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"Science for Sustainable Technology"

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ICONST NST 2025

International Conferences on Science and Technology
Natural Science and Technology
May 28-30, 2025 in Budapest - HUNGARY

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Dear Readers;

The 8th of ICONST organizations was held in Budapest-HUNGARY between May 28-30, 2025 with the theme of 'science for sustainable technology' again. In recent years, weather changes due to climate change have reached a perceptible level for everyone and have become a major concern. For this reason, scientific studies that transform technological progress into a sustainable one is seen as the only solution for humanity's salvation. Here we ask ourselves "which branch of science is responsible for sustainability?". Sustainability science is an interdisciplinary field of study that covers all basic sciences with social, economic, ecological dimensions. If we consider technology as the practical application of scientific knowledge, the task of scientists under these conditions is to design products that consume less energy, require less raw materials, and last longer.

ICONST organizations organize congresses on sustainability issues of three main fields of study at the same time in order to present different perspectives to scientists. This year, 215 papers from 20 different countries presented by scientists in ICONST Organizations.

32 papers from 8 countries (Albania, Azerbaijan, Ethiopia, Iran, Kazakhstan, Kosovo, Morocco and Türkiye) presented in our International Conference on Natural Science and Technology organized under ICONST organizations. Turkey is the country with the highest participation with 47%, followed by Kazakhstan with 15.6%, Kosovo and Iran with %9.4, Ethiopia with 6.2, Albania, Azerbaijan Morocco with 3%. Outside of Türkiye participant rate is totally 53%.

As ICONST organizations, we will continue to organize organizations with the value you deserve in order to exchange ideas against the greatest threat facing humanity, to inspire each other and to contribute to science. See you at your future events.

ICONST Organizing Committee

Efficiency of Growing Leafy Green Vegetables in a Greenhouse and Their Role in the Educational Process

ALTYNZER ISSAGALI

Abstract: Modern trends in food security and sustainable development highlight the importance of implementing innovative methods for growing agricultural crops. Leafy green vegetables — such as lettuce, spinach, sorrel, dill, and parsley — hold a vital place in the human diet due to their high content of vitamins, minerals, and fiber. This paper explores the efficiency of cultivating these crops under greenhouse conditions, where optimal microclimatic parameters allow for year-round production.

The study includes an analysis of growth indicators, vegetation period, yield, and plant resistance to pests and diseases when using various agricultural techniques such as drip irrigation, organic fertilizers, and light culture. Special attention is given to a comparative evaluation of yields in open-field vs. greenhouse conditions.

The second part of the paper focuses on the role of greenhouse vegetable growing in educational and developmental activities. Involving students in agricultural projects fosters environmental awareness, promotes work ethic, and develops research and practical skills in biology, ecology, and agronomy. Regular observation of plant development stimulates analytical thinking and instills a scientific approach to learning.Thus, the cultivation of leafy greens in a greenhouse is not only an economically viable agricultural practice but also a powerful educational tool that contributes to the formation of environmentally responsible individuals.Moreover, the integration of greenhouse projects into school or university environments promotes an interdisciplinary approach, combining knowledge from biology, chemistry, geography, and technology. Hands-on activities in the greenhouse allow students to see the tangible results of their work, boosting their self-esteem and fostering sustained learning motivation. Greenhouse cultivation also provides an effective platform for conducting scientific observations and project-based learning. The implementation of such initiatives can serve as a foundation for school research projects, science fairs, and academic competitions. In the long term, this experience encourages responsible attitudes toward nature and supports the development of a sustainable lifestyle.

Keywords: leafy green vegetable, greenhouse cultivation, sustainable agriculture, educational process, environmental education, student engagement, practical learning

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Biologically active compounds of algae and their effects on higher plants

BALNUR MAKUYEVA

Abstract: The relevance of the topic. The distribution of blue-green algae in nature is diverse. Recently, these goals have become a research interest of scientists. Algae secrete exometabolites into their nutrient medium. Exometabolites are biologically active compounds by their nature. Growth regulators and phytopreparations are obtained from algae as a promising target. There is a growing population and a shortage of products in the world. In this regard, the relevance of the research work lies in the development of research papers that stimulate optimal ways to increase grain yields using the growth-accelerating ability of algae extract. Algae and algae extracts have attracted more interest in recent years, as synthetic chemical products need to be replaced. A source of environmentally friendly products or a growing demand for environmentally friendly products leads to a search for a source of natural compounds. Due to the recent growing demand of the population for traditional sources of products, algae can be considered as a resource of natural compounds.

Microalgae synthesize various biologically active compounds and are morphologically, physiologically, and genetically diverse organisms. The biotechnology of cyanobacteria has become the subject of research based on various surfactants released into the environment during cultivation. While the growing trends of green and blue–green algae have been thoroughly studied, microalgae can be a source of environmentally friendly and economically interesting compounds, as they help optimize production-controlled crops. Microalgae are a natural source of very interesting biologically active compounds. In recent years, biologically active substances have attracted the attention of researchers and companies, as active substances obtained from agricultural crops can be used in a wide variety of industries. For example, for the food, feed, medical and pharmaceutical industries, it is recommended to obtain a large number of bioactive compounds, the composition of which is not medicinal, but is a source of biologically active compounds. Given the biodiversity of microalgae and recent advances in genetic engineering, it is important to obtain the most promising sources of new products and applications from organisms. Bioactive microalgae compounds have, among other things, anti-inflammatory, antimicrobial and antioxidant activity. In addition, these microorganisms are able to promote health and reduce the risk of developing degenerative diseases.

The biological resources present in human life are diverse, but the sources of natural raw materials are divided into resources that, as you know, are depleted and inexhaustible. The period of rapid development of science and education in the age of technology is causing an increase in demand for high-quality refined products. For example, a product from the underwater flora is rich in active biological compounds. Their biological and medical significance is enormous. Recently, scientists have been developing various medical labels to be able to quickly record or correct an injury. Medical labels have been found to have antimicrobial, antitumor, and antiviral effects. Algae extracts are the basis of dietary supplements used as immunostimulants, thyroid function correctors, and oncoprotectors. Algae is used in commercial and traditional food technologies.

Exometabolites released by algae directly affect higher plants. When growing some plants in model practice, algae extracts are added as a top dressing and conditions are created to accelerate and accelerate growth. Metabolites released in the environment by some algae contained in the soil under natural conditions have an optimal effect on the growth of nearby higher plants in mutualistic relationships, can promote growth, favorably affecting the microflora of mycorrhizae living in that place. The purpose of the research work Determination of the accelerating and growth-enhancing properties of certain plant extracts isolated from algae.
Objectives of the research work
1. To determine the effect of algae exometabolites on seed germination.
2. Investigation of the growth rate of Raphanus sativus seeds treated with algae extract.;
3. Determination of the effect of algoextract on the pigment content in wheat germ.
The object of research: cereals, algae extract.
Research methods
- Methods for determining the effect of algae exometabolites on seed germination;
- Determination of the strength of water absorption by crops treated with algae extract.
Scientific novelty and practical significance
The algicidal activity of the algae extract promotes the pretreatment of grains, obtaining a high-quality, abundant harvest in order to increase resistance to natural growth stimulators of some higher plants and environmental factors.

Keywords: Algae extract, cereals, germination.

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Effect of Magnetic Point Source on Fe_3O_4-Water Nanofluid in an Open Cavity

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Abstract: This study analyzes the impact of a magnetic point source on the natural convection flow of Fe_3O_4-water nanofluid in a unit square cavity with an open top wall. The magnetic point source is located under the heated bottom wall and the other walls are adiabatic. The governing dimensionless equations containing stream function, vorticity and temperature are solved by radial basis function (RBF) approximation. In this approximation, cubic spline (r^3) RBF is implemented with the use of nonuniform discretization since better results are obtained by using a small number of nonuniform points compared to the results in the uniform discretization. Streamlines and isotherms are depicted, and the average Nusselt number along the hot bottom wall is evaluated for the variation of Rayleigh (*Ra*), Hartmann (*Ha*), and magnetic (*Mn*) numbers and the position of the magnetic point source. It is found that the average Nusselt number is an increasing function of the Rayleigh and magnetic numbers, while it is a decreasing function of the Hartmann number. The convective heat transfer weakens as the *x*-coordinate of the point source increases from 0 to 0.50, after this point it rises. Hence, it shows that the location of the magnetic point source has a significant influence on the heat transfer.

Keywords: nanofluid, open cavity, Fe_3O_4-water, magnetic point source

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Adsorption of Remazol Turquoise and Remazol Navy Blue Reactive Textile Dyes on Magnetic Resin and Removal from Aqueous Media

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Abstract: The rapid growth of the industry has led to an increase in textile dye usage, resulting in water pollution. Dye-containing wastewater consists of complex mixtures of harmful chemicals that threaten aquatic life. Dyes can reduce oxygen levels in water by blocking sunlight and consequently inhibiting photosynthesis (1,2). The removal of textile dyes from urban and industrial wastewater is essential for the continuity of living organisms. In this study, a novel method was developed for the removal of two different reactive textile dyes, widely used in the textile and dyeing industries, using a commercial resin. Magnetic resin (DowFeO) was synthesized by precipitating iron (II-III) oxide onto Dowex 1x16 resin. The efficiency and extraction conditions of this material were investigated separately for Remazol Turquoise and Remazol Navy reactive textile dyes. Characterization of DowFeO was performed using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Dye quantification was conducted using Ultraviolet-Visible Spectrophotometry (UV-Vis). For Remazol Turquoise, the optimal removal efficiency was found to be 98±1% under conditions of 0.05 g adsorbent, pH 8, temperature 50°C, and extraction time of 30 minutes. For Remazol Navy, the optimal removal efficiency was 96±2% under conditions of 0.05 g adsorbent, pH 4, temperature 50°C, and extraction time of 30 minutes. Matrix effects were evaluated, and the applicability of the method to real samples was demonstrated.

Keywords: Dowex 1X16, Remazol Turquoise, Remazol Navy Blue, Removal, Adsorption

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On mappings and topological structure of Intuitionistic fuzzy parameter soft

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Abstract: Intuitionistic fuzzy (IF) sets, an important tool in uncertainty modelling, were introduced by Atasanov in 1986. In this theory, in addition to the membership function in fuzzy sets, there is a non-membership function that characterizes the non-membership of an element in the universe. Unlike traditional fuzzy sets that assign a single membership degree to each element in an intuitionistic fuzzy set, the non-membership function includes a degree of hesitation and provides a more comprehensive representation of uncertainty and imprecision. Therefore, for suitable problems IF sets can be used as a more effective tool than fuzzy sets. Another theory used in uncertainty modelling is soft set theory. This theory was introduced by Molodtsov in 1999 as a powerful mathematical tool for handling imprecise, uncertain and incomplete information. Unlike traditional mathematical models, soft sets do not rely on rigid membership functions, but instead use a flexible approach based on a set of linguistic parameters, making them highly adaptable to real-world problems. This advantage of the theory quickly made it popular in the scientific community and has been successfully applied to many fields. The increasing complexity of real-world problems has led to the development of hybrid mathematical models that combine the strengths of different uncertainty theories. Among these, hybrid theories based on soft sets have attracted significant attention due to their ability to integrate with other mathematical approaches such as fuzzy sets, rough sets, and IF sets. By combining the flexibility of soft sets with the improved uncertainty representation of other theories, these hybrid models are providing more robust decision-making tools, especially in areas such as artificial intelligence, pattern recognition, multi-criteria decision analysis, and mathematics. One of these theories is intuitionistic fuzzy parameter soft (IFPS) sets, which are based on the principle that parameter sets are IF sets. In addition, topology is one of the important areas of mathematics. Therefore, many researchers have studied the topological structures of these hybrid theories. Intuitionistic Fuzzy Parameter Soft (IFPS) sets are introduced in this study. Following this, the topological structure defined on these sets is presented and an introduction to the basic topological concepts it provides is given. In addition, the IFPS soft function is defined and its algebraic properties are investigated. Finally, the properties of a function between IFPS topological spaces are defined and the concept of continuity is introduced.

Keywords: ntuitionistic Fuzzy Sets, Intuitionistic Fuzzy Parametrized Soft Sets, Topology

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Electron transfer in a periodic electronic system containing two defects with high performance

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Abstract: This article presents a theoretical analysis of the impact of defect layers on electronic states in CdTe/**CdMn**Te multi-quantum wells (MQWs). The approach employed is based on transfer matrices. The incorporation of one or two defect layers into periodic structures comprising quantum wells (CdTe) and barriers (CdMnTe) is demonstrated to alter the band structure, resulting in the formation of localized states within the band gaps. The influence of defect thickness and concentration on the formation of localized states within the band gaps is also demonstrated. The results revealed that thicker defects tend to shift the energies of localized states towards lower values, while increased concentrations of defects have the effect of raising these energies. It is evidenced that configurations comprising identical defect types are capable of producing highly transmissive localized states under specific conditions of thickness and concentration. Furthermore, structures incorporating different defect types are shown to yield distinctive patterns of localized states, particularly when the parameters of the defects are optimized. This study highlights the potential for MQWs to be utilized in applications such as frequency-selective filters, waveguides, and photonic devices, emphasizing the significance of defect engineering in tailoring electronic properties for advanced technologies.

Keywords: Multi Quantum Wells; Defect thickness; Localized states; Transmission coefficient

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Improvement in Mean Estimation using Logarithmic Function under Simple Random Sampling

CEREN ÜNAL*[1]

Abstract: Sampling theory consists of the process of selecting a sample from a population using the most appropriate sampling method, and of estimating the parameters of the population from the sample. It is important to use the most efficient estimator among existing estimators in the literature. At this point, various estimators have been widely proposed to estimate population parameters under different sampling methods, including regression, product, ratio, and exponential estimators in sampling theory. Alternatively, by adding logarithmic functions to the estimators, a new type of estimator for estimating population parameters has been proposed in the literature recently. In this context, this study proposes a new mean estimator of the study variable based on the logarithmic function for simple random sampling without replacement (SRSWOR) by incorporating information on auxiliary variable. The expressions for the bias, mean square error (MSE), and minimum mean square error of the proposed logarithmic type of estimator are derived to the first degree of approximation. In addition, the proposed estimator is compared theoretically with the main estimators in literature, and it is shown that the proposed estimator is always more efficient than the classical mean estimator, classical ratio estimator, and exponential type estimator in all conditions. These theoretical comparison results are supported numerically by an empirical study. Considering both the theoretical and numerical results obtained, it is seen that the proposed logarithmic estimator always gives more efficient results than the compared estimators.

Keywords: Sampling Theory, Population Mean, Logarithmic Type Estimator, Auxiliary Variable, Efficiency.

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A Comparative Analysis of The Quality Criteria of Dried Saffron According to Conducted Different Drying Methodologies

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Abstract: This study compares the quality properties of saffron after different drying methods. Proper drying is crucial for the longevity and storage of saffron. Crocin, picrocrocin, and safranal are bioactive compounds unique to saffron that have numerous pharmacological benefits, including antioxidant, anti-cancer, and memory-enhancing effects. In this study, saffron agriculture began in 2019 in Fethiye, Muğla. The SUDUM center conducted quality control studies during saffron production. Selecting the appropriate drying method holds a crucial role in determining the quality of saffron, as measured by crocin, picrocrocin, and safranal levels. The study tested four drying methods, which included conventional room temperature drying, electric oven drying, a dehydration method using liquid nitrogen with oven, and lyophilization. Conventional drying at room temperature was used for saffron samples over a period of 2 days. Samples of saffron were also subjected to electric oven drying at a temperature of 50 ºC for 6 hours. A dehydration process using liquid nitrogen with an oven was applied to saffron samples for 3 hours at a temperature of 50 ºC. 10 g of saffron samples were mixed with 100 mL of liquid nitrogen and then dried at a temperature of 50 ºC for 3 hours in an oven. Lyophilization, also known as freeze-drying, is a process that combines the best of freezing and drying methods to produce a dry, active, long-lasting, and easily soluble product. The saffron samples were lyophilized at a temperature of -55 ºC and a pressure of 0.2 mbar for 24 hours. The findings revealed that the best color intensity and quality criteria were achieved through the freeze-drying process. Furthermore, a higher quantity of safranal and crocin were obtained. The greatest reduction in crocin content was observed in the liquid nitrogen-based dehydration method, whilst conventional drying also performed well as the second most effective option.

Keywords: Saffron, Drying methods, Lyophilization, Spectrophotometer

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Phylogenetic Analysis of Powdery Mildew Pathogens Belonging to the Erysiphaceae Family Isolated from Various Angiosperm Species

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Abstract: This study investigates five powdery mildew pathogens belonging to the genera *Erysiphe* and *Microsphaera*, which cause significant disease in angiosperm plants and result in substantial economic losses. In addition to macroscopic diagnosis of powdery mildew infections, the research aims to examine the phylogenetic relationships among the species. For molecular analyses, the internal transcribed spacer (ITS1, 5.8S rRNA, and ITS2) and 28S ribosomal RNA (rRNA) regions were amplified, sequenced, and analyzed.Plant species used in the study included *Platanus sp.*, *Euonymus japonicus*, *Mahonia aquifolium*, *Syringa vulgaris*, and *Convolvulus arvensis*. Powdery mildew samples were collected from infected leaves using adhesive tape and scraping methods. Mature cleistothecia and conidia (oidia) were examined microscopically. The number of asci and morphological features such as appendages of the cleistothecia were recorded. Phylogenetic analyses revealed that genetic variation and nucleotide diversity were higher in the ITS regions compared to the 28S regions. Both ITS- and 28S-based analyses showed clustering of *Microsphaera syringae* and *Microsphaera platani* together, while *Microsphaera berberidis*, *Erysiphe euonymicola*, and *Erysiphe convolvuli* formed a separate cluster. These findings demonstrate that ITS and 28S rRNA regions are effective molecular markers for phylogenetic studies in the *Erysiphe* genus and provide valuable insight into species differentiation within the Erysiphaceae family.

Keywords: ITS, 28S rRNA, fungi, powdery mildew, DNA sequencing

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Development and Characterization of Thyme Essential Oil-Loaded PLA Microspheres Coated with Alginate

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Abstract: The integration of natural antimicrobials into biodegradable polymer matrices offers promising solutions for the development of active food packaging systems. This study investigated the effectiveness of alginate coating in the enhancement of the structural stability of polylactic acid (PLA)-based microspheres, including thyme essential oil (TEO) which is recognized for its strong antimicrobial properties. Each phase of the microsphere production process was evaluated which including four different formulations: PLA emulsion in water, PLA microspheres coated with alginate, PLA loaded TEO emulsion in water, and PLA–TEO microspheres coated with alginate. Alginate coatings were applied using ionic gelation in a calcium chloride solution following the production of microspheres via a solvent evaporation method enhanced by ultrasonic gelation. Zeta sizer, optical microscopy, scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR) were used to characterize the resultant microspheres for zeta potential, size distribution, morphological properties, and chemical changes. The size distribution analysis demonstrated that the alginate coating had an impact on the resultant particle size. The spherical morphology was confirmed by SEM and optical microscopy as well as showing uniformly distributed structures. The coating efficacy of alginate was indicated by the characteristic peaks of PLA, alginate, and TEO in FTIR spectrums. The obtained microspheres showed promising potential on the biodegradable platforms loaded with natural active compounds for food packaging materials likely to enhance food preservation through passive antimicrobial activity.

