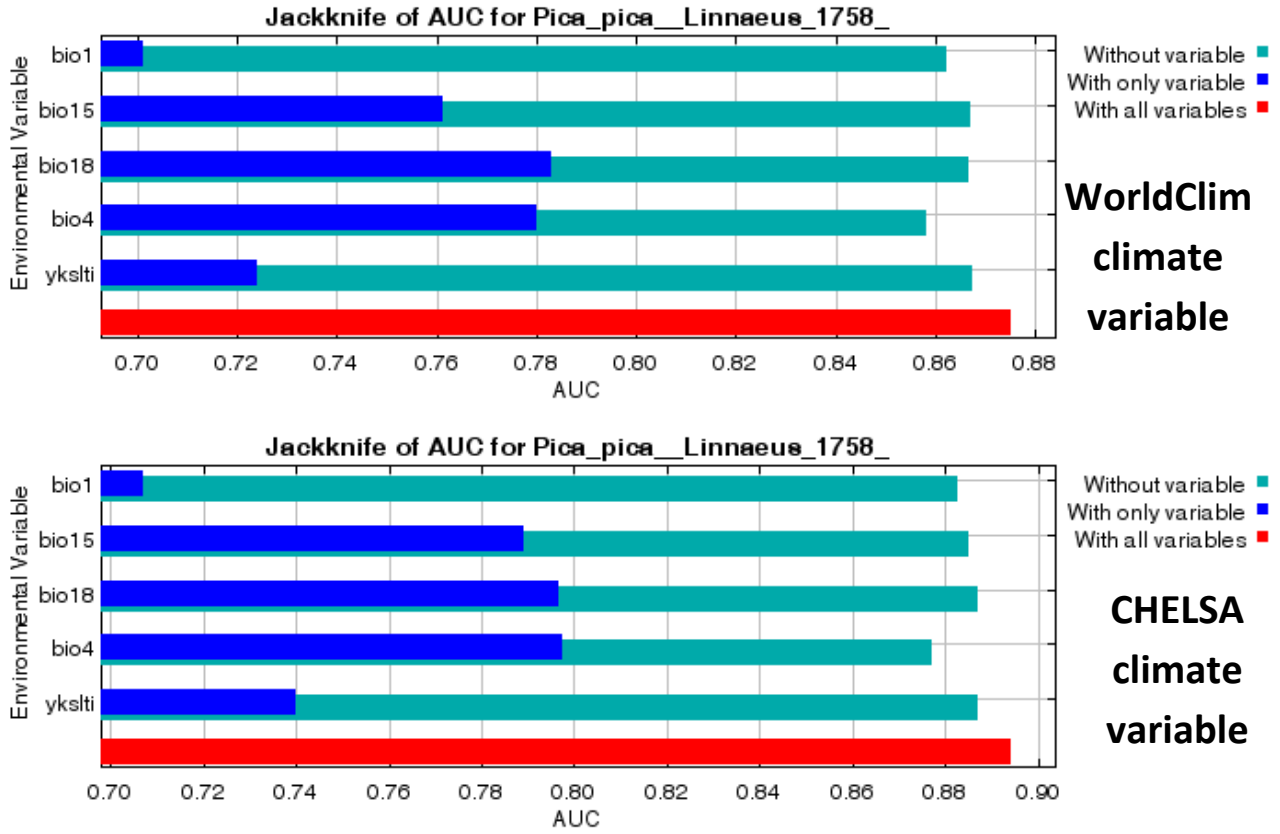


Figure 4. Jackknife graph of models produced with **a)** WorldClim and b) CHELSA climate data