Keywords: Polylactic acid, Sodium alginate, Thyme essential oils, Microspheres, Ultrasonication, Emulsion

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Drug Design: A Potential Drug Active Ingredient in the Pharmaceutical Industry Otolith - Teak Extract Powder Combination

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Abstract: Fish ear stones (this term is often called "otoliths") and teak tree extract powder are very interesting and potentially valuable components in the pharmacology industry. These two substances can be associated with scientific research and various pharmaceutical applications regarding their effects on human health, which are found in nature. Otoliths are small calcium carbonate crystals found in the ears of fish, which provide balance. Otoliths, also known as ear stones, are found in all fish except rays, jawless fish and sharks. Otoliths are white in color and have a calcium carbonate (CaCO3) structure. Otoliths, which are not attached to the skull, float in the soft, transparent inner ear canal under the brain. Otolith is also a detection organelle in determining the age of the fish. There are 3 pairs of otoliths in fish: Sacitta, Lapilli, Asterisk. This component has not yet been widely studied pharmacologically. However, this precious stone should be investigated immediately. Otoliths contain large amounts of calcium and magnesium. These minerals are important for bone health and can be evaluated as a potential treatment especially for bone diseases (osteoporosis etc.) and musculoskeletal disorders. It can be transformed into a new source that can be used in the treatment of osteoporosis. It is also thought that otoliths, especially those extracted from the skull of the kingfisher (Sciaena umbra), can break down kidney stones. However, this issue should be further investigated and studied. It can be included in products such as calcium and magnesium supplements. Some minerals and elements contained in otoliths are effective against microorganisms. Studies show that such minerals can reduce inflammation and have positive effects on the immune system. It can play a supporting role in the treatment of conditions such as skin infections and wound healing processes, and joint disorders. The microscopic structure of otolith is suitable for nanotechnological applications. The nanometric design of mineral particles makes their biological effects more apparent. Studies can be conducted on drug delivery systems and nanoparticle therapy. Teak tree, especially the "Tectona grandis" species, is known for its versatile use and high durability. Teak tree extract and compounds obtained from this tree are used in antimicrobial pharmacology against skin infections and some internal organ infections. Teak tree extract provides anti-inflammatory and analgesic (pain relieving) effects, especially in conditions such as joint pain, muscle tension and arthritis, and can be included in cream formulations. Teak tree extract powder reduces cell damage, delays aging, prevents acne, eczema, skin infections, inflammatory skin diseases and cardiovascular diseases thanks to some antioxidant compounds it contains. In addition, research on cell renewal and tissue repair is ongoing. In short, with extraction, standardization, combinations with natural compounds, nanotechnology and advanced formulation studies, the effective combination of teak tree extract powder and otolith is recommended as a potential drug active ingredient in the pharmacological industry.

Keywords: Otolith, Teak tree extract powder, Pharmacology, Pharmacological industry, Drug design

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Comparison of Antiproliferative Effects of *Elaeagnus angustifolia* Fruit and Seed Extracts on Different Cell Lines

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Abstract: Different parts of the *Elaeagnus angustifolia* plant are widely used in traditional medicine for the treatment of various diseases. The literature reports biological effects of this plant such as antioxidant, anti-inflammatory, antimicrobial and anticancer. The aim of this study was to investigate the antiproliferative effects of aqueous extracts obtained from *Elaeagnus angustifolia* fruits and seeds on various cell lines. For this purpose, aqueous extracts were prepared from fruits and seeds and the effects of these extracts on cell viability were evaluated by crystal violet staining method. The obtained data revealed that the plant extracts showed dose-dependent cytotoxic effects on the tested cell lines. IC_{10} values for fruit extract were 13.28 ± 0.90 µg/mL, 16.52 ± 0.58 µg/mL and 18.92 ± 0.93 µg/mL in lung cancer cell line (A549), breast cancer cell line (MDA-MB-231) and human embryonic kidney cell line (HEK293) cell lines, respectively; IC_{50} values were determined as 121.72 ± 8.42 µg/mL, 124.71 ± 7.93 µg/mL and 237.03 ± 10.47 µg/mL. Seed extract showed stronger cytotoxic effect; IC_{10} values were determined as 13.19 ± 0.26 µg/mL, 14.52 ± 0.92 µg/mL and 17.11 ± 0.31 µg/mL; IC_{50} values were determined as 109.15 ± 2.99 µg/mL, 95.62 ± 4.05 µg/mL and 143.43 ± 7.22 µg/mL, respectively. Both extracts showed higher toxicity against cancer cell lines compared to HEK293. These results show that *Elaeagnus angustifolia* extracts may show different cell type-specific effects and that target cell-based evaluations should be made for potential therapeutic applications.

Keywords: *Elaeagnus angustifolia*, antiproliferative, A549, MDA-MB-231, HEK293

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A Comparative Efficiency Analysis with DEA and Integrated AHP–DEA Methods: A Case Study in the Kosovo Food Sector

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Abstract: This study analyzes the relative efficiency of a selected firm operating in the food processing sector in Kosovo by applying classical Data Envelopment Analysis (DEA) and an integrated DEA-AHP approach. The analysis is based on nine product groups treated as Decision Making Units (DMUs), with three input variables (raw materials, energy and water consumption, labor) and two output variables (net profit and value added). Initially, input-oriented DEA models (CCR and BCC) were used to determine the efficiency levels. To overcome the limitations of the DEA method, particularly the weight flexibility issue that may lead to unrealistic efficiency scores, the Analytic Hierarchy Process (AHP) was employed to define input and output weights using expert opinions.

Both Saaty's traditional scale and a balanced scale were used in the pairwise comparisons to evaluate the consistency and impact of weighting. The findings show that while several DMUs were found fully efficient under the classical DEA model, efficiency scores significantly declined when weight restrictions were introduced through AHP, revealing more accurate and realistic efficiency assessments. The average efficiency score dropped from 91.23% in the DEA model to 63.94% in the DEA-AHP model using Saaty's scale and to 67.01% using the balanced scale.

The study concludes that incorporating expert-based weight constraints improves model sensitivity and reliability. The results can be used to guide firms in identifying resource optimization targets for improving performance. Regular repetition of such efficiency assessments is recommended to support sustainable operational improvement.

Keywords: Data Envelopment Analysis (DEA), Analytic Hierarchy Process (AHP), Efficiency Measurement, Balanced Scale.

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Determination of Antioxidant Activity and Vitamin Content in Four Types of Nuts

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Abstract: Considered as good sources of unsaturated fats, nuts are gaining interest worldwide. Nuts are regarded as a source of active and functional compounds that benefit the body, help improve health and reduce the risk of various diseases. In Albania too, over the recent decades, their inclusion in diets has increased. In this study, we have selected the four most commonly used nuts in the country: walnuts, hazelnuts, almonds, and peanuts. Samples were purchased from public markets and were selected with their shells, to ensure that they were untreated. Using the SPECORD 40 spectrophotometer, we determined the antioxidant activity, as well as the content of vitamins B1 (thiamine), B2 (riboflavin), C (ascorbic acid), E, and D. The antioxidant activity, was measured using the free radical inhibition methods for the radicals ABTS and DPPH. From our study, walnuts showed the highest antioxidant activity with both radicals, with IC50ABTS= 89µg/mL and IC50DPPH=154µg/mL. Vitamin D3 was not detected in our measurements. The content of vitamins B1, B2, and C in the nuts was found to be low. Good values were observed for vitamin E, ranging from 4.8mg/100g in peanuts to 10.64mg/100g in walnuts. From our study, we conclude that among the selected nuts, walnuts are the best source of nutrients and antioxidants.

Keywords: nuts, antioxidant activity, thiamin, vitamin E.

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Cryopreservation in Plant and Animal Biology: Methods and Applications

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Abstract: Cryopreservation represents a fundamental technique in contemporary biological science, enabling the long-term storage of living biological materials under ultra-low temperatures, typically in liquid nitrogen (–196°C). As a Master of Biological Sciences, I emphasize the significance of this method for the conservation of biological diversity, preservation of genetic resources, and support of biotechnological and ecological research. In the context of accelerating biodiversity loss and increasing demands for sustainable management of biological materials, cryopreservation offers a reliable and efficient approach to ensuring biological continuity.

In biological research, cryopreservation is widely applied to a variety of biological specimens, including plant seeds, meristems, pollen, somatic and germ cells, microbial cultures, and tissues. In plant biology, it is instrumental in conserving rare and endangered species through the storage of in vitro cultures and germplasm. In zoological and veterinary sciences, cryopreservation supports ex situ conservation programs and facilitates assisted reproductive technologies. Microorganism repositories rely on cryogenic storage to maintain the viability and genetic stability of strains used in biotechnology and medicine. Furthermore, in cell biology and molecular research, cryopreserved cell lines ensure experimental reproducibility and reduce risks associated with continuous subculturing.

Recent advances in cryoprotective agents, vitrification methods, and molecular assessment of post-thaw viability have significantly improved the efficacy and reliability of cryopreservation protocols. Establishing cryobanks and genetic repositories based on standardized cryogenic techniques is now considered a cornerstone in both fundamental research and applied biological sciences. The continued development of cryopreservation technologies is essential for the long-term safeguarding of biological resources and for addressing challenges in conservation, agriculture, and regenerative medicine.

Keywords: cryopreservation, biodiversity conservation, genetic resources, cryoprotectants, plant and animal biology, biobanks, cell viability.

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Conversion of Food Waste into Bioenergy

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Abstract: The increasing volume of food waste represents both an environmental challenge and an untapped resource for renewable energy production. This study evaluates current technologies for converting food waste into bioenergy, focusing on anaerobic digestion, fermentation, and thermochemical processes such as pyrolysis and gasification. Anaerobic digestion is highlighted as an effective method for producing biogas rich in methane, which can be utilized for heat and power generation. The research also examines process optimization strategies including co-digestion with other organic wastes and pre-treatment techniques to enhance yield and stability. Fermentation processes for bioethanol production from carbohydrate-rich food residues are analyzed with regard to microbial strain selection and substrate preparation. Thermochemical conversion is discussed as a versatile approach capable of producing syngas, bio-oil, and biochar, which can be further processed into various biofuels and soil amendments. Environmental benefits such as reduced landfill use and greenhouse gas emissions are quantified, alongside economic considerations like cost, scalability, and policy incentives. The paper advocates for integrated waste management systems combining multiple conversion technologies to maximize bioenergy recovery and contribute to circular bioeconomy goals.

Keywords: Food Waste, Bioenergy, Anaerobic Digestion, Fermentation, Pyrolysis, Circular Bioeconomy.

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Environmental Impacts of Next-Generation Biodegradable Materials

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Abstract: The development of next-generation biodegradable materials aims to address the environmental challenges posed by conventional plastics. This paper analyzes the lifecycle environmental impacts of novel biodegradable polymers derived from renewable resources such as polylactic acid (PLA), polyhydroxyalkanoates (PHA), and starch-based composites. It compares their biodegradability, mechanical properties, and suitability for various applications. The environmental benefits, including reduced landfill accumulation and lower greenhouse gas emissions, are quantified through lifecycle assessment (LCA) studies. The paper also discusses potential risks related to biodegradation products and interactions with natural ecosystems. Manufacturing processes and feedstock sourcing are evaluated for sustainability. Regulatory frameworks and market trends influencing the adoption of biodegradable materials are examined. Challenges such as higher production costs, limited performance under certain conditions, and end-of-life management are addressed. The paper concludes by emphasizing the importance of holistic assessment and innovation to maximize the environmental advantages of biodegradable materials while minimizing unintended consequences.

Keywords: Biodegradable Materials, Lifecycle Assessment, Renewable Polymers, Plastic Pollution.

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Pasta Consumption Habits Among Students in Pristina: An Empirical Insight

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Abstract: This study explores the pasta consumption habits of university students living in the city of Pristina. A structured survey was conducted with 500 students from the University of Pristina to examine their frequency of consumption, preferences, and perceptions regarding the potential impact of pasta on weight gain. The results indicate that 89.1% of the participants consume pasta regularly, while 10.9% do not. Students consume on average 3 to 5 portions (600–1000 grams) of pasta per month. Although advertisements, price, and new pasta types have limited influence on their consumption choices, the shape of the pasta plays a significant role. The study found that 67.2% of the students believe that pasta contributes to weight gain, mainly due to its high carbohydrate content. In contrast, 32.8% do not associate pasta with increased body weight. Most students reported choosing pasta for its taste, affordability, and ease of preparation. These findings shed light on youth dietary behaviors and may offer valuable insights for both food producers and public health stakeholders aiming to promote more informed eating habits among young adults.

Keywords: Pasta, Students, Pristina, Eating habits, Weight gain

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Green Bioprocessing of *Rosa canina* Fruits Via Deep Eutectic solvents and Enzymes

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Abstract: The transition to sustainable bioprocesses is essential for converting regional botanicals into high-value functional ingredients, while also reducing the solvent footprint in the food sector. This study integrates deep eutectic solvents (DES) with enzyme-assisted extraction (EAE) to enhance the value of Rosa canina fruits and convert their phenolic-rich extracts into solutions ready for use in apple-based products susceptible to enzymatic browning. Using a design-of-experiments framework, we optimised the composition of the DES and the process variables (solid–liquid ratio, temperature and time), benchmarking them against aqueous–ethanolic controls. Targeted and untargeted metabolite profiling (UHPLC–DAD–MS/MS) identifies major phenolic acids and flavonoids, and antioxidant capacity is evaluated using multiple assays to reduce bias from a single method. Antimicrobial activity is also screened against common foodborne strains. Based on our knowledge of the enzyme-focused process, we evaluate the effectiveness of selective pectinase/amyloglucosidase pretreatments in enhancing yield and deconstructing the matrix. We also assess the recyclability of the solvent across multiple extraction cycles. We validate functionality in a model apple juice system by quantifying polyphenol oxidase (PPO) inhibition kinetics, tracking colour stability and haze formation during refrigerated storage and running small-panel sensory checks for acceptability. Finally, a streamlined life-cycle assessment compares DES/EAE scenarios with conventional solvents in terms of global warming potential and E-Factor indicators. This integrated approach shows how DES and EAE can be optimised together to produce bioactive extracts with good chemical characterisation that inhibit PPO activity while maintaining sensory quality, offering a practical, lower-impact way to stabilise apple-based foods with clean labels.

Keywords: Deep eutectic solvents (DES); enzyme-assisted extraction; Rosa canina; polyphenol oxidase (PPO) inhibition.

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Food Waste Valorization: From Bioactive Compounds to Bioenergy through Green Technologies

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Abstract: Food waste is a serious global problem that causes environmental pressure but also offers an opportunity as a renewable resource. In this study, a cascade approach is proposed to use food waste more efficiently. The process consists of two main stages: (1) recovery of valuable bioactive compounds, and (2) conversion of the remaining material into bioenergy. In the first stage, fruit and vegetable wastes such as apple pomace, citrus peel, and Rosa canina residues were treated with deep eutectic solvents (DES), which are environmentally friendly alternatives to chemical solvents. To improve efficiency, enzyme-assisted extraction was also applied. This combination allowed higher recovery of natural antioxidants such as phenolics and flavonoids. Some extracts were tested in apple juice as natural inhibitors of polyphenol oxidase (PPO), showing potential to reduce browning and replace synthetic additives. In the second stage, the solid residues after extraction were used for anaerobic digestion (AD) to produce biogas, mainly methane. Co-digestion with other organic wastes increased process stability and gas yield. In addition, part of the digested material was converted into biochar by pyrolysis, which can be used as a soil amendment. The results show that this integrated method can create multiple products: (i) natural antioxidants for food and health applications, (ii) renewable bioenergy in the form of biogas, and (iii) biochar for agricultural use. Compared with only producing energy, the cascade approach adds more value and supports the goals of the circular bioeconomy by reducing waste, greenhouse gas emissions, and dependence on non-renewable resources.

Keywords: bioactive compounds; green extraction; anaerobic digestion; circular bioeconomy.

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The Effect of Tourism And Recreation Activities on the Spread of Invasive Alien Species: A Compilation from the World and Türkiye Perspective

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Abstract: Tourism and recreation significantly contribute to the global economy and enhance human well-being, but they also play an increasingly critical role in the introduction and spread of invasive alien species (IAS). These species pose substantial threats to biodiversity, ecosystems, and economic sustainability. Activities such as hiking, camping, boating, and fishing inadvertently facilitate the movement of IAS across diverse ecosystems by transporting organisms through boats, recreational firewood, footwear, equipments or pets. Türkiye, located at the intersection of Europe and Asia and possessing diverse climatic zones alongside a rapidly growing tourism sector, exemplifies this vulnerability. Coastal tourism has accelerated marine invasions by species such as lionfish and pufferfish, leading to ecological damage and economic losses. Freshwater lakes are also affected by invasive fish introduced through sport fishing, which threatens endemic species. Moreover, urban wetlands and rivers have been impacted by invasive pets and recreational activities, highlighting shortcomings in national biosecurity policies and monitoring programs. Addressing these challenges necessitates integrated strategies that include robust monitoring, public education, targeted biosecurity measures, and effective management policies. This review synthesizes global and regional knowledge to illuminate management challenges and opportunities for enhancing biosecurity, using Türkiye as a case study for deriving global insights and informing practical policy development.

Keywords: Tourism, non-native species, outdoor recreational activities, introduction pathways, Invasive Alien Species

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1. INTRODUCTION

Tourism and recreation are among the fastest-growing global sectors, significantly contributing to economic growth and improving quality of life (Lee & Chang, 2008). However, the rapid expansion of tourism, especially nature-based and outdoor recreational activities, has introduced unintended ecological impacts. One critical but often overlooked consequence is the spread of invasive alien species (IAS), organisms introduced outside their natural habitats that negatively influence local biodiversity and ecosystems. IAS, including various plants, animals, fungi, and microorganisms, are recognized as one of the greatest threats to biodiversity globally, second only to habitat destruction (IUCN & SSC, 2000).

Tourism and recreation activities like hiking, camping, boating, and fishing unintentionally facilitate the spread of these species by transporting seeds, spores, and living organisms across ecological and geographic boundaries (Anderson et al., 2015; Chapman et al., 2020; Clarke Murray et al., 2011; Davidson et al., 2010; Sbragaglia et al., 2022). As tourism grows, billions of visitors worldwide unknowingly contribute to this ecological challenge, posing threats to both environmental integrity and the economic sustainability of tourism itself (Anderson et al., 2014, 2015; Hall, 2015; Sardar & Islam, 2025).

Despite growing recognition of the link between tourism and IAS spread, significant gaps remain in our understanding of how these processes vary across ecosystems and geographic regions. Türkiye, a biodiversity hotspot experiencing rapid tourism growth, is particularly vulnerable yet remains underrepresented in global research. Addressing these gaps is essential not only for protecting Türkiye's unique biodiversity but also for contributing valuable insights applicable to other regions facing similar challenges.

This review synthesizes global and regional evidence to clarify the role tourism and recreation play in spreading invasive alien species. It highlights current knowledge gaps, management challenges, and opportunities for improving biosecurity, particularly focusing on Türkiye as a case study.

2. TOURISM AND RECREATION AS INVASION PATHWAYS

The pathways through which alien species are introduced into new areas are diverse and complex. While trade and transport are well-known drivers (Hulme, 2009, 2021, 2024, 2025), tourism and recreation are increasingly recognized as significant unintentional pathways which also represent increasingly significant yet underregulated vectors (Anderson et al., 2014, 2015; Hulme, 2024; Robinson & McNeill, 2022). Unlike trade or transport pathways, which often fall under

phytosanitary controls, tourism-related movements are diffuse, decentralized, and difficult to monitor (Anderson et al., 2014, 2015; Britton et al., 2023; Robinson & McNeill, 2022).