4. CONCLUSIONS

In this study, the accuracy of the WorldClim and CHELSA global climate models was examined on the Eurasian magpie. For this purpose, it was carried out in the Mediterranean region, one of the hot regions of Türkiye where the global climate difference may be the highest. According to the model results obtained, it was determined that the habitat suitability model AUC value results presented by the CHELSA global climate models were higher than those of the WorldClim climate variables. However, since the AUC difference of the models was not very high, it is recommended to present the habitat suitability model on a larger scale. In addition, WorldClim and CHELSA climate data are two different sources, each of which has advantages and areas of use. CHELSA especially by providing high-resolution climate data, allows for more detailed and local-scale analyses. On the other hand WorldClim by providing a wider historical time range, includes different climate scenarios to be used in climate change studies. In addition, CHELSA's data is generally based on more up-to-date and dynamic climate models, which makes it more suitable for certain

applications, while WorldClim is preferred in terms of providing a wide database and historical background. In summary, which global climate data is better depending on your intended use. If you are looking for historical data and a large scale, WorldClim is recommended, whereas if you need up-to-date and high-resolution data, CHELSA is recommended. Both datasets can be valuable and complementary in certain contexts.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization: A.A.; Investigation: A.A.; Material and Methodology: A.A.; Supervision: A.A.; Visualization: A.A.; Writing-Original Draft: A.A.; Writing-review & Editing: A.A.; Other: Author have read and agreed to the published version of manuscript.

Conflict of Interest
The author has no conflicts of interest to declare.

Funding
The author declared that this study has received no financial support.

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Comparison of Three Analytical Methods to Determine the Amount of Thymoquinone in Black Cumin Seed Oil (*Nigella sativa* Linn)

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Abstract: Thymoquinone is a natural chemical found in the plant Nigella sativa. It can also be found in some cultivated Monarda fistulosa plants, which can be steam distilled to produce an essential oil. It has been classified as a pan-assay interference compound, which binds to many proteins indiscriminately. It is currently being researched to identify its possible biological properties (Enoli and Hugh, 2018).
In this study, essential oil was obtained from Nigella sativa by distillation of water vapour. The amount of thymoquinone in the essential oil was analyzed by three different analytical methods using a high purity reference analytical standard of thymoquinone in order to compare the methods. In the present study, three chromatographic methods based on direct n-hexane extraction were validated for the thymoquinone quantification in Black Cumin Seed Oil samples, the first one by high-performance liquid chromatography combined with tandem mass spectrometry (UHPLC-MS/MS), the second one is by gas chromatography mass spectrometry (GC-MSD) and the third one is by gas chromatography flame ionization detector (GC-FID). All analytical methods showed good linearity in the calibration range ($R^2 > 0.99$). The recoveries of thymoquinone were greater than 95 % with three techniques. The methods showed good precision, repeatability, and reproducibility. The LOD and LOQ values achieved for UHPLC-MS/MS were 0,299 and 0,996 µg/mL, for GC-MSD were 0,348 and 1.161 µg/mL, while for GC-FID were 0,456 and 1,519 µg/mL.

The results demonstrate that all three methods exhibit high selectivity for the determination of thymoquinone. It can be concluded that all three methods are suitable for the determination of thymoquinone content in black cumin oil, although analyses by UHPLC-MS/MS are one step ahead.

Keywords: Black Cumin, Nigella sativa, thymoquinone, UHPLC-MS/MS, GC-FID, GC-MSD

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1. INTRODUCTION

Thymoquinone is a compound found in the seeds of the plant Nigella sativa, commonly known as black cumin, and is associated with a number of health benefits. Black cumin oil is obtained through the process of cold pressing the seeds, whereby the active ingredient thymoquinone is extracted. The compound has anti-inflammatory properties. Thymoquinone has been demonstrated to possess anti-inflammatory properties, thereby reducing inflammation within the body. This may contribute to the prevention and treatment of various diseases. Immune System Support: Black cumin oil has been demonstrated to possess immune system strengthening properties, with the potential to protect the body against infections.

Antioxidant Effect: Thymoquinone has been shown to reduce cellular damage by neutralising free radicals in cells, which can slow down the ageing process.

Diabetes Management: Some studies have indicated that thymoquinone may assist in stabilising blood sugar levels.
Respiratory Health: Black cumin oil has been observed to relieve symptoms of respiratory diseases such as asthma and allergic rhinitis.

Black cumin oil is typically consumed as a dietary supplement or incorporated into meals. Topically, black cumin oil can be used to treat various skin conditions and to promote hair health. However, as with any oil, it should be used with caution. While generally considered safe, it is advisable for pregnant women or those with underlying health conditions to consult a medical professional before use. Additionally, excessive consumption should be avoided.

Thymoquinone and black cumin oil are known for their health-promoting properties. However, as with any supplement, they should be used in accordance with individual health needs.

Several different methods of analysis can be used for the quantification of thymoquinone (Haidy and Sherweit, 2018). Gas Chromatography (GC) is a frequently used method for the analysis of thymoquinone. It is based on the principle of separating samples in the gas phase and analysing them with a specific detector (usually FID- Flame Ionisation Detector). Advantages are high sensitivity and selectivity, low detection limits, fast analysis time. Disadvantages are that non-volatile components are difficult to analyse, sample preparation and separation steps can be complex.

Liquid Chromatography (HPLC) is a technique used for the separation of thymoquinone and other components in the liquid phase. It is usually coupled with UV or fluorescence detectors (Rajat and Kiran, 2024) (Nida and Shahnaz, 2021). Its advantages are that it is suitable for non-volatile components, provides high resolution and simultaneous analysis of different components is possible. As for the disadvantages, the analysis time may be longer than GC and may require high-cost equipment (Erick et al., 2016).

The amount of thymoquinone in the essential oil was analyzed by three different analytical methods using a high purity reference analytical standard of thymoquinone in order to compare the methods. In the present study, three chromatographic methods based on direct n-hexane extraction were validated for the thymoquinone quantification in Black Cumin Seed Oil samples, the first one by high-performance liquid chromatography combined with tandem mass spectrometry (UHPLC-MS/MS), the second one is by gas chromatography mass spectrometry (GC-MSD) and the third one is by gas chromatography flame ionization detector (GC-FID).

2. MATERIAL AND METHOD

2.1. Reference Standard of Thymoquinone and Black Cumin Seeds (Nigella sativa Linn)

In this study, reference analytical standard of thymoquinone was obtained from Sigma-Aldrich company (Burlington, Massachusetts, USA). Essential oil was obtained from Nigella sativa by distillation of water vapour. The amount of thymoquinone in the essential oil was analyzed by three different analytical methods using a high purity reference analytical standard of thymoquinone in order to compare the methods.

2.2. GC–System (GC–FID and GC–MS) and Used Method

The analyses were performed using a Thermo Scientific Trace 1300 GC gas chromatograph instrument (FID) (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) and a Thermo Scientific-ISQ7000 single quadrupole mass spectrometer detector (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) system to determine the presence of thymoquinone in Nigella sativa oil. The chromatographic evaluations were conducted using the Xcalibur software. The analytical column employed for chromatographic separation was the TraceGOLD TG-624SilMS GC (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). The inlet temperature of the instrument was set to 250 °C. An injection volume of 2 µL was employed. A split ratio of 1/5 was employed. Helium was employed as the carrier gas, with a flow rate of 1.5 mL/min. The oven temperature was programmed from 35 °C (2 min) to 100 °C at a rate of 2 °C/min, then from 100 °C (1 min) to 250 °C at a rate of 5 °C/min. The detector temperature was set at 280 °C.

A 50 µL sample of the essential oil was taken and diluted with 15 mL of n-hexane solvent. Following a three-minute vortexing process, a 1/40 dilution was prepared with acetone in a 2 mL vial and subsequently injected into the GC-MS instrument.

2.3. LC-MS/MS System and Used Method

The samples were subjected to analysis by ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). The homogenised 0.1 g sample was extracted with 10 mL solution of methanol and acetonitrile (50:50, v/v) containing 0.1% formic acid. The mixture was vortex-mixed for a period of five minutes and then subjected to centrifugation at 4000 rpm at 4°C for 15 minutes (Chung et al. 2023). The supernatant obtained after centrifugation was passed through a 0.2 µm PTFE membrane filter and subsequently injected into the UHPLC-MS/MS instrument. The total analysis time was seven minutes.

Analysis and determination of thymoquinone levels were performed using the Thermo Scientific Ultimate 3000 UPLC, Thermo Scientific- TSQ Fortis system (Thermo Fisher Scientific Inc. Waltham, Massachusetts, USA). Chromatographic analyses were made within the Xcalibur software. The analytical column used for the chromatographic separation was a

Hypersil Gold RP C18 (1.9µm), 50x2.1 mm, (Thermo Fisher Scientific Inc. Waltham, Massachusetts, USA) UHPLC column.