Tourism and recreation introduce IAS both directly through propagule transfer and indirectly by disturbing habitats and weakening ecosystem resistance (Liedtke et al., 2020; Sardar & Islam, 2025). Recreational activities such as hiking, camping, off-road driving, and boating facilitate species movement through contaminated clothing, footwear, boats, trailers, fishing gear, recreational firewood and pets (Davidson et al., 2010; Koch et al., 2012; Liedtke et al., 2020; Mount & Pickering, 2009; Pickering & Ansong, 2022; Pickering & Mount, 2010; South et al., 2022). These vectors operate across ecosystems, bypassing natural dispersal barriers and enabling cross-border introductions. These activities also create physical and ecological disturbances that promote invasion (Monz et al., 2013; Pickering et al., 2007; Schafft et al., 2021). Tourism and recreational activities unintentionally facilitate the introduction and spread of IAS across different environments. The relative importance of specific vectors varies; for example, boats may be more significant in aquatic systems while hiking trails influence mountain plant invasions moderately (Anderson et al., 2015; Kelly et al., 2013; Liedtke et al., 2020).

Recreational activities on land, such as hiking, camping, cycling, and the presence of pets, often unintentionally transport invasive species. Seeds of invasive plants frequently attach to clothing, footwear, pet fur, and camping gear, later being deposited in new locations (Pickering & Mount, 2010). Similarly, off-road vehicles and bicycles disturb soil and vegetation, inadvertently spreading invasive plants and small animals along trails and recreational routes (Liedtke et al., 2020). Mountainous areas are particularly vulnerable due to frequent visitor traffic and associated disturbances. Trails in the European Alps and Andes Mountains have become conduits for invasive plants such as *Taraxacum officinale* (common dandelion) and *Poa annua* (annual bluegrass). These plants thrive in disturbed soils commonly found along hiking paths and around tourist facilities (Liedtke et al., 2020).

Aquatic recreational activities significantly contribute to invasive species spread. Coastal tourism contributes significantly to marine invasive species introductions. Boating, fishing, kayaking, and diving equipment commonly harbour invasive aquatic organisms such as algae, molluscs, and crustaceans, which are transported from one water body to another (Davidson et al., 2010). Boats and trailers are particularly problematic, as they can carry organisms in ballast water or attached to hulls and propellers, facilitating the rapid spread of invasive species across previously isolated aquatic ecosystems (Britton et al., 2023). Areas like the Mediterranean and Caribbean have experienced substantial impacts from invasive species facilitated by recreational boating, diving, and fishing. Notable examples include the spread of invasive lionfish (*Pterois miles*) and pufferfish (*Lagocephalus sceleratus*), which threaten local marine biodiversity and fishing economies due to their rapid population growth and ecological dominance (Kaplan et al., 2022). Island ecosystems, such as the Galápagos Islands and New Zealand, also face severe threats from invasive species due to tourism and recreation. For instance, invasive algae (*Didymosphenia geminata*) spread through contaminated fishing gear have significantly altered freshwater ecosystems in New Zealand. Efforts to mitigate these invasions include extensive educational campaigns and gear-cleaning stations, though compliance remains challenging (Davidson et al., 2010).

Tourism infrastructure, including hotels, resorts, roads, and recreational trails, plays a substantial role in facilitating the establishment and spread of invasive species (Barros et al., 2022). Construction activities disturb native habitats, creating conditions favourable for invasive species to establish and thrive (Anderson et al., 2015). Additionally, landscaping associated with tourism infrastructure often introduces non-native ornamental plants that may escape and become invasive. Roads and trails further fragment ecosystems and act as corridors, enabling invasive species to spread deeper into natural areas, thereby threatening local biodiversity and ecological stability (Barros et al., 2022).

3. TÜRKİYE: A CRITICAL INTERSECTION FOR INVASIVE ALIEN SPECIES

Türkiye's unique geographical location at the intersection of Europe and Asia, coupled with its diverse climatic zones ranging from temperate to subtropical, creates ideal conditions for the introduction and establishment of invasive alien species (Arslan et al., 2015). Rapid expansion of domestic and international tourism, especially along coastlines, inland lakes, rivers, and urban wetlands, significantly accelerates biological invasions by facilitating species introductions through human-mediated pathways such as recreational activities and pet trade (Arslan et al., 2015; Bilecenoğlu & Çınar, 2021; İnnal, 2020; Kaplan et al., 2022; Tarkan et al., 2024; Uludag et al., 2017).

Marine environments in Türkiye are particularly vulnerable due to coastal tourism activities such as boating, fishing, and diving. The proliferation of invasive marine species like lionfish (*Pterois miles*) and pufferfish (*Lagocephalus sceleratus*) exemplifies this issue. These species have rapidly expanded along Türkiye's Aegean and Levantine coasts, including popular tourism destinations like Fethiye-Göcek Bay, Kaş-Kekova, and Gökova Bay, causing ecological and economic disruptions (Bilecenoğlu & Çınar, 2021). Lionfish threaten reef biodiversity by preying on native fish populations, whereas pufferfish pose direct threats to human health due to their potent toxin and also negatively impact fisheries by damaging fishing gear (Bilecenoğlu & Çınar, 2021; Çinar et al., 2021; Kaplan & Yildirim, 2023). Freshwater ecosystems in Türkiye face significant pressure from invasive piscivorous fishes introduced primarily via recreational angling. Ecological niche modeling highlights alarming expansions of invasive species such as largemouth bass (*Micropterus*

salmoides), perch (*Perca fluviatilis*), and pikeperch (*Sander lucioperca*), particularly affecting lakes such as Eğirdir and Beyşehir. These invasions pose substantial threats to endemic small-bodied fishes through predation and habitat competition, exacerbated by climate-driven habitat alterations (Emiroğlu et al., 2023). Urban wetlands and recreational waterbodies are affected by invasive pet species, notably the red-eared slider turtle (*Trachemys scripta elegans*). Released by pet owners, these turtles have established populations in urban and peri-urban wetlands like İstanbul's Belgrad Forest wetlands, İzmir's Gediz Delta, and Ankara's Mogan Lake, competing with native turtle species and altering ecosystems. Despite their recognized invasive status, regulation and public awareness remain limited (Uludağ et al., 2017). River systems supporting tourism activities such as rafting and recreational fishing have facilitated the spread of the Chinese mitten crab (*Eriocheir sinensis*). Sightings of this species have increased notably in rivers such as the Sakarya and Kızılırmak. The crab's burrowing behavior threatens riverbanks and infrastructure, disrupting biodiversity and local fisheries.

Economic impacts from these invasions are significant, with agricultural and fisheries sectors bearing the highest costs. Türkiye's economic losses attributed to invasive species reached approximately 4.1 billion USD between 1960 and 2022, with annual costs increasing steadily (Tarkan et al., 2024). A comprehensive assessment of marine protected areas (MPAs) in Türkiye further underscores these vulnerabilities, revealing substantial gaps in conservation effectiveness against bioinvasions. Despite nominal protection, many MPAs exhibit high levels of established invasive species, reflecting the inadequacy of current management strategies (Bilecenoğlu & Çınar, 2021; Çinar et al., 2021). Moreover, climate change also acts as an accelerator of marine biological invasions in the region (Emiroğlu et al., 2023; Tarkan et al., 2017; Toklu Alıçlı et al., 2024). These insights collectively highlight the urgent need for integrated and proactive biosecurity measures, enhanced public awareness, targeted regulatory frameworks, and coordinated monitoring programs to mitigate the introduction and spread of invasive alien species across Türkiye.

4. CONCLUSIONS

Tourism and recreation activities significantly contribute to the introduction and spread of invasive alien species (IAS), posing substantial threats to biodiversity, ecosystems, and local economies worldwide. Türkiye, with its rich biodiversity, unique geographic position, and rapidly growing tourism sector, is particularly vulnerable. The country's diverse ecosystems, including coastal areas, freshwater lakes, urban wetlands, and rivers are increasingly affected by biological invasions driven by tourism and recreation.

To effectively address this challenge, Türkiye must develop and implement targeted management strategies. Enhanced public education, robust biosecurity protocols, and comprehensive monitoring and rapid response systems are essential components. Collaboration among governmental agencies, tourism operators, conservation organizations, and the broader community is crucial for successfully mitigating the impact of IAS. These integrated efforts will not only protect Türkiye's exceptional biodiversity but also sustain the ecological and economic benefits derived from its tourism and recreation sectors.

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The Importance of Aramid Fibers and The Economic Status of Aramid Fibers In The World

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Abstract: High-performance fibers have begun to emerge from composite materials depending on the desired properties. Aramid fibers are one of the fibers used as reinforcement elements in high-performance composite materials, which are considered engineering materials. Aramid fibers have various properties such as high strength, lightness and flame retardation. Due to these properties, aramid fibers are used in different areas such as aviation, weapons, vehicle industries, as well as bulletproof body armor, individual protection and sports equipment. The annual production of aramid fibers, which have many application areas, is expected to be around 100 thousand tons and this rate is expected to increase steadily in the coming years, and as a result, it is anticipated that they will have a significant market share. This increase in production can be attributed to the use of personal protective equipment, ballistic vests and helmets, tire production and other products. These fibers, which were first produced in the 1960s, have survived to the present day with improved properties, and intense efforts are still being made to improve their properties. Today, the largest producer countries in the production of aramid fibers are the USA, Japan, Russia, South Korea and China. In this study, export and import data and rates of major aramid fiber producing countries and Türkiye were compared using foreign trade data. In order to access these data, Harmonized System (H.S.) codes were determined and foreign trade data for the years 2022, 2023 and 2024 were found from the H.S. codes. Data obtained from the databases of the official statistical institutions of the countries were used to find these export and import rates. As aramid fibers have three different H.S. codes, foreign trade data for each product was examined and compared on a country basis. As a result of the shared data, the largest producer country and the most produced aramid fiber type were determined.

Keywords: Aramid fiber, import, export, harmonized system codes.

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1. GİRİŞ

Teknolojinin sürekli gelişmesi ile birlikte mühendislik malzemeleri ön plana çıkmaya başlamıştır. Mühendislik malzemelerinden biri olan kompozit malzemeler ise günümüzde geliştirilmesi devam eden bir tür olarak karşımıza çıkmaktadır. Kompozit malzemeler, geleneksel malzemelere yenilikçi çözüm kazandırması, verimliliği artırmasından dolayı çokça kullanılmaya başlanmıştır (Clyne & Hull, 2019). Kompozit malzemeleri en az iki farklı malzemeden oluştuğundan, tasarımları yapılırken üretim yöntemleri, kalite kontrol, malzemelerin birbiri ile uyumluluğu en önemli kriterler olarak düşünülebilir. Metal malzemeler ile kıyaslandığında yüksek dayanım sergilemeleri, düşük yoğunlukların dolayı ağırlık kazanımı avantaj olarak gösterilebilir (Mallick, 2007). Bu malzemeler elektronik kartlar, robotik kollar gibi elektronik, rüzgâr tribünleri gibi enerji, dişliler, rulmanlar gibi makine, havacılık ve savunma gibi birçok sektörde ürün üretiminde kullanılmaktadır (Sathishkumar et al., 2014).

Kompozit malzemeler farklı tür malzemelerden oluştuğundan kendi içerisinde çeşitlenmektedir. Kompozit malzemelerin temel bileşenleri olan matris ve takviye elemanlarından oluşması bu çeşitlenmede rol oynamaktadır. Fakat günümüzde en çok kullanılan kompozit malzemelerden polimer matrisli elyaf takviyeli olanlar tercih edilmektedir (Şişecam, 2010). Bu tercihin sebebinde ise savunma, havacılık, otomotiv ve enerji gibi sektörlerde yaygın olarak kullanılması gösterilebilinir. Üretim yöntemlerinin kolay, çok çeşitli polimerler olması ve elyaflardan gelen üstün dayanımı birçok sektörde yaygınlaşmasını sağlamıştır. Takviye elemanı olarak tercih edilen elyaflar selüloz gibi, kendiliğinden doğada var olan doğal elyaflar ve üretimi yapılan sentetik elyaflar olmak üzere ikiye ayrılırlar (Wang et al., 2011). Günümüzde en çok kullanılan sentetik elyaflar cam, karbon ve aramid olarak karşımıza çıkmaktadır.

Sentetik elyafların geliştirilmesinde dönüm noktası niteliğinde olan aramid elyaf, üretimi büyük bir başarı ve artan bir ilgi görmüştür (He et al., 2024). Aramid elyaflar ve bu elyaflardan üretilen kompozitlerin, yüksek mekanik özellikler, hafiflik, yüksek sıcaklık direnci, alev geciktirici, kimyasal stabilite ve yalıtım gibi özellikleri bulunmaktadır. Bu nedenle havacılık, silah ve otomotiv endüstrilerinde, kurşun geçirmez vücut zırhı, sporları araçları, elektrik yalıtımı, endüstriyel filtreler gibi uygulamalarda yaygın olarak kullanılmaktadır (Amesimeku et al., 2019; Lv et al., 2015; Wang et al., 2018). İlk aramid türü olarak ortaya çıkan Para-aramid, otomobil lastikleri içinde yer alan tellerin yerine kullanılacak bir malzeme arayışı sırasında 1971 yılında Dupont tarafından geliştirilmiştir (He et al., 2024). Aramid, naylon ailesinin bir üyesi olup naylon türevlerinden farklı özellikler göstermektedir. Aramidler yüksek mukavemete ve modüle sahip bir elyaftır (Ercümen, 2024). Dupont firması tarafından üretilen ilk Para-aramid elyafı ise "Kevlar" ticari adıyla tescil edilmiştir. Farklı uygulama alanları için geliştirilmiş Kevlar çeşitleri mevcuttur.

ABD Federal Ticaret Komisyonu tarafından 'Aramid' kelimesi, elyafı oluşturan hammaddenin uzun zincirli sentetik poliamid olduğu ve amid bağlantılarının en az %85'inin doğrudan iki aromatik halkaya bağlı olarak üretilmiş bir elyaf olarak tanımlanmıştır (Commission, 2025). Bu nedenle aramid ailesi Kevlar, Nomex, Technora, Teijinconex, Twaron gibi ticari isimlerle adlandırılan elyafları da kapsamaktadır (Jassal & Ghosh, 2002). Küresel aramid elyaf pazarı 2023 yılında 3,9 Milyar ABD doları değerine ulaşmıştır (Group, 2025). IMARC Group tarafından yapılan analize göre, otomotiv endüstrisinden araç parçaları için hafif ve yüksek mukavemetli malzemelere yönelik artan talep, pazara önemli ölçüde yön vermektedir (Group, 2025). Buna paralel olarak, aramid elyafların yüksek mukavemet/ağırlık oranı nedeniyle havacılık ve uzay endüstrisinde uçak parçaları için giderek daha fazla benimsenmektedir. 2032 yılına kadar pazar değerinin 5 Milyar ABD doları değerine ulaşacağı ve 2024-2032 döneminde %2,9'luk bir bileşik yıllık büyüme oranı (CAGR) ile büyüyeceği düşünülmektedir (Group, 2025). Küresel aramid elyaf pazarında en büyük 10 üretici firma; Teijin (Japon), DuPont (ABD), Yantai Tayho Advanced Materials Company (Çin), Hyosung Corporation (Güney Kore), Toray Chemical Korea (Güney Kore), Kolon Industries (Güney Kore), Huvis (Güney Kore), China National BlueStar Group Company (Çin), Taekwang Industrial (Güney Kore) ve JSC Kamenskvolokno (Rusya)'dır (Group, 2025). Dünya üzerindeki ikinci büyük aramid üreticisi ise Japon Teijin'dir. Ardından üçüncü büyük oyuncu olarak Çin de aramid piyasasına yer almaktadır.

Bu çalışmanın amacı takviye elemanı olarak tercih edilen elyaf türlerinden biri olan aramid elyafın ikincil verilerden elde edilen bilgiler doğrultusunda değerlendirmesi yapılmaktadır. Birçok sektörde kullanılan farklı türlerdeki aramid elyaf ürünlerinin 2022, 2023 ve 2024 yıllarında G-7 ülkelerinin ve Türkiye'nin ithalat ve ihracat rakamlarının kıyaslanması yapılmıştır. Bu elyafın kullanım miktarı ülkeler bazında incelenmiştir.

2. MATERYAL VE METOT

Dünya gümrük sisteminde malların ihracat, ithalat ve gümrük rejimi uygulamalarındaki operasyonların düzgün bir şekilde yürütülmesi ana hedeflerden biridir. Bu doğrultuda Dünya Gümrük Örgütü, uluslararası ticaretin kolay bir şekilde yürütülmesi için Armonize Mal Tanımı ve Kodlama Sistemi geliştirilmiştir. Bu sisteme uyum sağlamak için ülkeler kendi kurallarını geliştirmişlerdir. Türkiye bu sisteme ilk olarak 1958 yılımda geçmiş ve kodlama uygulaması Gümrük Tarife İstatistik Pozisyon (GTİP)'tir. GTİP terimi; T.C. Ticaret Bakanlığı tarafından Türk Gümrük Tarife Cetvelinde kullanılan 12 rakamlı kod olarak belirtilmektedir. Bu kodun ilk altı hanesi Dünya Gümrük Örgütü'ne üye tüm ülkelerce kullanılan Armonize Sistem (HS: Harmonized System) Nomanklatürü kodunu, yedinci ve sekizinci haneleri Avrupa Birliği (AB) ülkeleri tarafından kullanılan Kombine Nomanklatür (Combined Nomenclature-CN) kodunu, dokuzuncu ve onuncu haneleri farklı vergi uygulamaları nedeniyle açılan pozisyonları gösteren kodlarını, on birinci ve on ikinci haneler ise ilgili ülkelerdeki istatistik kodlarını göstermek için kullanılmaktadır (Ticaret Bakanlığı, 2025).

Uluslararası Ticaret Merkezi (UTM) tarafından oluşturulmuş ve koordine edilen "trademap.org" internet sitesi, 200'den fazla ülkenin ve bölgenin harmonize sistemde kayıtlı bulunan 5.000'den fazla ürününü içerecek şekilde tasarlanmış ve kurgulanmıştır. "Trademap.org" internet sitesi sisteme dâhil olan ülkelerin resmi istatistik kurumlarının veri tabanlarından ilgili ürünlerin ihracat ve ithalat rakamlarını çeşitli ölçütler (aylık, 3 aylık, 6 aylık, yıllık, kg/ton/lt, para birimi vb.) doğrultusunda kendi bünyesinde bulunan veri tabanına aktarmaktadır. Bu internet sitesini kullanan araştırmacılar, ülkelerin ilgili ürünlerdeki ticari verilerine, dünya payına, yeni pazarların ekonomik ve ticari yapısına, rekabetçi pazarlara, ihracatçı-ithalatçı detaylarına sayısal veriler, tablolar, grafikler ve haritalar şeklinde ulaşabilmektedir. Kısaca; "trademap.org" isimli ticaret temelli internet sitesi, uluslararası ticaretin ve iş olanaklarının geliştirilebilmesi için kullanılan istatistiki bilgiler sağlayan veri tabanı niteliğinde bir ara yüz sağlayıcısı ve sistemidir. Veri tabanı uluslararası ticarette ekonomik ve ticari bilgi elde etmek amacıyla birçok araştırmacı tarafından kullanılmakta ve piyasa çalışanlarına tavsiye edilmektedir.

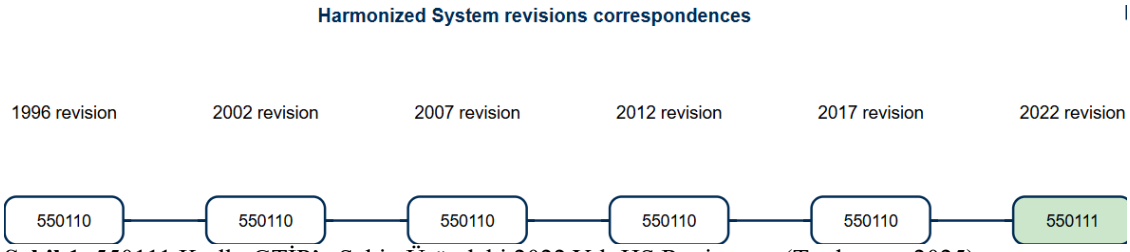
Bu çalışmada yer alan kompozit malzemelerde takviye elemanı olarak kullanılan aramid elyafların ticari olarak sınıflandırılması yapılmıştır. Uluslararası piyasada aramid elyafların sınıflandırılması ve Gümrük Tarife İstatistik Pozisyon (GTİP) numaraları sırası ile Tablo 1'de sunulmuştur.

Tablo 1. Aramid Elyaf Ürünlerinin GTİP'i ve Ürün Tanımları (Trademap, 2025)

GTİP	**Ürün Tanımı**
550111000000 (5501.11)	Kırpılmış Aramid Filament İplik
550311000000 (5503.11)	Kesikli Aramid Elyaf
540211000000 (5402.11)	Yüksek Mukavemetli Aramid Filament İplik

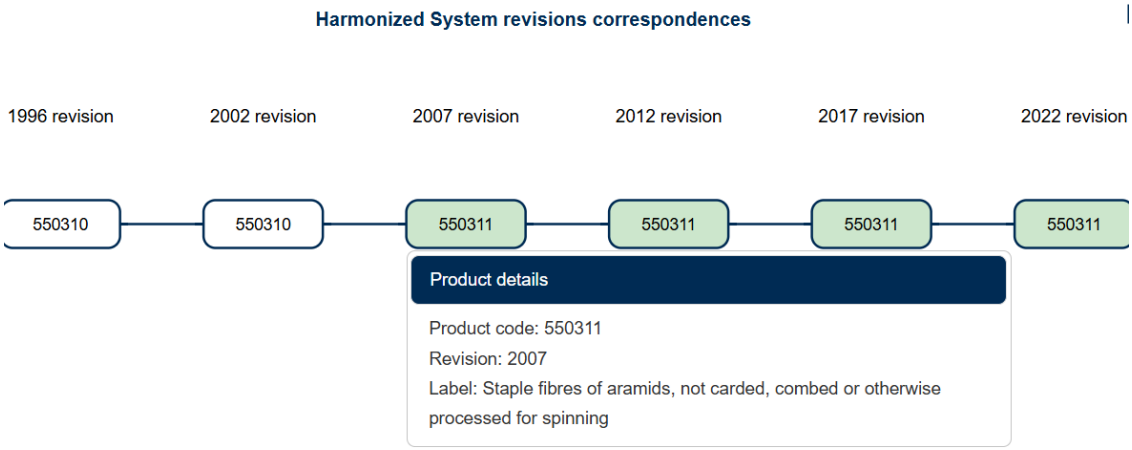
Günün değişen ekonomik koşullarına ve ticari şartlarına istinaden Dünya Gümrük Örgütü (DGÖ), GTİP'ler üzerinde değişikliğe giderek hesaplamaları ve verileri daha doğru şekilde sonuçlandırmaya gayret göstermektedir. Aramid elyaflar bu doğrultuda GTİP'lerinde değişikler göstermiştir. Şekil 1, Şekil 2 ve Şekil 3'te belirtilen açıklamaya örnek bir durum bulunmaktadır. 550111 GTİP kodlu aramid elyaf türü ise 2022 yılı başına kadar farklı mal GTİP'nin altında hesaplanmış ve verileri farklı kodlar doğrultusunda paylaşılmıştır. 550111 GTİP kodlu ürün, 2022 yılı başına kadar 550110 (XXX) GTİP kodlu malın içerisinde hesaplanmıştır. 2022 yılında yapılan HS revizyonu ile birlikte 550111 kodlu GTİP'e sahip

olan mal bireysel GTİP'i üzerinden hesaplanmaya başlanmıştır (Şekil 1). Belirtilen bu duruma istinaden 550111 GTİP kodlu malın 2022 yılına kadar ki ihracat ve ithalat rakamları tam anlamı ile hesaplanamamakta ancak bireysel hesaplamaya geçilen 2022 yılı itibari ile kesin rakamlara ulaşılmaktadır.

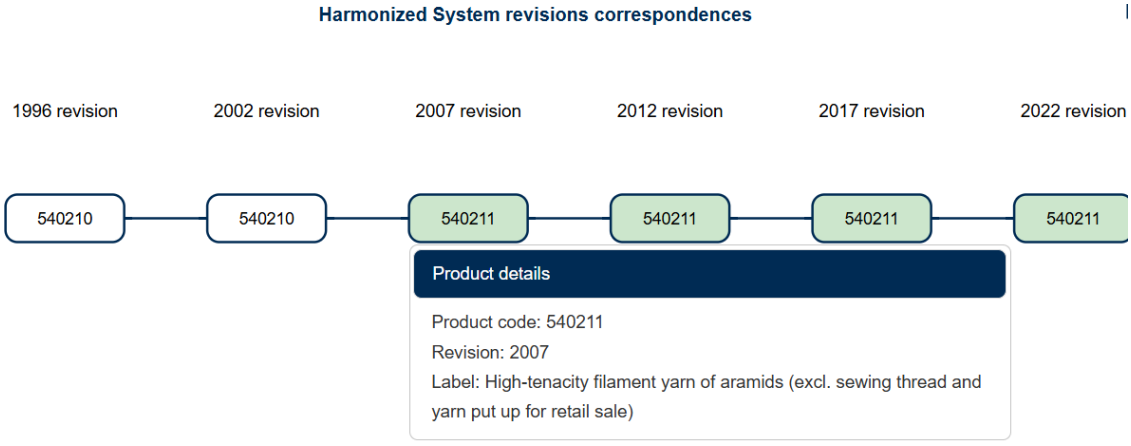


Şekil 1. 550111 Kodlu GTİP'e Sahip Üründeki 2022 Yılı HS Revizyonu (Trademap, 2025)

Çalışma kapsamında ele alınan 550311 ve 540211 GTİP kodlu diğer aramid elyaf çeşitleri 2007 yılına kadar sırasıyla 550310 (XXX) ve 540210 (XXX) GTİP kodlu malların içerisinde hesaplanırken, 2007 yılından itibaren HS revizyonuna uğrayarak (Şekil 2 ve Şekil 3) bireysel GTİP kodlarına sahip olmuştur. Bu GTİP kodları ile son on yedi yıla ilişkin ticari verileri trademap internet sitesi ara yüzünden elde edilebilmektedir. 2007 ve 2022 yıllarında HS revizyonu olan GTİP'lere ilişkin görseller Şekil 2 ve Şekil 3'te sunulmuştur.



Şekil 2. 550311 Kodlu GTİP'e Sahip Üründeki 2007 Yılı HS Revizyonu (Trademap, 2025)



Şekil 3. 540211 Kodlu GTİP'e Sahip Üründeki 2007 Yılı HS Revizyonu (Trademap, 2025)

Çalışmanın kapsamında; 550111, 550311 ve 540211 GTİP koduna sahip olan malların Türkiye'den Dünya ülkelerine ve en gelişmiş 7 ülke ekonomisi ülkelerinden (G-7) Dünya ülkelerine yapmış olduğu ihracat ve ithalat rakamlarını 2022, 2023 ve 2024 yıllarını milyon USD para birimi cinsinden içermektedir (bazı veriler bin USD-$ şeklindedir).

3. BULGULAR

Çalışmada aramid elyaf ve çeşitlerinin ilgili GTİP kodları doğrultusunda en çok üretim yapan firmaların bulunduğu ülkeler ile Türkiye bazında ele alınmıştır. Konu kapsamına giren ürünlerin ihracat ve ithalat rakamları Amerikan doları cinsinden 2022, 2023 ve 2024 yıllarını kapsayacak şekilde Türkiye açısından, sonrasında ise üretici ülkeler ile G-7 açısından araştırılmıştır. Tablo 2'de Türkiye'nin ilgili ürünlerdeki dış ticaret verileri paylaşılmıştır.

Tablo 2. (5501.11), (5503.11), (5402.11) GTİP Koduna Sahip Ürünlerin Türkiye'deki Dış Ticaret Verileri (Milyon USD) (Trademap, 2025)

Ürün GTİP Kodu	Türkiye'den Dünyaya İhracat Verileri			Türkiye'nin Dünyadan İthalat Verileri		
	2022	2023	2024	2022	2023	2024
550111000000 (5501.11)	4 bin $	2 bin $	1 bin $	67 bin $	0	17 bin $
550311000000 (5503.11)	129 bin $	42 bin $	244 bin $	40,617	39,118	46,402
540211000000 (5402.11)	3,126	2,933	1,870	39,635	47,191	44,122

Tablo 2'den elde edilen veriler doğrultusunda; Türkiye 5501.11 GTİP kodlu üründen 2022 yılında yaklaşık 4 bin dolar, 2023 yılında 2 bin dolar ve 2024 yılında sadece bin dolar ihracat gerçekleştirmiştir. Bu duruma karşın 2022 ve 2024 yıllarında Türkiye'nin 5501.11 GTİP kodlu üründe ithalatı 67 bin dolar ve 17 bin dolar düzeyindedir. Türkiye 5503.11 GTİP kodlu üründen 2022, 2023 ve 2024 yıllarında sırasıyla 129 bin, 42 bin ve 244 bin dolar ihracat gerçekleştirmiştir. Bu duruma karşın 2022, 2023 ve 2024 yıllarında yaklaşık 41 milyon, 39 milyon ve 46 milyon dolarlık ithalat gerçekleştirmiştir. Türkiye 5402.11 GTİP kodlu üründen 2022, 2023 ve 2024 yıllarında sırasıyla 3 milyon, 2.9 milyon ve 1.8 milyon ihracat gerçekleştirmiştir. Bu duruma karşın 2022, 2023 ve 2024 yılında sırasıyla 39 milyon, 47 milyon ve 44 milyon dolarlık ithalat gerçekleştirmiştir. Bu durumlar göstermektedir ki; Türkiye'de 5501.11, 5503.11 ve 5402.11 GTİP kodlu malların dış ticaretinde açık bulunmaktadır.

Türkiye 2022, 2023 ve 2024 yıllarında 5402.11 GTİP kodlu malda en çok ithalatı Birleşik Krallık'tan gerçekleştirmiştir. 5503.11 GTİP kodlu ürünü 2022, 2023 ve 2024 yıllarında sırasıyla İspanya'dan, Çin'den ve yine İspanya'dan ithal etmiştir. 5501.11 GTİP kodlu ürünü 2022 ve 2024 yıllarında Çin'den ithal etmiştir. Türkiye 2022, 2023 ve 2024 yıllarında 5402.11 GTİP kodlu malda en çok ihracatı Bulgaristan'a, ABD'ye ve Belçika'ya gerçekleştirmiştir. 5503.11 GTİP kodlu ürünü 2022, 2023 ve 2024 yıllarında sırasıyla en çok Hindistan'a, Çin'e ve Hollanda'ya gerçekleştirmiştir. 5501.11 GTİP kodlu ürünü 2022, 2023 ve 2024 yıllarında ABD'ye çok küçük miktarlar ile ihraç etmiştir.

Çalışma kapsamında ele alınan ürünler hem özel hem de endüstriyel alanda kullanılan ürünlerdir. Dolayısı ile bu tarz ürünlerin üretiminin, ihracatının ve ithalatının gelişmiş ve gelişmekte olan ülkelerde daha çok döngüsünün olacağına kanaat getirilmesine istinaden bu ülkelerin de araştırılması çalışmaya dâhil edilmiştir. Araştırma kapsamında yapılan incelemeler Tablo 3, Tablo 4 ve Tablo 5'te paylaşılmıştır.

Tablo 3. G-7 Ülkelerinin 550111000000 GTİP Kodlu Üründeki Dış Ticaret Verileri (Milyon USD) (Trademap, 2025)

GTİP: 5501.11						
Ülkeler	İhracat ($)			İthalat ($)		
	2022	2023	2024	2022	2023	2024
Almanya	0	0	15 bin $	2,151	1,176	1,681
ABD	30,534	50,360	41,133	28 bin $	284 bin $	287 bin $
Birleşik Krallık	100 bin $	56 bin $	98 bin $	496 bin $	157 bin $	120 bin $
Fransa	897 bin $	29 bin $	25 bin $	243 bin $	46 bin $	46 bin $
İtalya	325 bin $	572 bin $	252 bin $	954 bin $	387 bin $	227 bin $
Japonya	307 bin $	184 bin $	59 bin $	35,908	52,505	50,442
Kanada	19 bin $	50 bin $	37 bin $	16 bin $	51 bin $	5 bin $
Rusya	86 bin $	*Veriler tam ve net değildir	*Veriler tam ve net değildir	1 bin $	*Veriler tam ve net değildir	*Veriler tam ve net değildir
Çin	5,168	3,611	4,415	535 bin $	449 bin $	696 bin $
Güney Kore	96 bin $	142 bin $	109 bin $	34 bin $	1 bin $	142 bin $
Dünya	39,153	58,582	*Veriler tam ve net değildir	45,267	60,053	*Veriler tam ve net değildir

(*) Trademap.org sitesinde bazı ülkelerin ilgili üründe 2024 yılı ithalat ve ihracat verileri paylaşılmadığından dolayı veri bilgisine tam olarak ulaşılamamış olup ticari verilere eklenmemiştir.

Tablo 3'ten anlaşılabileceği üzere; 550111000000 GTİP kodlu üründe belirtilen ülkelerinin 2022, 2023 ve 2024 yıllarını kapsayacak şekilde ihracat ve ithalat rakamları milyon dolar cinsinden paylaşılmıştır. G-7 ülkeleri içinde 2022, 2023 ve 2024 yıllarında sırası ile en fazla ihracat 30, 50 ve 41 milyon dolar ile ABD tarafından gerçekleştirilmiştir. G-7 ülkeleri içinde 2022, 2023 ve 2024 yıllarında sırası ile en fazla ithalat 35, 52 ve 50 milyon dolar ile Japonya tarafından gerçekleştirilmiştir. Tüm dünya ülkeleri içerisinde de 2022, 2023 ve 2024 yıllarında Amerika en fazla ihracat sırasıyla 30 ve 50, 41 milyon dolar ile 2022, 2023 ve 2024 yıllarında en fazla ithalat Japonya tarafından sırasıyla 35, 52 ve 50 milyon dolar olacak şekilde gerçekleşmiştir. Hem ihracatta hem ithalata 2023 ve 2024 yılı verileri Rusya'daki istatistik kurumu tarafından paylaşılmadığından dolayı veriler elde edilememiştir. Ayrıca Rusya'nın 2019, 2020 ve 2021 yıllarında da ilgili malda ihracat ve ithalat gerçekleştirmediği veri tabanı araştırması neticesinde anlaşılmıştır.

Tablo 4. G-7 Ülkelerinin 550311000000 GTİP Kodlu Üründeki Dış Ticaret Verileri (Milyon USD) (Trademap, 2025)
(*) Trademap.org sitesinde bazı ülkelerin ilgili ürünlerde 2024 yılı ithalat ve ihracat verileri paylaşılmadığından dolayı veri bilgisine ulaşılamamış olup ticari verilere eklenmemiştir.

GTİP: 5503.11						
Ülkeler	**İhracat ($)**			**İthalat ($)**		
	2022	**2023**	**2024**	**2022**	**2023**	**2024**
Almanya	10,405	8,928	7,525	61,559	54,496	45,445
ABD	47,051	45,015	55,018	84,544	66,007	62,890
Birleşik Krallık	3,352	3,925	2,757	6,732	6,024	3,702
Fransa	31,914	23,045	29,015	7,138	5,390	6,290
İtalya	3,743	2,340	2,826	27,230	20,862	12,088
Japonya	3,011	2,725	85 bin $	8,814	8,814	6,619
Kanada	563 bin $	974 bin $	200 bin $	15,523	16,022	14,112
Rusya	189 Bin $	345 Bin $	*Veriler tam ve net değildir	2,427	4,374	*Veriler tam ve net değildir
Çin	52,695	52,446	46,183	45,186	39,085	41,218
Güney Kore	67,130	58,717	53,390	6,575	8,609	10,129
Dünya	517,758	512,003	*Veriler tam ve net değildir	602,489	563,340	*Veriler tam ve net değildir

Tablo 4'ten anlaşılabileceği üzere; 550311000000 GTİP kodlu üründe G-7 ülkelerinin 2022, 2023 ve 2024 yıllarını kapsayacak şekilde ihracat ve ithalat rakamları milyon dolar olarak paylaşılmıştır. G-7 ülkeleri içinde 2022, 2023 ve 2024 yıllarında sırası ile en fazla ihracat 47 milyon dolar, 45 milyon dolar ve 55 milyon dolar ile ABD tarafından gerçekleştirilmiştir. G-7 ülkeleri içinde 2022, 2023 ve 2024 yıllarında sırası ile en fazla ithalat sırası ile 84 milyon dolar, 66 milyon dolar ve 62 milyon dolar ile ABD tarafından gerçekleştirilmiştir. 2022 ve 2023 yıllarında Belçika en fazla ihracatı sırasıyla 140 milyon ve 132 milyon dolar ile ve 2024 yılında İspanya 77 milyon dolar ile gerçekleştirmiştir. 2022 ve 2023 yıllarında Belçika en fazla ithalatı sırasıyla 145 milyon ve 143 milyon dolar ile ve 2024 yılında en fazla ithalat Amerika tarafından 62 milyon dolar ile gerçekleştirmiştir. Ek olarak Rusya 2019'da 18 bin $, 2020'de 36 bin $ ve 2021'de 96 bin $'lık ihracat gerçekleştirmiştir. Ayrıca 2019'da 3.4 milyon $, 2020'de 4,1 milyon $ ve 2021'de yaklaşık 3.1 milyon $'lık ithalat gerçekleştirmiştir.

Tablo 5. G-7 Ülkelerinin 540211000000 GTİP Kodlu Üründeki Dış Ticaret Verileri (Milyon USD) (Trademap, 2025) (*) Trademap.org sitesinde bazı ülkelerin ilgili ürünlerde 2024 yılı ithalat ve ihracat verileri paylaşılmadığından dolayı veri bilgisine ulaşılamamış olup ticari verilere eklenmemiştir.

GTİP: 5402.11						
G-7 Ülkeleri	**İhracat ($)**			**İthalat ($)**		
	2022	**2023**	**2024**	**2022**	**2023**	**2024**
Almanya	0	0	0	146,136	131,937	123,021
ABD	140,146	143,120	110,946	193,230	144,586	128,049
Birleşik Krallık	8,053	67,189	139,192	28,293	28,420	26,119
Fransa	1,855	3,173	3,006	46,388	51,929	56,862
İtalya	15,237	15,633	12,704	109,766	132,289	99,494
Japonya	39,678	42,311	34,365	7,175	6,492	1,979
Kanada	1,689	1,137	1,012	3,884	2,611	2,739
Rusya	774 bin $	11 bin $	*Veriler tam ve net değildir	18,766	28,527	*Veriler tam ve net değildir
Çin	64,058	75,102	66,279	141,888	124,637	112,090
Güney Kore	206,266	188,483	148,982	9,045	6,826	5,536
Dünya	876,486	1,260,299	*Veriler tam ve net değildir	1,385,195	1,441,957	*Veriler tam ve net değildir

Tablo 5'ten anlaşılabileceği üzere; 540211000000 GTİP kodlu üründe G-7 ülkelerinin 2022, 2023 ve 2024 yıllarını kapsayacak şekilde ihracat ve ithalat rakamları milyon dolar cinsinden paylaşılmıştır. G-7 ülkeleri içinde 2022, 2023 ve 2024 yıllarında sırası ile en fazla ihracat 140 milyon dolar, 143 milyon dolar ile ABD ve 139 milyon dolar ile Birleşik Krallık tarafından gerçekleştirilmiştir. G-7 ülkeleri içinde 2022, 2023 ve 2024 yıllarında sırası ile en fazla ithalat 193 milyon dolar, 144 milyon dolar ve 128 milyon dolar ile ABD tarafından gerçekleştirilmiştir. 2022 yılında Belçika 268 milyon dolar, 2023 ve 2024 yılında ise Hollanda 355 milyon ve 343 milyon dolar ile en fazla ihracat gerçekleştiren ülkeler olmuştur. 2022 yılında Amerika 193 milyon dolar, 2023 yılında Belçika 162 milyon dolar, 2024 yılında Amerika 128 milyon dolar ile ithalatta ilk sırada yer alan ülkelerdir. Ek olarak Rusya 2019'da 3,5 milyon $, 2020'de 2,5 milyon $ ve 2021'de yaklaşık 6,8 milyon $ ihracat gerçekleştirmiştir. Ayrıca 2019'da 25.4 milyon $, 2020'de 21,6 milyon $ ve 2021'de yaklaşık 18.1 milyon $ ithalat gerçekleştirmiştir.