The mobile phases used for the analysis consisted of two variants: organic and inorganic based. Mobile phase A consisted of a 95:5 mixture of water and acetonitrile containing 0.1% formic acid and 4 mM ammonium formate. Mobile phase B was prepared by mixing acetonitrile and water in a 95:5 ratio with the addition of 0.1% formic acid and 4 mM ammonium formate (Kausar et al., 2017).

Two distinct mobile phase solutions were prepared and the thymoquinone was subjected to analysis via the application of a gradient flow programme utilising an analytical column comprising both organic and inorganic components (Iqbal and Anwer, 2013). The mass detector of the system is a triple quadrupole spectrometer, which operates using a combination of three consecutive quadrupoles. The ionisation technique employed is referred to as soft ionisation, which is typically based on the principle of the conservation of the primary mass of the analyte. The method operates on the basis of positive ionisation, whereby a proton is added to the main mass (M+1), or negative ionisation, whereby a proton is removed from the main mass (M-1) (Rabab et al., 2020). The analytical purity standard of thymoquinone at a known concentration was analysed, and the data obtained with the device were compared with the analytical results of the samples. Quantitative results were obtained by comparing the data obtained with the instrument.

3. RESULTS

In this study, 6 (0, 0.1, 0.2, 0.5, 1.0 and 2 mg/kg) point calibration curves were drawn on each analyser for comparison with the thymoquinone analytical reference standard.

The methods showed good precision, repeatability and reproducibility.

All analytical methods demonstrated satisfactory linearity within the calibration range, with a correlation coefficient (R^2) exceeding 0.99. The recovery of thymoquinone was greater than 95% for three of the methods employed (Table 1). The recovery of thymoquinone was conducted using the spike method, whereby the analytical reference standard was spiked into the blank sample, which was sesame oil.

Table 1: Recovery of thymoquinone

	Spike	Recovery 1	Recovery 2	Recovery 3	Recovery 4	Recovery 5	Recovery 6	Rec Average	% Average
	µg/mL								% (m/V)
GC-FID	5	4,65	4,77	4,85	4,74	4,82	4,79	4,770	95,40
GC-MS	5	4,85	4,66	4,9	4,86	4,91	4,83	4,835	96,70
LC-MS/MS	5	4,95	4,84	4,84	4,87	4,69	4,93	4,853	97,07

The methods showed good precision, repeatability and reproducibility. The LOD and LOQ values obtained were 0,299 and 0,996 µg/mL for UHPLC-MS/MS, 0,348 and 1.161 µg/mL for GC-MSD and while for GC-FID were 0,456 and 1,519 µg/mL for GC-FID (Table 2).

Table 2: LOD – LOQ of thymoquinone

	Spike	Recovery 1	Recovery 2	Recovery 3	Recovery 4	Recovery 5	Recovery 6	Average	SD	LOD	LOQ
	µg/mL										
GC-FID	1	0,772	0,941	1,115	0,861	0,759	1,080	0,921	0,152	0,456	1,519
GC-MS	1	0,838	1,082	1,045	0,795	0,863	0,918	0,869	0,116	0,348	1,161
LC-MS/MS	1	1,092	0,924	0,926	0,837	0,836	0,838	0,909	0,100	0,299	0,996