4. TARTIŞMA VE SONUÇLAR

Kompozit malzemelerde yaygın olarak kullanılan aramid elyaf ürünlerinin ülkeler bazında 2022, 2023 ve 2024 yılına ait ihracat ve ithalat rakamlarının incelenmesi yapılmıştır. 2024 yılına ait verilerin Dünya genelinde bazı eksikliklerin olduğu görülürken, bunun temelinde Rusya'dan gerekli bilgilerin alınamamasından kaynaklanmaktadır. Dünya genelinde rakamlar incelendiğinde 5501.11 ve 5402.11 GTİP kodlu aramid elyaf çeşitlerinde ihracat ve ithalat rakamlarında artış olduğu belirlenmiştir. Fakat 5503.11 GTİP koduna sahip aramid elyaf çeşidinde ise bir düşüş yaşandığı görülmektedir. Bu daralmanın sebebi olarak G-7 ülkelerinin ihracat ve ithalat rakamlarının düşmesi olarak yorumlanabilir. Dünya Gümrük Örgütü tarafından belirlenmiş ve ticarete konu olan aramid elyaf çeşitlerini üreten en büyük firmaların bulunduğu ülkeler sırasıyla ABD, Japonya, Çin ve Güney Kore'nin ihracat ve ithalat verileri incelendiğinde firmaların etkinliğinin önemli olduğu görülmektedir. Benzer şekilde Dünya genelinde üçüncü büyük üretici konumunda olan Çin'in Türkiye üzerinde etkisi olduğu ve bu aramid elyafları Çin'den ithal edildiği belirlenebilmektedir. Bu üç aramid elyaf çeşidinin yaklaşık olarak 90 milyon dolarlık bir ithalat rakamına ulaşan Türkiye'de bu ürünlerde önemli bir pazar payına olduğu düşünülebilinir.

Author Contributions
Conceptualization: S.C.A., T.Ü.; Material and Methodology: S.C.A.; Supervision: T.Ü; Writing-Original Draft: T.Ü.; Writing-review & Editing: S.C.A., T.Ü.; 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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GC-MS Analysis of Volatile Compounds in Commercial *Rosa damascena* Mill. Products: Water, Oil, Yeast, Concrete, and Absolute

OSMAN-BODUR*[1], TANER-ERKAYMAZ[1]

Abstract: *Rosa damascena* Mill., widely known as Damask rose, is a valuable aromatic plant cultivated primarily for its high value-added products such as rose oil, rose water, rose yeast, rose concrete, and rose absolute. These products are extensively utilized in perfumery, cosmetics, food, and pharmaceutical industries due to their rich volatile compound profiles. The quality, yield, and aromatic characteristics of these derivatives are highly dependent on the extraction technique employed, which significantly affects the chemical composition and sensory attributes of the final product. This study aims to commercially available *Rosa damascena* products and to analyze their volatile constituents using gas chromatography-mass spectrometry (GC-MS). While the densest sample in terms of terpene compounds was water oil from rose oils, the product with the highest paraffinic content was rose concrete extracted with n-hexane. It was observed that the oil content and relative percentage values of rose yeast were higher than rose water samples. Rose absolute was close to rose water in terms of the phenyl ethyl alcohol content, while it was observed that it was close to rose oil in terms of terpene values. The methods of obtaining these samples result in differences in the types of volatile components such as citronellol, geraniol, nerol, phenylethyl alcohol and various hydrocarbons. This study contributes to the growing body of knowledge on rose product standardization by linking extraction parameters with chemical outcomes. The integration of GC-MS in the evaluation of commercial *Rosa damascena* derivatives provides a robust framework for quality control, authenticity assessment, and product optimization in rose-based industries.

Keywords: Rosa damascena Mill., Rose Products, Distillation, Extraction, GC-MS.

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1. INTRODUCTION

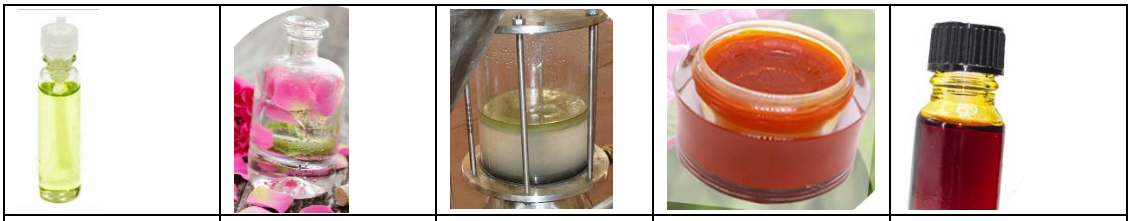
The Damask rose (*Rosa damascena* Mill.) is one of the most economically significant aromatic plants globally, renowned for its rich essential oil content and distinctive fragrance (Figure 1). Traditionally cultivated in countries such as Turkey, Bulgaria, Iran, and India, *R. damascena* is the principal source for the production of a wide array of rose-based commercial products (Figure 2) including rose oil, rose water, rose yeast, rose concrete, and rose absolute (Akram et al., 2020; Widrlechner, 1981). These derivatives are widely used in perfumery, cosmetics, aromatherapy, pharmaceuticals, and the food industry due to their diverse volatile compound profiles and therapeutic potentials (Kurkcuoglu and Baser, 2003; Aycı et al., 2005).

Figure 1. *Rosa damascena* Mill.

The extraction method employed in obtaining these products plays a critical role in determining both the yield and the qualitative attributes of the volatile constituents (Baydar, 2006). Water distillation, the oldest and most traditional method, is still widely used to produce rose oil and rose water. In this process, rose yeast a valuable by product accumulates beneath the oil layer (Chemat et al., 2012). Recent developments in steam distillation technology have enhanced efficiency and yield, making it more suitable for industrial-scale production (Katekar et al., 2022). Alternatively, solvent extraction using non-polar solvents such as *n*-hexane enables the production of rose concrete, a semi-solid waxy extract. Further purification of concrete using ethanol at low temperatures yields rose absolute, which retains a more complete aromatic profile compared to distilled products (Fierascu et al., 2021).

The chemical composition of these products primarily consists of volatile compounds such as citronellol, geraniol, nerol, linalool, and phenylethyl alcohol, which contribute to their fragrance and bioactivity (Rasooli et al., 2021). However,

these compositions vary significantly depending on the extraction technique, storage conditions, geographic origin, and plant genotype (Bayrak and Akgul, 1994). Gas chromatography–mass spectrometry (GC-MS) is a widely accepted analytical tool for profiling and quantifying volatile components in rose products, enabling both quality control and authenticity verification (Koksal et al., 2015; Najem et al., 2011).



Rose Oil	Rose Water	Rose Yeast	Rose Concrete	Rose Absolute

Figure 2. Rose-based commercial products

Recent studies have focused on optimizing extraction techniques to improve the retention of key aroma compounds and reduce degradation during processing (Chemat & Cravotto, 2012). Moreover, the commercial differentiation of *Rosa damascena* products based on their volatile composition is gaining importance in global markets that demand consistency, purity, and traceability (Boskabady et al., 2011). Thus, a comprehensive evaluation of extraction methodologies coupled with detailed GC-MS analysis is essential for understanding the relationships between processing techniques and chemical profiles.

This study aims to assess the volatile composition of commercially available *Rosa damascena* derivatives using GC-MS and to examine how different extraction techniques influence the aromatic and chemical characteristics of rose water, rose oil, rose yeast, concrete, and absolute. The findings are expected to contribute to the standardization, quality assurance, and further industrial application of rose-derived products.

2. MATERIAL AND METHOD

Five samples of each rose product (Water, Yeast, Oil, Concrete, and Absolute) from various brands were randomly selected from retail outlets. These samples were assigned random numbers from 1 to 5 for identification purposes (Table 1).

Table 1. Labeling of commercially available samples

Sample No	Rose Water	Rose Yeast	Rose Oil[a]	Rose Oil[b]	Rose Concrete	Rose Absolute
1	RW-1	RY-1	ROF-1	ROW-1	RC-1	RA-1
2	RW-2	RY-2	ROF-2	ROW-2	RC-2	RA-2
3	RW-3	RY-3	ROF-3	ROW-3	RC-3	RA-3
4	RW-4	RY-4	ROF-4	ROW-4	RC-4	RA-4
5	RW-5	RY-5	ROF-5	ROW-5	RC-5	RA-5

[a]: Flower Oil or First Oil
[b]: Water Oil or Second Oil

Commercially available samples were procured using two primary methods of extraction:

- **Water Distillation**: This method involved the use of both traditional (copper still) and industrial (stainless steel boiler with steam generator) systems for the production of rose oil, yeast, and rose water.
- **Solvent Extraction**: Rose concrete was produced through n-hexane extraction, followed by ethanol-based processing to obtain rose absolute.

2.1. GC–MS Analysis

Analyses were carried out with Thermo Scientific Trace 1300 GC gas chromatograph instrument, Thermo Scientific-ISQ7000 single quadrupole mass spectrometer detector (Thermo Fisher Scientific Inc. Waltham, Massachusetts, USA) system. Chromatographic evaluations were made using Xcalibur software. TraceGOLD TG-624SilMS GC (Thermo Fisher Scientific Inc. Waltham, Massachusetts, USA) column was used as the analytical column for chromatographic separation. The inlet temperature of the instrument was 250 °C. The injection volume was 2 µL. 1/5 split ratio was used. Helium gas was used as the carrier gas and the gas flow was 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 280 °C.

3. RESULTS

3.1. GC-MS Analysis of Rose Waters

GC-MS spectra of rose water samples are given in Figure 3. It is seen that the major peaks in the spectra for phenylethyl alcohol, the active compound of rose water, occur at approximately 16.06 minutes. Nerol, α-citronellol and geraniol compounds representing the oils in it are formed between 23.00-25.50 minutes. Percentage values of rose water samples are given in detail in Table 2.

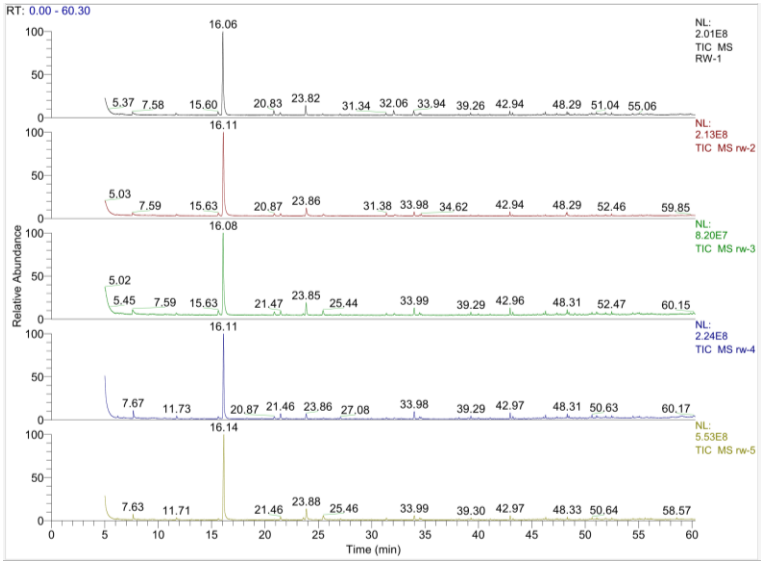


Figure 3. GC-MS spectrum of rose water samples

3.2. GC-MS Analysis of Rose Yeasts

The most obvious feature that distinguishes rose yeast from rose water is its high oil content. The GC-MS spectrum of rose yeast samples is given in Figure 4. When looking at the spectrum, it is seen that especially nerol, α-citronellol and gerainol compounds are significantly higher compared to rose water. Percentage values of the rose yeast samples are given in detail in Table 2.

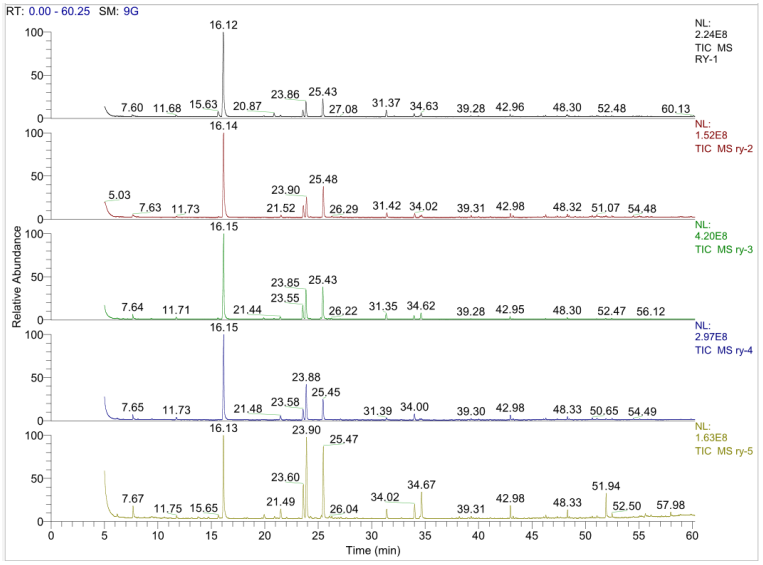


Figure 4. GC-MS spectrum of rose yeast samples

3.3. GC-MS Analysis of Rose Oils

Since flower oil is the oil that comes in the first part, paraffinic compounds are more. GC-MS spectrum of flower oil samples is given in Figure 5. When looking at the spectrum, it is seen that paraffinic compounds are high between 46.00-58.00 minutes. Percentage values of the rose oil (flower oil) samples are given in detail in Table 2.

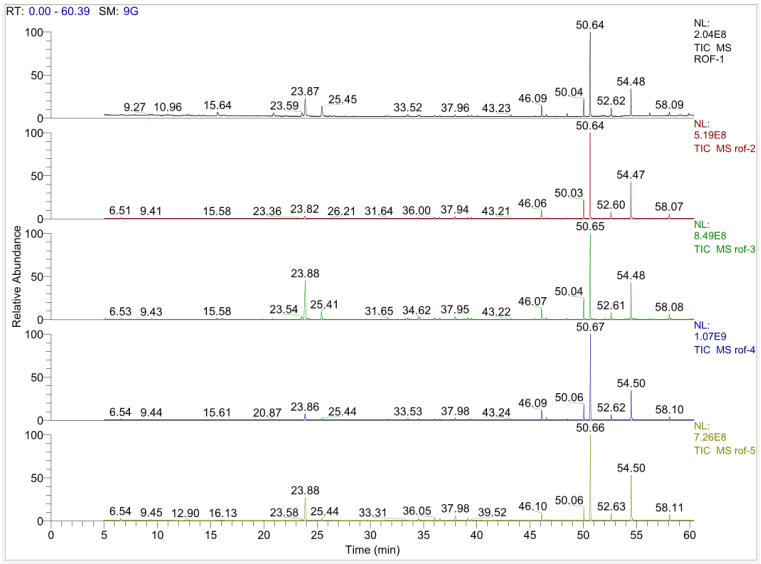


Figure 5. GC-MS spectrum of rose oil (flower oil) samples

Since water oil is the oil obtained by processing it for the second time, paraffinic compounds are lower than flower oil. GC-MS spectrum of water oil samples is given in Figure 6. When looking at the spectrum, it is seen that paraffinic compounds are lower and nerol, α-citronellol and gerainol compounds are higher. Percentage values of the rose oil (water oil) samples are given in detail in Table 2.

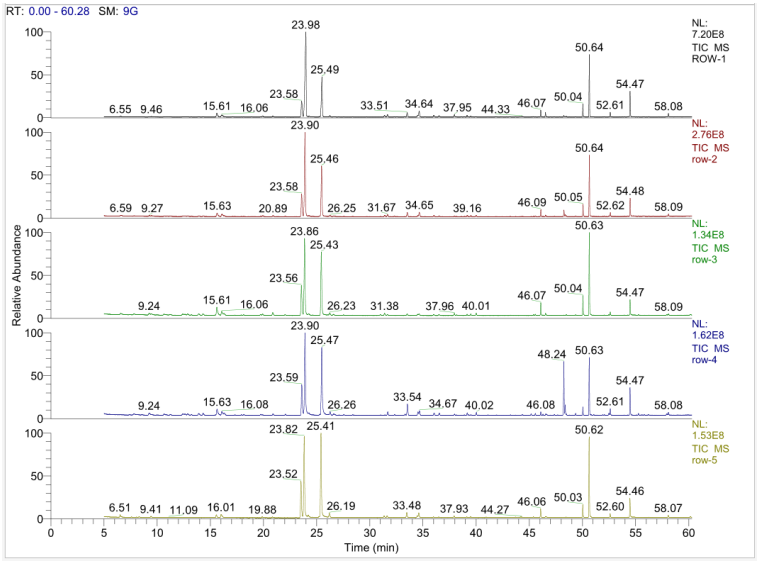


Figure 6. GC-MS spectrum of rose oil (water oil) samples

3.4. GC-MS Analysis of Rose Concretes

Since n-hexane is used as a solvent in obtaining rose concrete, it dissolves paraffinic compounds very well and takes them from the rose. The GC-MS spectrum of rose concrete samples is given in Figure 7. When the spectrum is examined, it is seen that paraffinic compounds are the major peak between 45.00-58.00 minutes. Percentage values of the rose concrete samples are given in detail in Table 2.

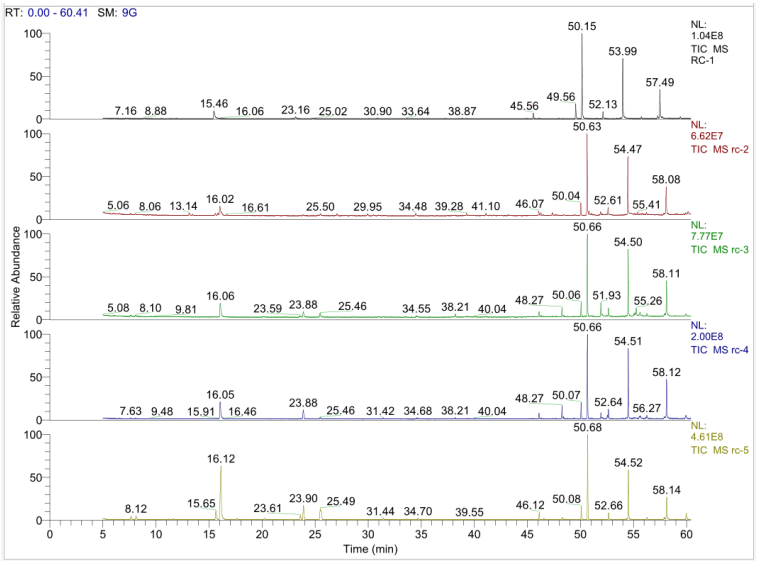


Figure 7. GC-MS spectrum of rose concrete samples

3.5. GC-MS Analysis of Rose Absolutes

Since the rose absolute is obtained by taking ethanol as a second process after n-hexane, the paraffinic compounds coming from the first part are separated here and the volatile structures come to the fore. The GC-MS spectrum of the rose absolute samples is given in Figure 8. When the spectrum is examined, volatile compounds in the form of alcohols such as phenylethyl alcohol, nerol, α-citronellol and gerainol come to the fore with the low amount of paraffinic compounds. Percentage values of the rose absolute samples are given in detail in Table 2.