Figure 1: GC-FID and GC-MS System

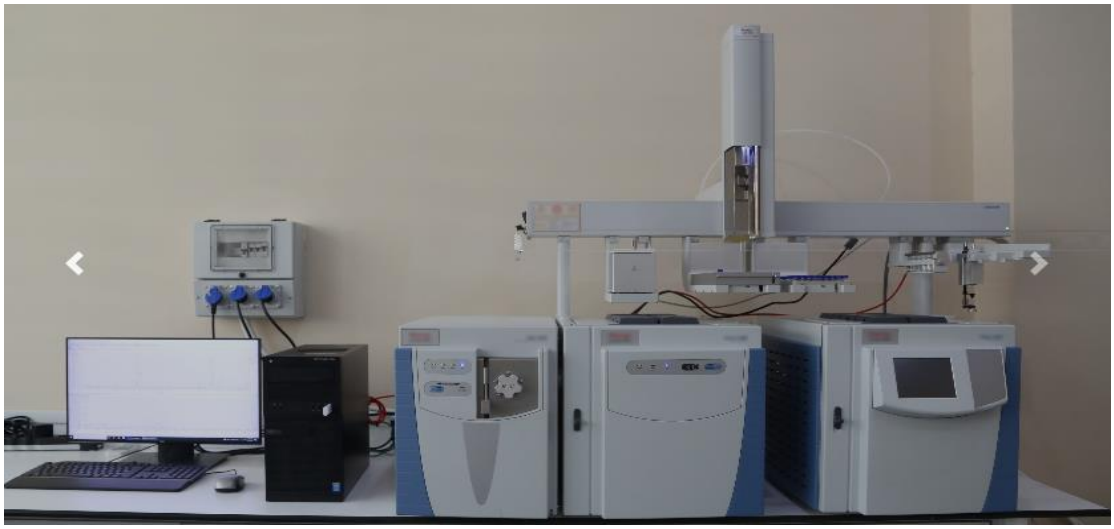

Figure 2: LC-MS/MS System

The essential oil obtained from Nigella sativa by water vapour distillation was subjected to analysis by three distinct analytical techniques and instruments employed for comparison in this study. The quantity of thymoquinone present in the essential oil was analysed by three separate analytical techniques utilising a high-purity reference analytical thymoquinone standard. The findings are presented in the table below (Table 3). As can be observed, the results obtained are in alignment with one another.

Table 3: Thymoquinone Amount of Nigella Sativa Oil

	Analyze 1	Analyze 2	Analyze 3	Analyze 4	Analyze 5	Analyze 6	Average
				µg/mL			
GC-FID	1,876	1,860	1,908	1,94	1,896	1,928	1,901
GC-MS	1,916	1,972	1,940	1,864	1,960	1,944	1,933
LC-MS/MS	1,964	1,932	1,980	1,936	1,936	1,948	1,949

4. DISCUSSION AND CONCLUSIONS

It is possible for LC-MS/MS (liquid chromatography/ tandem mass spectrometry) and GC/FID (gas chromatography / flame ionization detection) to yield disparate results with regard to the quantity of thymoquinone content in black cumin seed oil present in a given sample.

The principal reason for this discrepancy is the divergence in the underlying principles and techniques employed by these two analytical methods.

Liquid chromatography/mass spectrometry (LC/MS) is a technique that combines the principles of liquid chromatography and mass spectrometry to separate and identify compounds in a sample. It is particularly efficacious for the analysis of polar and non-volatile compounds, such as fatty acids. LC/MS provides high sensitivity and specificity, thereby enabling accurate identification and quantification of individual fatty acids.

Conversely, GC/FID is a technique that combines gas chromatography with flame ionization detection for the separation and detection of volatile compounds. It is a commonly employed method for the analysis of volatile fatty acids. GC/FID provides effective separation of individual fatty acids based on their volatility; however, it may not offer the same level of sensitivity or specificity as LC/MS.

The discrepancies in separation mechanisms, detection principles, and instrument sensitivities can result in variations in the quantification of fatty acids between LC/MS and GC/FID methods. Furthermore, differences in sample preparation techniques may also contribute to these inconsistencies.

It is therefore essential to consider these differences when comparing results obtained from LC/MS and GC/FID analyses of fatty acids in the same sample.

In conclusion, the results of the aforementioned studies and data demonstrate that all three methods employed for the determination of thymoquinone exhibit high selectivity. Consequently, it can be posited that all three methods are appropriate for the assessment of thymoquinone content in black cumin oil.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization: T.E. O.B.; Investigation: O.B.; Material and Methodology: O.B., T.E.; Supervision: O.B., T.E.; Visualization: O.B.; Writing-Original Draft: O.B.; Writing-review & Editing: T.E.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

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