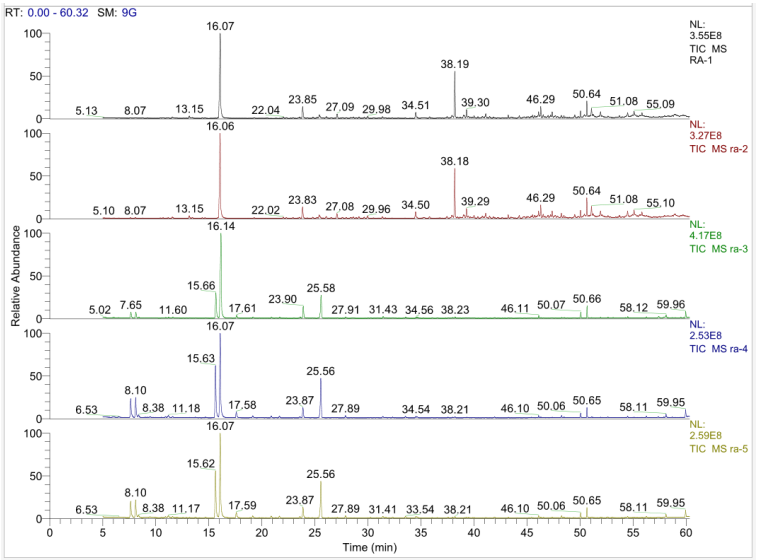


Figure 8. GC-MS spectrum of rose absolute samples

The relative percentage values of GC-MS results of all samples were calculated separately. Then, the average values within each sample group were calculated and the percentage values of significant compounds are given in Table 2.

Table 2. Average of GC-MS relative percentage values (%)

Compounds	Average of RWs	Average of RYs	Average of ROFs	Average of ROWs	Average of RCs	Average of RAs
α-Linalool	1,57	0,74	2,92	1,71	0,95	tr
Phenylethyl alcohol	72,66	51,14	0,77	1,24	3,28	65,76
Nerol	0,64	5,41	2,87	8,67	0,87	1,62
α-Citronellol	3,68	18,31	7,69	32,94	1,46	8,86
Geraniol	1,59	14,29	5,23	19,29	1,10	4,11
Geranyl acetate	0,55	0,74	0,85	2,47	0,23	1,06
trans-Caryophyllene	0,21	0,63	0,97	1,27	tr	0,92

Methyleugenol	0,94	1,88	0,61	1,72	tr	0,56
n-Hexadecane	1,13	0,61	1,38	0,33	2,36	1,52
n-Heptadecane	1,86	0,92	5,97	1,52	2,92	2,37
n-Octadecane	0,94	0,48	2,19	0,51	1,55	1,91
1-Nonadecene	1,78	0,18	9,44	2,77	4,62	1,97
n-Nonadecane	2,57	0,22	28,41	12,79	24,62	2,64
n-Eicosane	0,82	0,18	6,03	0,88	1,49	1,17
n-Heneicosane	1,29	0,13	11,61	4,59	12,78	1,28
n-Docosane	0,47	tr	2,15	0,18	1,72	0,55
n-Tricosane	tr	tr	1,54	0,59	7,23	0,68
n-Pentacosane	tr	tr	1,98	0,11	5,58	tr
n-Heptacosane	tr	tr	3,69	tr	17,19	tr

tr: trace (< 0.1)

4. DISCUSSION AND CONCLUSIONS

While the densest sample in terms of terpene compounds was water oil from rose oils, the product with the highest paraffinic content was rose concrete extracted with n-hexane. It was observed that the oil content and relative percentage values of rose yeast were higher than rose water samples. Rose absolute was close to rose water in terms of the phenyl ethyl alcohol content, while it was observed that it was close to rose oil in terms of terpene values. The methods of obtaining these samples result in differences in the types of volatile components such as citronellol, geraniol, nerol, phenylethyl alcohol and various hydrocarbons.

The two-step distillation process also influenced oil composition. First distillation yielded oils with lower monoterpene alcohol content, resulting in a milder aroma. Second distillation produced oils with higher concentrations of these key aromatic compounds, offering a more intense and refined fragrance.

Rose yeast, a byproduct of distillation, was found to contain residual oil with valuable aromatic compounds, indicating its potential for secondary product recovery. Similarly, the quality of rose water was closely linked to its monoterpene alcohol content, affecting its fragrance strength.

Solvent extraction methods, particularly for producing rose concrete and absolute, preserved a broader range of volatile compounds. Rose absolute, in particular, offered a concentrated and potent aroma suitable for perfumery. However, extraction efficiency was highly dependent on temperature and solvent management, which are critical for preserving aromatic integrity.

In conclusion, both traditional and modern extraction techniques offer clear advantages. Further research can be focused on environmentally friendly extraction methods, quality standardization, and expanding the use of rose-derived products in pharmaceuticals and nutraceuticals.

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

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Some Basic Topological Properties of Graph Non-Adjacency Topological Spaces

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Abstract: Non-adjacency topological spaces have been constructed using a subbasis on simple graphs and were first defined by Lalithambigai and Gnanachandra in 2023. In our study, fundamental properties of non-adjacency topological spaces, such as neighborhood, connectedness, compactness, and separation axioms (T_{0-}, T_{1-}, T_{2-}), have been analyzed in detail with examples provided. The study consists of eight sections, including the conclusion. It has been shown that the non-adjacency topological space is an Alexandroff space. Open and closed sets in the non-adjacency topological space have been defined. The concept of neighborhood has been explained through the open neighborhoods and families of vertices, with their responses to intersection, union, and superset operations supported by examples. It has been proven that the non-adjacency topological space is second countable and separable, and compact when the graph is finite. It has been stated that if the graph is not connected, the topological space is also not connected; however, the connectedness of the topological space of connected graphs may vary. It has been demonstrated with examples that non-adjacency topological spaces of isomorphic graphs are homeomorphic, but the converse is not always true. In conclusion, this article introduces new concepts (neighborhood, isomorphism, connectedness, compactness) in non-adjacency topological spaces and investigates their fundamental properties. The study emphasizes that this new structure is only a starting point, and further research on non-adjacency topological spaces is needed. Future studies may aim to explore the advanced properties and potential applications of this structure.

Keywords: Graphs, non-adjacency topological space, neighborhood, connectivity, compactness

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1. INTRODUCTION

The interdisciplinary relationship between graph theory and topology has attracted significant interest from researchers in recent years.Some researchers have established a topology on the vertex set of graphs and studied their properties. Others have established a topology on the edge set and studied their properties. Graphs are divided into two categories: directed graphs and undirected graphs.We studied undirected simple graphs. Abdulkalek and Kılıcman (Abdulkalek and Kılıcman,2018). defined an incidence topological space on undirected simple graphs and showed that this topology is an Alexandroff space.In response to this, In response to this, Ahmad has also constructed a topology on the vertex set of a graph through a sub-basis, called it the non-incidence topology, and examined its basic properties. Amiri et al. (Amiri et al, 2013) also defined a graphic topo- logical space on undirected simple graphs and showed that this topology is an Alexandroff space. Lalithambigai and Gnanachandra (Gnanachandre and Lalithambigai, 2023) has also con- structed a topology on the vertex set of a graph through a sub-basis and named it the non-adjacency topological space. This is the definition we will use in our study.We have examined the basic properties of this topology. Additionally, we defined nehigborhood, compactness, connectedness, and some separation axioms. We provided examples.

2. PRELIMINARIES

Fundamental definitions and preliminaries of graph theory and topological spaces can be found in (Chartrand and Lesniak,2016), (Dugundjı,1996), (Munkres, 2006)

Definition 2.1: A graph is defined as a binary structure $G = (V, E)$ con- sisting of a finite, non-empty set of points $V = \{(1, 2, \ldots, n)\}$, where the elements are called vertices, and a finite set of edges E, where the elements are called edges. It is denoted as $G = (V, E)$ or G.

Definition 2.2: A topology τ on a set A is a collection of subsets of A, denoted as (A, τ). Each element of the family τ is called an open set. The union of any subsets of τ belongs to τ. The intersection of finite subsets of τ also belongs to τ, and additionally, both the empty set and the set A are elements of τ. If $\tau = P(A)$, then the topology τ is called the discrete topology; if $\tau = \{A, \emptyset\}$, then the topology τ is called the trivial (or indiscrete) topology.

Definition 2.3: (Gnanachandre and Lalithambigai, 2023) Let $G = (V(G), X(X))$ be a graph. For $v \in V(G)$, The neighborhood family N_v is defined as $N_v = \{u \in V(G): uv \in X(G)\}$. The non-neighborhood set NA_v is defined as $NA_v = \{u \in V(G): uv \in X(G)\}$.

Definition 2.4: (Gnanachandre and Lalithambigai, 2023) For every $v \in V(G)$ where $|V(G)| = n$ and $0 \leq d(v) \leq n-2$ The family $S_{NA} = \{NA_v : v \in V(G)\}$ forms a subbase for the topology T_{NA}.The pair $(V(G), T_{NA})$ is called the graph non-adjacency topological spaces.

Throughout the paper, the graph under discussion is the simple undirected graph and without isolated vertices.
Example 2.5:

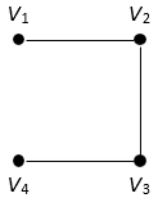


Figure 1: $G = (V, E)$

For the simple graph (G, E), let $V = \{v_1, v_2, v_3, v_4\}$ and the non-adjacency topology; The subbase family is: $S_{Iv_1} = \{v_3, v_4\}, S_{Iv_2} = \{v_4\}, S_{Iv_3} = \{v_1\}, S_{Iv_4} = \{v_1, v_2\}$. Therefore, $S_{IV} = \{\{v_3, v_4\}, \{v_4\}, \{v_1\}, \{v_1, v_2\}\}$ is the subbase family. From the finite intersections of the subbase family elements, we obtain the base family:$\{\emptyset, \{v_3, v_4\}, \{v_4\}, \{v_1\}, \{v_1, v_2\}\}$ Taking any union of the base family elements, we obtain the non-adjacency topology:

$T_{NA} = \{\{\emptyset, \{v_3, v_4\}, \{v_4\}, \{v_1\}, \{v_1, v_2\}, \{v_1, v_2, v_3, v_4\}, \{v_1, v_4\}, \{v_1, v_3, v_4\}, \{v_1, v_3, v_4\}, \{v_1, v_2, v_4\}$

We use notations K_n , $K_{m,n}$, P_n and C_n for a complete graph with n vertices, the complete bipartite graph when partite sets have sizes m and n, the path on n vertices and the cycle on n vertices, respectively.

3. PRELIMINARY RESULTS

In this section, the basic properties of a non-adjacency topological space obtained on a graph have been examined.

Definition 3.1: Let $(V(G), T_{NA})$ be a graph non-adjacency topological sapaces. Each element of the family T_{NA} is called an open set. Therefore, both the empty set $\emptyset$ and the set of all vertices $V(G)$ are open sets.

Definition 3.2: Let $(V(G), T_{NA})$a graph adjacencey topological sapaces. $U(G) \subseteq V(G)$, if $V(G) - U(G)$ is an open set, then $U(G)$ is a closed set.

Example 3.3:

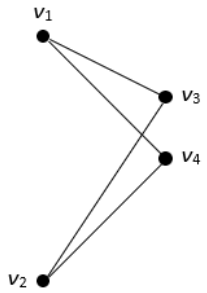


Figure 2: $V = (\mathrm{G}, \mathrm{E})$

For the simple graph (G, E), let $V = \{v_1, v_2, v_3, v_4\}$ and the non-adjacency topology;

T_{NA}=$\{\emptyset, V, \{v_1\}, \{v_2\}, \{v_3\}, \{v_4\}, \{v_1, v_2\}, \{v_1, v_3\}, \{v_1, v_4\}, \{v_2, v_3\}, \{v_2, v_4\}, \{v_3, v_4\}, \{v_1, v_2, v_3\}, \{v_1, v_2, v_4\}, \{v_2, v_3, v_4\}, \{v_1, v_3, v_4\}\}$. Let $U(G) = \{v_1, v_2\}$.

Since $V(G) - U(G) = \{v_3, v_4\} \in T_{NA}$, $U(G)$ is a closed set.

Proposition3.4: Let $G = (V, E)$ be a graph without isolated vertices. The set $S = \{T_{NA_v} : v \in V\}$is a subbase for the non-adjacency topological space on E.

Proof: We need to show that $V \subseteq \bigcup_{v \in V} S_{NA}$. For any $v \in V$, there exists $u \in V$ such that $uv \notin E$. Thus, $u \in S_{NA}$ Therefore, $u \in \bigcup_{v \in V} S_{NA}$.

Definition 3.5: Let $G = (V(G), X(G))$ be a graph and let its non- adjacency topological space be $(V(G), T_{NA})$. Let W be a vertex-induced subgraph of G. In this case, the closure of $V(W)$ is defined as;

$$Cl(V(W)) = V(W) \cup \{v \in V(G) : NA_v \cap V(W) \neq \emptyset\}$$

and the interior of $V(W)$ is defined as;

$$Int(V(W)) = \{v \in V(G) : NA_v \subseteq V(W)\}$$

4. IN NON-ADJACENCY TOPOLOGICAL SPACES NEIGHBORHOOD

Definition 4.1: Let $(V(G), T_{NA})$ be the non-adjacency topological space of a graph, and let v be a vertex in $V(G)$. Every subset $A \subset V(G)$ that contains the vertex v is called the open neighborhood of the vertex v.

Example 4.2:

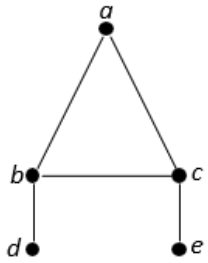


Figure 3: $V = (G, E)$

For the simple graph $V = (G, E)$, let $V = \{a, b, c, d, e\}$ and the non-adjacency topology;

$T_{NA} = \{\emptyset, \{a\}, \{d\}, \{e\}, \{a, d\}, \{a, e\}, \{d, e\}, \{a, c, e\}, \{a, b, d\}, \{a, d, e\}, \{a, b, d, e\}, \{a, c, d, e\}, \{a, b, c, d, e\}\}$.

The neighborhoods of the vertex a are: $V, \{a\}, \{a, e\}, \{a, d\}, \{a, b, d\}, \{a, d, e\}, \{a, c, e\}, \{a, b, d, e\}, \{a, c, d, e\}$

Definition 4.3: Let $(V(G), T_{NA})$ be the non-adjacency topological space of a graph. Every subset $W \subset V(G)$ that contains an open neighborhood of the vertex v is called a neighborhood of the set v. We will denote the family of all neighborhoods of the vertex v by $\mathcal{N}(v)$ and refer to this as the family of neighborhoods of vertex v.

Example 4.4: According to Example 4.2., let's find the family of neighborhoods $\mathcal{N}(b)$ for the vertex b. The open neighborhoods of the vertex $\mathcal{N}(b)$ are $V, \{a, b, d\}, \{a, b, d, e\}$. $\mathcal{N}(b)$ $\{V, \{a, b, d\}, \{a, b, d, e\}, \{a, b, d, c\}\}$

Theorem 4.5: Let $(V(G), T_{NA})$ be the non-adjacency topological space of a graph and let $v \in V(G)$ be a vertex. The family of neighborhoods $\mathcal{N}(v)$ of the vertex v has the following properties:

1. For every neighborhood $W \in \mathcal{N}(v), v \in W$.
2. The intersection of any finite number of neighborhoods of the vertex v is a neighborhood of v.
3. Any superset of a set belonging to the family $\mathcal{N}(v)$ is also in $\mathcal{N}(v)$.
4. For every $W \in \mathcal{N}(v)$, there exists an $A \in \mathcal{N}(v)$ such that for every $w \in A, W \in \mathcal{N}(w)$.

Proof:

1. By definition, for every neighborhood $W \in \mathcal{N}(v)$, there exists $T \subset V$ such that $v \in$ W.
2. Given a finite number of neighborhoods $W_1, W_2, \ldots, W_n \in \mathcal{N}$ (v). Since $W_1 \in \mathcal{N}$ (v), by definition, there exists $A_1 \in T_{NA}$ such that $v \in A_1 \subset W_1$. Since $W_1 \in N(v)$ by definition, there exists $A_2 \in T_{NA}$ such that $v \in A_2 \subset W_2$.

Since $W_n \in \mathcal{N}(v)$ by definition, there exists $A_n \in T_{NA}$ such that $v \in A_n \subset W_n$.From the intersection operation, we obtain:

$$v \in A_1 \cap A_2 \cap \ldots \cap A_n \subset \bigcap_{n=1}^{n} W_i$$

$i = 1$ Since $A_1 \cap A_2 \cap \ldots \cap A_n \in T_{NA}$, by definition:

$$\bigcap_{n=1}^{n} W_i \in \mathcal{N}(v)$$

3. Given a neighborhood $W \in \mathcal{N}(v)$, if $W \subset U \subset V$, then $U \in \mathcal{N}(v)$Therefore, any set that contains any neighborhood of vertex v is itself a neighborhood of vertex v.
4. Given a neighborhood $W \in \mathcal{N}(v)$ there exists by definition an $A \in \mathcal{N}(v)$such that $v \in A \subset W$. Since the set A is open, for any point $w \in W$, A is a neighborhood of w. Because $w \in A \subset W$ and A is an open set, we obtain $W \in \mathcal{N}(w)$.

Example 4.6:

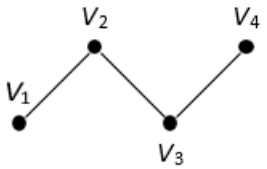


Figure 4: $V = (G, E)$

For the simple graph $V = (G, E)$, let $V = \{v_1, v_2, v_3, v_4\}$ and the non-adjacency topology;

$T_{NA} = \{\{\emptyset, \{v_3, v_4\}, \{v_4\}, \{v_1\}, \{v_1, v_2\}, \{v_1, v_2, v_3, v_4\}, \{v_1, v_4\}, \{v_1, v_3, v_4\}, \{v_1, v_3, v_4\}, \{v_1, v_2, v_4\}$

of a graph, let's find the family of neighborhoods for the vertex v_1;

$\mathcal{N}(v_1) = \{V, \{v_1\}, \{v_1, v_2\}, \{v_1, v_4\}, \{v_1, v_4\}, \{v_1, v_2, v_3\}, \{v_1, v_2, v_4\}, \{v_1, v_3, v_4\}\}$.

Among these neighborhoods, the following are elements of T_{NA}; $\{v_1\}, \{v_1, v_2\}, \{v_1, v_4\}, \{v_1, v_4\}, \{v_1, v_3, v_4\}, \{v_1, v_2, v_4\} \in T_{NA}$

Proposition 4.7: Let $(V(G), T_{NA_1})$ and $(V(G), T_{NA_2})$ be non-adjacency topological spaces. The necessary and sufficient condition for $T_{NA_1} = T_{NA_2}$ is that the neighborhoods of every vertex $u \in V(G)$ are the same in both topologies.

Proof:

⇒: Let $T_{NA_1} = T_{NA_2}$. For any $u \in V(G)$, there exists some $U \in T_{NA_1}$ such that $u \in U$. Since $T_{NA_1} = T_{NA_2}$, it follows that $U \in T_{NA_2}$. By definition, for any $v \in V(G)$, there is a unique neighborhood $N \subset V(G)$ such that $v \in U \subset N$.

⇐: For every $v \in V(G)$, there exist sets $M \in \mathcal{N}(v)$ and $N \in \mathcal{N}(v)$ such that $v \in G \subset M$ and $v \in H \subset N$ where $G \in T_{NA_1}$ and $H \in T_{NA_2}$. Then, there is a set L that contains both M and N .
$v \in G \subset M \subset L$ and $v \in H \subset N \subset L$
Thus, we obtain $T_{NA_1} = T_{NA_2}$

Example 4.8: Let $(V(G), T_{NA})$ be the non-adjacency topological space of a graph and let $v \in V(G)$. For neighborhoods $V_1, V_2 \in \mathcal{N}(v)$, the union $V_1 \cup V_2$ is also a neighborhood in $\mathcal{N}(v)$, By definition, there exist $W_1 \in T_{NA}$ such that $v \in W_1 \subset V_1$and $W_2 \in T_{NA}$ such that $v \in W_2 \subset V_2$. From the union operation, there exist W_1, $W_2 \in T_{NA}$ such that $v \in W_1 \cup W_2 \subset V_1 \cup V_2$. Since $V_1 \cup V_2 \in T_{NA}$,by definition, $V_1 \cup V_2 \in \mathcal{N}(v)$.

Proposition 4.9: Let $(V(G), T_{NA})$ be a non-adjacency topological space. The space $(V(G), T_{NA})$ is an Alexandroff space.

Proof: To show that $(V(G), T_{NA})$ is an Alexandroff space, it suffices to demonstrate that any intersection of base elements of the space, denoted by S_{NA} is open. Given $S \subseteq V$, for any $u \in S$, if $v \in u \in S$, if $\bigcap_{u \in S} A_u$ then $v \in A_u$. Thus, $u \in A_v$ and for each $u \in S$, $S \subseteq A_v$. Since G is finite, A_v and S are finite sets. If S is infinite, $\bigcap_{u \in S} A_u = \emptyset$. If S is finite, then the intersection of finitely many open sets is open. Let $G = (V(G), X(G))$ be a graph without isolated vertices. From the proposition, for each $v \in V$, the intersection of all open sets containing v is the smallest open set containing v. Let's denote this as V_v. The family $V_G = \{V_v : v \in V\}$ ...(*) will be a minimal base for $(V(G), T_{NA})$.

Definition 4.10: If the non-adjacency topological space $(V(G), T_{NA})$ has a countable dense subset, this space is called a separable space.

Definition 4.11: If the non-adjacency topological space $(V(G), T_{NA})$ has a countable base, this space is called a second-countable space.

Example 4.12: Let $G = (V(G), X(G))$ be a graph where the number of edges and vertices are natural numbers. Therefore, the base of the non- adjacency topological space $(V(G), T_{NA})$ derived from this graph is count- able, making it a second-countable space.

Example 4.13: Since the non-adjacency topological space $(V(G), T_{NA})$ is second-countable, it is separable.

Let $\mathcal{B}$ be a countable base for the non-adjacency topological space T_{NA}. Form the set A by choosing one point from each non-empty set in the family $\mathcal{B}$. The set A is also countable. We will show that $cl(A) = V(G)$. Suppose $cl(A) \neq V(G)$. Then,$V(G) - cl(A) \neq \emptyset$ and $V(G) - cl(A) = int(V(G) - A) \neq \emptyset$. There exists a $v \in int(V(G) - A)$. Since $B \subset T_{NA}$ and there exists $B \in \mathcal{B}$, such that $v \in B \subset V(G) - cl(A)$, then $v \in B \subset int(V(G) - A) \subset V(G) - A$ implies $v \notin A$, which is a contradiction. Therefore, $(V(G), T_{NA})$ is dense everywhere. Since it has a countable dense subset, it is separable.

Definition 4.14: Let $(V(G), T_{NA})$ be a non-adjacency topological space, if for $u, v \in V(G)$ (where $u \neq v$), there exists $A \in \mathcal{N}(u)$ such that $v \notin A$ or there exists $B \in \mathcal{N}(v)$ such that $u \notin B$ then the space $(V(G), T_{NA})$ is called a T_0 −space.

Definition 4.15: Let $(V(G), T_{NA})$ be a non-adjacency topological space, if for any $u, v \in V(G)$ (where $u \neq v$) there exists $A \in \mathcal{N}(u)$ such that $v \notin A$ and there exists $B \in \mathcal{N}(v)$ such that $u \notin B$, then the space $(V(G), T_{NA})$ is called a T_1 −space.

Definition 4.16: Let $(V(G), T_{NA})$ be a non-adjacency topological space, if for any $u, v \in V(G)$ (where $u \neq v$) there exists $A \in \mathcal{N}(u)$ and there exists $B \in \mathcal{N}(v)$ such that $A \cap B = \emptyset$, then the space $(V(G), T_{NA})$ is called a T_2 −space.

Proposition 4.17: Let $(V(G), T_{NA})$ be a non-adjacency topological space. The topological space $(V(G), T_{NA})$ is a T_1 −space if and only if for each pair (x, y) of distinct points of V, it is satisfied that $y \notin \mathcal{N}(x)$ and $\notin \mathcal{N}(y)$.

Proof: Suppose that $(V(G), T_{NA})$is a T_1 −space. Let $x, y \in V$. Then there exist open sets U_1 and U_2 such that $x \in U_1$, $y \notin U_1$ and $y \in U_2$, $y \notin U_2$. Since $\mathcal{N}(x) \in T_{NA}$, it is seen that $x \in \mathcal{N}(x) \subseteq U_1$. Similarly, it is seen that $y \in \mathcal{N}(y) \subseteq U_2$. Accordingly, we obtain that $y \notin \mathcal{N}(x)$ and $x \notin \mathcal{N}(y)$. Conversely, suppose $y \notin \mathcal{N}(x)$ and $x \notin \mathcal{N}(y)$, for each pair $x, y \in V$. Then there exist open sets $\mathcal{N}(x)$ and $\mathcal{N}(y)$such that $x \in \mathcal{N}(x)$, $y \notin \mathcal{N}(x)$ and $y \in \mathcal{N}(y)$, $x \notin \mathcal{N}(y)$. Thus, $(V(G), T_{NA})$is a T_1 −space.

Proposition 4.18: Let $(V(G), T_{NA})$be a non-adjacency topological space. The topological space $(V(G), T_{NA})$ is a T_1 −space if and only if for each pair (x, y) of distinct points of V, it is satisfied that $y \notin \mathcal{N}(x)$ and $x \notin \mathcal{N}(y)$.

Proof: Suppose that $(V(G), T_A)$ is a T_1 -space. Let $x, y \in V$. Then there exists open sets U_1, and U_2 such that $x \in U_1$, $y \notin U_1$, and $y \in U_2$, $x \notin U_2$. Since $\mathcal{N}(x) \in T_{NA}$. it is seen that $x \in \mathcal{N}(x) \subseteq U_1$, Similarly, it is seen that $y \in \mathcal{N}(y) \subseteq U_2$. Accordingly, we obtain that $y \notin \mathcal{N}(x)$ and $x \notin \mathcal{N}(y)$. Conversely, suppose $y \notin \mathcal{N}(x)$, and $x \notin \mathcal{N}(y)$, for each pair $x, y \in V$. Then there exists open sets $\mathcal{N}(x)$ and $\mathcal{N}(y)$ such that $x \in \mathcal{N}(x)$, $y \notin \mathcal{N}(x)$, and $y \in \mathcal{N}(y)$, $x \notin \mathcal{N}(y)$. Thus, $(V(G), T_A)$ is a T_1 -space.

Proposition 4.19: Let $G = (V(G), X(G))$ be a graph without isolated vertices and $(V(G), T_A)$ be its non-adjacency topological space. The topological space $(V(G), T_A)$ is a Hausdorff space if and only if $\mathcal{N}(x) \cap \mathcal{N}(y) = \emptyset$, for each pair (x, y) of distinct points of V .

Proof: Let $(V(G), T_{NA})$ be a Hausdorff space and $x, y \in V$. Then there exists open sets U_1 and U_2 such that $x \in U_1$, $y \in U_2$, and $U_1 \cap U_2 = \emptyset$, for each pair of (x, y) of distinct points of V . Since $\mathcal{N}(x) \in T_A$, it is seen that $x \in \mathcal{N}(x) \subseteq U_1$. Similarly, it is seen that $y \in \mathcal{N}(y)$, $\subseteq U_2$. Accordingly, we obtain that $\mathcal{N}(x) \cap \mathcal{N}(y) = \emptyset$ .Conversely, suppose $\mathcal{N}(x) \cap \mathcal{N}(y) = \emptyset$, for each pair (x, y) of distinct points of V . Then, since there exists open sets $\mathcal{N}(x)$ such that $x \in \mathcal{N}(x)$, $y \in \mathcal{N}(y)$, and $\mathcal{N}(x) \cap \mathcal{N}(y) = \emptyset$, $(V(G), T_{NA})$ is a Hausdorff space

Remark 4.20:

1. If the non-adjacency topological space $(V(G), T_{NA})$ is a T_2-space, then it is a T_1-space. However, the converse is not true.
2. If the non-adjacency topological space $(V(G), T_{NA})$ is a T_1-space, then it is a T_0-space. However, the converse is not true.

Example 4.21: According to Example 3.3.,

$T_{NA}=\{\emptyset, V, \{v_1\}, \{v_2\}, \{v_3\}, \{v_4\}, \{v_1, v_2\}, \{v_1, v_3\}, \{v_1, v_4\}, \{v_2, v_3\}, \{v_2, v_4\}, \{v_3, v_4\}, \{v_1, v_2, v_3\}, \{v_1, v_2, v_4\}, \{v_2, v_3, v_4\}$ $\{v_1, v_3, v_4\}\}$.

T_{NA} non-adjacency topological space is T_2 −space. It is also a T_1 −space, and therefore a T_0 −space.

Example 4.21:

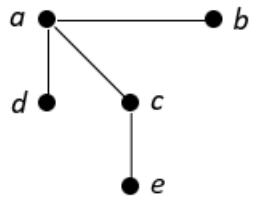


Figure 5: $G = (V, E)$

$G = (V, E)$ be a simple graph with the vertex set $V(G) = \{a, b, c, d, e\}$.

$T_{NA} = \{\emptyset, V, \{e\}, \{d\}, \{b, d\}, \{e, d\}, \{c, d, e\}, \{b, d, e\}, \{a, b, d\}, \{a, b, d, e\}, \{c, d, b, e\}\}$.

T_{NA} non-adjacency topological space is a T_0 −space but it is neither a T_1 −space nor a T_2 −space.

5. IN NON-ADJACENCY TOPOLOGICAL SPACES ISOMORPHISM

Definition 5.1: Let $G_1 = (V_1, E_1)$ and $G_2 = (V_2, E_2)$ be two graphs. If there exists a injective transformation between the vertices and edges of G_1 and G_2that preserves their properties, then G_1 and G_2 are called isomorphic graphs, and this is denoted as $G_1 \cong G_2$**.** If $f : V_1 \to V_2$ is a graph isomorphism, then:f is injective and surjective. For all $a, b \in V_1$ such that $\{a, b\} \in E_1$, then $\{f(a), f(b)\} \in E_2$.

Definition 5.2: Let (V_1, τ_1) and (V_2, τ_2)be two topological spaces. For a function $f : V_1 \to V_2$, if for every $U \in \tau_2$, $f^{-1}(U) \in \tau_1$, then the function f is called continuous. If the function f is bijective, f is continuous, and f^{-1} is also continuous, then $V_1$and V_2 are said to be homeomorphic.

Proposition 5.3: If $G_1 = (V_1, E_1)$ and $G_2 = (V_2, E_2)$ are isomorphic graphs, then the non-adjacency topological spaces (G_1, T_{NA_1}) and (G_2, T_{NA_2}) are homeomorphic.

Example 5.4:

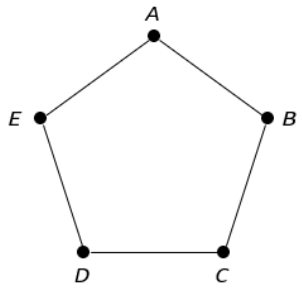


Figure 6 : $G_1 = (V_1, E_1)$

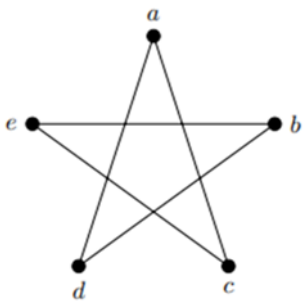


Figure 7: $G_2 = (V_2, E_2)$

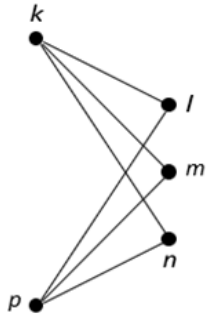
The graphs $G_1 = (V_1, E_1)$ and $G_2 = (V_2, E_2)$ given in the figures are isomorphic. Let's find the non-adjacency topological spaces of these graphs. Given $V_1 = \{A, B, C, D, E\}$, the non-adjacency topological space T_{NA_1} is defined as:

$T_{NA_1} = \{\emptyset, V1, \{A\}, \{B\}, \{C\}, \{D\}, \{E\}, \{A, B\}, \{A, C\}, \{A, D\}, \{A, E\}, \{B, C\}, \{B, D\}, \{B, E\}, \{C, D\}, \{C, E\}, \{D, E\}, \{A, B, C\}, \{A, B, D\}, \{A, B, E\}, \{A, C, D\}, \{A, C, E\}, \{A, D, E\}, \{B, C, D\}, \{B, C, E\}, \{B, D, E\}, \{C, D, E\}, \{A, B, C, D\}, \{A, B, C, E\}, \{A, B, D, E\}, \{A, C, D, E\}, \{B, C, D, E\}\}$

Let $V_2 = \{a, b, c, d, e\}$, then the non-adjacency topological space T_{NA_2} is defined as:
$T_{NA_2} = \{\emptyset, V_2, \{a\}, \{b\}, \{c\}, \{d\}, \{e\}, \{a, b\}, \{a, c\}, \{a, d\}, \{a, e\}, \{b, c\}, \{b, d\}, \{b, e\}, \{c, d\}, \{c, e\}, \{d, e\}, \{a, b, c\}, \{a, b, d\}, \{a, b, e\}, \{a, c, d\}, \{a, c, e\}, \{a, d, e\}, \{b, c, d\}, \{b, c, e\}, \{b, d, e\}, \{c, d, e\}, \{a, b, c, d\}, \{a, b, c, e\}, \{a, b, d, e\}, \{a, c, d, e\}, \{b, c, d, e\}\}$

The topologies T_{NA_1} and T_{NA_2} are discrete topologies and are homeomorphic.

Example 5.5:



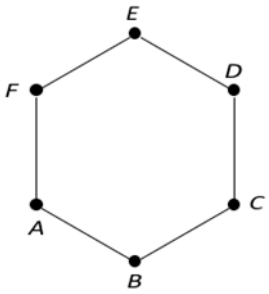


Figure 8: $G_1 = (V_1, E_1)$ **Figure 9:** $G_2 = (V_2, E_2)$

Let $G_1 = (V_1, E_1)$ and $G_2 = (V_2, E_2)$ be as given in the figures. For $V_1 = \{k, l, m, n, p\}$, the non-adjacency topological space T_{NA_1} is defined as:

$T_{NA_1} = \{\emptyset, V_1, \{k\}, \{l\}, \{m\}, \{n\}, \{p\}, \{k, l\}, \{k, m\}, \{k, n\}, \{k, p\}, \{l, m\}, \{l, n\}, \{l, p\}, \{m, n\}, \{m, p\}, \{n, p\}, \{k, l, m\}, \{k, l, n\}, \{k, l, p\}, \{k, m, n\}, \{k, m, p\}, \{k, n, p\}, \{l, m, n\}, \{l, m, p\}, \{l, n, p\}, \{m, n, p\}, \{k, l, m, n\}, \{k, l, m, p\}, \{k, l, n, p\}, \{k, m, n, p\}, \{l, m, n, p\}\}$.

For $V_2 = \{A, B, C, D, E, F\}$, the non-adjacency topological space T_{NA_2} is defined as:

$T_{NA_2} = \{\emptyset, V_2, \{A\}, \{B\}, \{C\}, \{D\}, \{E\}, \{F\}, \{A, B\}, \{A, C\}, \{A, D\}, \{A, E\}, \{A, F\}, \{B, C\}, \{B, D\}, \{B, E\}, \{B, F\}, \{C, D\}, \{C, E\}, \{C, F\}, \{D, E\}, \{D, F\}, \{E, F\}, \{A, B, C\}, \{A, B, D\}, \{A, B, E\}, \{A, B, F\}, \{A, C, D\}, \{A, C, E\}, \{A, C, F\}, \{A, D, E\}, \{A, D, F\}, \{A, E, F\}, \{B, C, D\}, \{B, C, E\}, \{B, C, F\}, \{B, D, E\}, \{B, D, F\}, \{B, E, F\}, \{C, D, E\}, \{C, D, F\}, \{C, E, F\}, \{D, E, F\}, \{A, B, C, D\}, \{A, B, C, E\}, \{A, B, C, F\}, \{A, B, D, E\}, \{A, B, D, F\}, \{A, B, E, F\}, \{A, C, D, E\}, \{A, C, D, F\}, \{A, C, E, F\}, \{A, D, E, F\}, \{B, C, D, E\}, \{B, C, D, F\}, \{B, C, E, F\}, \{B, D, E, F\}, \{C, D, E, F\}, \{A, B, C, D, E\}, \{A, B, C, D, F\}, \{A, B, C, E, F\}, \{A, B, D, E, F\}, \{A, C, D, E, F\}, \{B, C, D, E, F\}\}$.

T_{NA_1} and T_{NA_2} non-adjacency topological space are discrete topological spaces and are homeomorphic, but the graphs $G_1 = (V_1, E_1)$ and $G_2 = (V_2, E_2)$ are not isomorphic.

6. IN NON-ADJACENCY TOPOLOGICAL SPACES CONNECTEDNESS

Definition 6.1: Let $(V(G), T_{NA})$ be a non-adjacency topological space. If $V(G) = U \cup V$ where $U \cap V = \emptyset$, then the space $(V(G), T_{NA})$ is called a disconnected space.

Definition 6.2: Let $(V(G), T_{NA})$ be a non-adjacency topological space. If $V(G) = U \cup V$ where $U \cap V \neq \emptyset$, then the space $(V(G), T_{NA})$ is called a connected space.

Definition 6.3: In a graph $G = (V(G), X(G))$, if there is a path be- tween any two vertices $x, y \in V$, then the graph is called a connected graph.
Remark 6.4: If the graph $G = (V(G), X(G))$ is not connected, then the non-adjacency topological space $(V(G), T_{NA})$ is not connected. On the other hand, if the graph $G = (V(G), X(G))$ is connected, the non-adjacency topological space $(V(G), T_{NA})$ may be either connected or not connected.

Example 6.5:

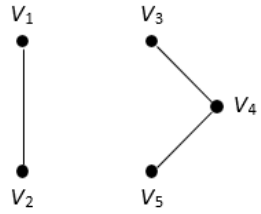


Figure 10:$G = (V, E)$

For the simple graph $V = (G, E)$, let $V = \{v_1, v_2, v_3, v_4, v_5\}$ and the non-adjacency topology;

$T_{NA} = \{\emptyset, V, \{v_3\}, \{v_5\}, \{v_1, v_2\}, \{v_3, v_5\}, \{v_1, v_2, v_3\}, \{v_1, v_2, v_5\}, \{v_1, v_2, v_3, v_5\}\}$

$U = \{v_1, v_2\}$ and $V = \{v_3, v_4, v_5\}$. If $U \cap V = \emptyset$ and $U \cup V = V(G)$,then the space $(V(G), T_{NA})$ is a disconnected space.

Example 6.6:

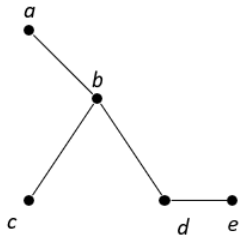


Figure 11: $V = (G, E)$

For the simple graph $V = (G, E)$, let V = {a, b, c, d, e} and the non-adjacency topology*;*

T_{NA}= $\{\emptyset, V, \{a\}, \{c\}, \{e\}, \{a, c\}, \{a, e\}, \{c, e\}, \{a, c, e\}, \{c, d, e\}, \{a, b, c\}, \{a, b, e\}, \{a, c, d, e\}, \{a, b, c,$ e$\}\}$

$U = \{a, c, d, e\}$ and $V = \{a, b, e\}$. Since $U \cap V = \{a, e\} \neq \emptyset$ and $U \cup V = V(G)$, the space $(V(G), T_{NA})$ is considered connected.

Example 6.7: If $(V(G), T_{NA})$ is discrete, the space is disconnected. Let $U = \{v_1\}$ and $V = \{v_2, v_3, \ldots\}$. If $U \cap V = \emptyset$ and $U \cup V = V(G)$, then the space $(V(G), T_{NA})$ is a disconnected space.

Example 6.8:

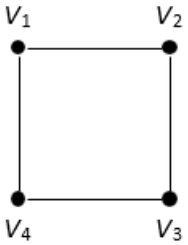


Figure 12: $G = (V, E)$

For the simple graph $V = (G, E)$, let $V = \{v_1, v_2, v_3, v_4, v_5\}$ and the non-adjacency topology*;*

T_{NA}=$\{\emptyset, V, \{v_1\}\{v_2\}\{v_3\}, \{v_4\}, \{v_1, v_2\}, \{v_1, v_3\}, \{v_1, v_4\}, \{v_2, v_3\}, \{v_2, v_4\}, \{v_3, v_4\}, \{v_1, v_2, v_3\}, \{v_1, v_2, v_4\}, \{v_2, v_3, v_4\}$ $\{v_1, v_3, v_4\}\}$.

Let $U = \{v_1\}$ and $V = \{v_2, v_3, v_4\}$. If $U \cap V = \emptyset$ and $U \cup V = V(G)$, then $(V(G), T_{NA})$ is disconnected. The non-adjacency topological space $(V(G), T_{NA})$of the graph is a discrete topology.

Conclusion 6.9: The graphs generated by the cycle graph C_n (where $n \geq 4$) and the complete bipartite graphs $K_{m,n}$ (where $m, n \geq 2$) are disconnected. This happens because the non-adjacency topological spaces obtained from them form a discrete topology.

7. IN NON-ADJACENCY TOPOLOGICAL SPACES COMPACTNESS

Definition 7.1: Let $(V(G), T_{NA})$ be a non-adjacency topological space. Let $(V_i)_{i\in I}$ be a family of subsets of the set $V(G)$. If $V(G) = \bigcup_{i\in I} V_i$then the family $(V_i)_{i\in I}$ is called a cover of the set $V(G)$. If $J \subset I$ is finite, then the cover $(V_i)_{i\in I}$ of the set $V(G)$ is called a finite cover of $V(G)$.

Definition 7.2: If every open cover of the set $V(G)$ in the space $(V(G), T_{NA})$ has a finite subcover, then the space $(V(G), T_{NA})$is called a compact space.

Proposition 7.3: Given that $G = (V(G), X(G))$ is a graph, the necessary and sufficient condition for the space $(V(G), T_{NA})$to be compact is that the set $V(G)$ is finite.

Proof:

⇒: Let $(V(G), T_{NA})$be compact. Given the minimal base denoted by $(*)$. $V_G = \{V_v : v \in V\}$ is an open cover of $V(G)$. Let U be a finite cover of $V(G)$. Since $V(G)$ has a minimal base, U must be V_G. From this, it follows that $V(G)$ is finite.

⇐: If V is finite, then every open cover has a finite subcover. This means that $(V(G), T_{NA})$ is compact.

Proposition 7.4: If V is finite, then every open cover has a finite subcover. This means that $(V(G), T_{NA})$ is compact.

Proof: Let $(V(G), T_{NA})$ be a space with a countable base $B = \{V_n : n \in N\}$. Let $(V_i)_{i\in I}$ be a family of open sets covering $V(G)$. In this case, each A_i in this family can be written as a union of sets from the base B. Since every countable open cover has a finite subcover, there exists a finite family $\{V_j : j = 1, 2, \ldots, n\}$ from B that covers $V(G)$. For each V_j, there are open sets U_j such that $V_j \subset U_j$. Thus, $\bigcup_{j=1}^{n} U_j = V(G)$ making $(V(G), T_{NA})$ a compact space.

Proposition 7.5: Every closed subset of the compact non-adjacency topological space $(V(G), T_{NA})$ is compact.

Proof: Let U be a closed subset of the space $(V(G), T_{NA})$. Let $(U_i)_{i\in I}$be a family of open subsets of $V(G)$ that form an open cover of U . Since U is closed, $V(G) - U$ is open. Thus, $V(G) = U \cup (V(G) - U)$. Given that $U = \bigcup_{i\in I} U_i$, the family $\{V(G) - U_i : i \in I\}$ forms an open cover of $V(G)$. Since $(V(G), T_{NA})$. is a compact space, this open cover has a finite subcover.

That is, the family $\{V(G) - U_j, : j = 1, 2, \ldots, n\ finite\}$ is a finite cover of $V(G)$. Therefore, $\{U_1, U_2, \ldots, v_n\}$ is also a finite cover of U . Hence, U is compact.

8. CONCLUSION

We have studied some new concepts in non-adjacency topological space such as neihgborhood, isomorphism, connectedness, compactness. Also, we inves- tigate many basic properties of these concepts. In the end, we must say that, this paper is just a beginning of a new structure and we have studied a few ideas only. Hence we expect that some research will be actively working on non-adjacency topological space.

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Cultural Branding and Cross-Industry Synergy: A Marketing Impact Analysis of the Influencer Marketing in Sport (Case Study FC Barcelona x Travis Scott Collaboration)

ADA GASHI[1*]

Abstract: Sports marketing has undergone significant changes during the last few years because of the growing participation of influencers and public figures in advertising campaigns and brand collaborations. Studying sports clubs teaming up with influential people from sport, art, music backgrounds who possess strong social influence power is a highly interesting field of research. The phenomenon has attracted significant attention from both sports marketing researchers and academic readers who study this field. This research paper examines the strategic partnership between FC Barcelona and rapper Travis Scott through their joint release of a restricted-edition jersey in 2025. I chose a mixed methods which combines quantitative and qualitative methods to study the effects on brand co-creation and cultural branding as well as fan engagement and influencer marketing and merchandising. The research combines quantitative data points such as sales numbers and engagement statistics with qualitative information including media coverage and sentiment analysis to demonstrate that the partnership generated substantial commercial and brand equity outcomes. The case serves as a reference point for modern sports marketing strategies that combine different industries.

Keywords: Sports marketing, cultural branding, influencer marketing, fan engagement, merchandising, co-creation.

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1. INTRODUCTION

Global sports brands are increasingly venturing into cultural domains, merging sports with music, fashion, and digital experiences. In May 2025, FC Barcelona collaborated with American rapper Travis Scott to unveil a "Cactus Jack" branded jersey during El Clásico. This study explores the strategic motives and marketing outcomes of this initiative, positioning it as a leading example of cultural branding in sports. Key questions include: How did this partnership influence brand identity and consumer perception? What were the measurable effects on sales and engagement? What lessons does it offer for future sports marketing campaigns?

2. LITERATURE REVIEW

Brand co-creation now represents a sophisticated multi-participant system where consumers engage actively to determine brand value instead of receiving brand messaging passively. Prahalad and Ramaswamy's (2004) initial ideas about co-creation have developed through Brodie et al.'s (2024) contemporary research which examines both direct and enabling brand co-creation performances. Social listening forms part of these actions which also include narrative development and platform interaction facilitation. Digital engagement tools together with platforms have become key facilitators for value co-creation in sports environments (Filieri et al., 2023). Virtual platforms help sports organizations create fan participation opportunities which leads to improved experiential value alongside stronger brand loyalty (Kabadayi et al., 2024, p. 9). These platforms allow immediate social interaction and feedback while enabling users to create content that integrates into official brand stories. Sports organizations use cultural branding to develop symbols which connect with societal identities and myths according to Holt (2004). Sports organizations employ cultural branding strategies more deliberately these days to match cultural developments which focus on gender equality alongside sustainability and community pride (Zhang, Lu, & O'Neill, 2022). This strategic alignment works best to engage both Gen Z consumers and multicultural audiences. Major League Soccer has adopted a repositioning approach which unites sport with urban culture and entertainment to build a diverse fan base (Post, 2025). The FIFA World Cup and Olympic Games have evolved into platforms where sponsors utilize them to link brand messages with cultural values (Dept Agency, 2024). Sports branding strategies heavily depend on influencer marketing as a vital component today. Research from Freberg et al. (2011) initially showed how influencers affect brand perception yet current studies focus on understanding platform-specific behavior and authenticity in their work. Athlete influencers have revolutionized sports endorsement practices through Name Image and Likeness (NIL) programs which have become widespread in college athletics (Wong & Frederick, 2023). Research shows that 86% of American marketers plan to use influencer marketing as part of their strategies during 2025 according to Sprout Social (2025). Sports branding strategies become more precise through the implementation of AI tools which help choose influencers while segmenting audiences and measuring content effectiveness (Influencer Marketing Hub, 2025). Sports branding relies heavily on experiential marketing because it helps build emotional connections between brands and their consumers. The accelerating digital transformation has led to enhanced fan engagement through data-

driven approaches across multiple communication channels. Sports companies implement AR/VR immersive technologies together with gamification elements to deliver individualized experiences according to Kamenidou et al. (2021, p. 524). Technology plays an increasingly significant role in sports consumer behavior according to Future Market Insights (2025) which predicts the global fan engagement market will reach $7.24 billion by 2025. Seedtag (2025) shows that brands achieve enhanced relevance and conversion through real-time contextual marketing strategies which enable fan engagement during live events.

3. METHODOLOGY

The research employed a mixed-methods design. The study collected quantitative data through jersey sales records and resale market prices and social media engagement metrics from Instagram and X (Twitter). The research collected qualitative data through media analysis of El País and Spotify and beIN Sports and thematic coding of fan sentiment.

4. RESULTS

Sales and Revenue

3,798 standard jerseys at €399.99 sold out in 30 minutes: €1,519,922

22 signed jerseys at €2,999.99: €65,999

Total revenue: €1,585,921

Secondary Market Impact

Resale platforms like StockX reported jersey prices exceeding $2,000.

Scarcity and hype elevated the product's symbolic and collectible status.

Fan Engagement Metrics

1M+ Instagram likes, 50K+ comments within 24 hours

X (Twitter) sentiment: 92% positive, 5% neutral, 3% negative

Over 100 news articles globally within 48 hours

Exclusive concert held at Sant Adrià drew international attention (El País, 2025)

Comparative Analysis of FC Barcelona’s Influencer Collaborations

FC Barcelona, in partnership with Spotify, has engaged in several high-profile collaborations with global music artists, integrating their branding into the club's jerseys during significant matches, notably El Clásico. These collaborations aim to merge music and football cultures, enhance global reach, and create unique merchandise offerings.

Comparative Table of Key Collaborations

Collaboration	Artist	Date	Jersey Feature	Limited Edition Details	Additional Activations
Travis Scott	Cactus Jack Logo	May 2025	Featured on men's and women's jerseys during El Clásico	1,899 standard jerseys; 22 signed matchday jerseys	Exclusive concert in Barcelona; capsule merchandise collection
Rosalía	MOTOMAMI Logo	March 2023	Featured on men's and women's jerseys during El Clásico	1,899 standard jerseys; 22 glow-in-the-dark signed jerseys	Viral social media campaigns; Cannes Lions Bronze Award
Coldplay	Moon Logo	October 2024	Featured on jerseys during El Clásico	1,899 standard jerseys; 22 signed jerseys	Collaboration with UNHCR; proceeds donated to charity
The Rolling Stones	Tongue and Lips Logo	October 2023	Featured on jerseys during El Clásico	Details not specified	Commemorated band's 60th anniversary
Karol G	Heart Logo	March 2024	Featured on jerseys during El Clásico	Details not specified	Celebrated International Women's Day
Drake	OVO Owl Logo	October 2022	Featured on jerseys during El Clásico	Details not specified	Celebrated 50 billion Spotify streams milestone

Each collaboration is evaluated by merchandise revenue, social media engagement, cultural impact, and fan sentiment.

Merchandise Revenue
Travis Scott: €1.58 million from jersey sales.

Others: Data not publicly disclosed.

Social Media Engagement

Rosalía: 669 million views and 56 million interactions across digital platforms.

Travis Scott: 1M+ Instagram likes, 50K+ comments within 24 hours.

Cultural Impact

Rosalía: Cannes Lions Bronze Award for the MOTOMAMI campaign.

Travis Scott: Exclusive concert in Barcelona; significant media coverage.

Coldplay: Charitable collaboration with UNHCR.

Others: Commemorative or celebratory themes.

Fan Sentiment

Travis Scott: 92% positive sentiment on X (Twitter).

Others: Data not publicly disclosed.

The Travis Scott collaboration stands as the most successful overall based on the analysis of merchandise revenue data and social media engagement and cultural impact and fan sentiment. The collaboration generated the highest merchandise revenue while achieving major social media engagement and creating substantial cultural impact through exclusive events and receiving widespread positive fan sentiment. The Rosalía collaboration achieved outstanding success through its strong performance in social media engagement and cultural impact by winning prestigious awards and gaining deep fan appreciation. The other collaborations helped FC Barcelona achieve brand diversification and cultural integration but specific metrics remain less accessible to the public.

5.CONCLUSION

The collaboration represents multidimensional co-creation, integrating design, narrative, and performance. Fans actively took part in the process through social media platforms and by creating their own content and participating in fan-driven resale activities. The jersey connected Catalan football to Houston hip-hop through its design which represented the fusion of worldwide identities. Through this initiative FC Barcelona transitioned from being a regional sports power to becoming a worldwide cultural brand. Travis Scott participated in the product design process while wearing the jersey during performances and incorporating it into his album visual content. The deep involvement strengthened the authenticity of the product. The product launch achieved success through its controlled scarcity levels and its high visual appeal and embedded storytelling elements. The model demonstrates how clubs can target Gen Z consumers through its implementation. The strategic collaboration between FC Barcelona and international artists enables the club to reach new audiences worldwide. The combination of football and music cultures through cultural integration produces distinctive fan experiences which build stronger brand identity. The company generates additional revenue through limited edition merchandise sales and exclusive events. FC Barcelona demonstrates innovative marketing through their artist collaborations which unite sports with music and fashion. The partnerships have achieved three major goals by increasing worldwide brand recognition and attracting multiple fan groups and producing substantial merchandise income. The club demonstrates how sports marketing can incorporate cultural elements through its successful approach.

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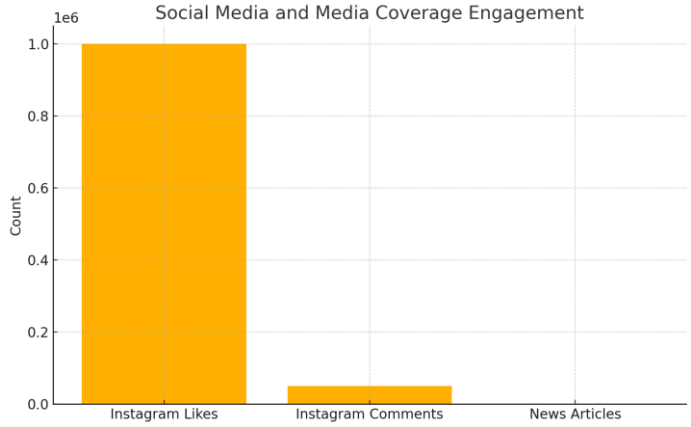
8. Appendices

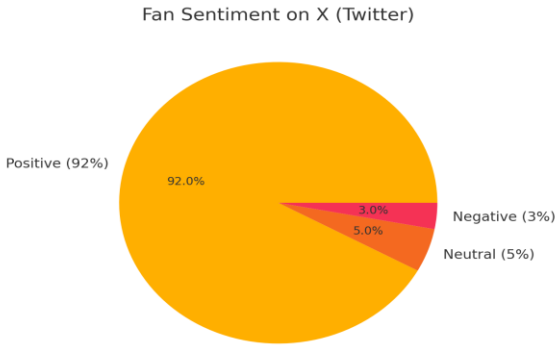
Appendix A: Sales Data Table

Item	Units Sold	Price (€)	Total Revenue (€)
Standard Jersey	3,798	399.99	1,519,922
Signed Jersey	22	2,999.99	65,999
Total	3,820	-	1,585,921

Appendix B: Social Media Engagement. The chart below presents the spike in social media and press engagement metrics following the collaboration announcement.



Appendix C: Sentiment Pie Chart, Fan sentiment on X (Twitter) captured within 24 hours of announcement.


Fan Sentiment on X (Twitter)
Positive (92%)
92.0%
3.0%
Negative (3%)
5.0%
Neutral (5%)

Bioactive Compounds In Cultivated And Wild Pomegranate Paste And Juice

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Abstract: To compare the phytochemical compositions of wild and cultivated pomegranate in the Behshahr region, two types of pomegranates were selected: sour (wild) and sweet (cultivated). A study was conducted to analyze the phytochemical parameters of pomegranate juices and pastes, with three replicates to ensure accurate results. The identification of polyphenolic compounds was performed using HPLC-DAD-ESI-MS. A total of six different compounds were identified, all belonging to the ellagitannin group: Punicalagin I, Punicalagin III, Punicalin, Galloyl-HHDP-glc, Peduncalagin, and HHDP-diglc. In the analysis, Punicalagin I, Punicalagin III, and Punicalin exhibited similar patterns, with the highest concentrations found in the sweet (cultivated) variety, specifically in the pomegranate juice. The highest amount of Galloyl-HHDP-glc was detected in the paste of the sour (wild) pomegranate, followed by the paste from the sweet variety. Trace amounts of this compound were also identified in the juice from the sweet pomegranate. Additionally, Peduncalagin was found in higher quantities in the paste of the sour variety compared to the sweet variety. Significant differences were observed in the levels of HHDP-diglc across the different samples (juice and paste) of both varieties. In both types of pomegranates, the amount of HHDP-diglc was higher in the juice than in the paste; however, no significant differences were noted between the two varieties within the same sample type. Our results underscore the significance of cultivating wild varieties, which also play a crucial role in the local economy.

Keywords: Behshahr, *Punica granatum*, Wild edible plants, Food industry

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1. INTRODUCTION

Pomegranate (*Punica granatum*) has long attracted attention due to its rich content of bioactive compounds, including polyphenols, flavonoids, and anthocyanins. These compounds contribute to the fruit's antioxidant, anti-inflammatory, and antimicrobial properties. Various parts of the pomegranate—such as seeds, peel, juice, and leaves—are rich in these bioactive substances, making the fruit beneficial for health applications such as cardiovascular protection, diabetes management, and cancer prevention (Ranjha et al., 2019).

Wild and cultivated pomegranate varieties show significant differences in morphology and phytochemical composition. Wild pomegranates, often characterized by their sour taste, generally contain higher concentrations of phenolic compounds and antioxidants compared to their sweeter, cultivated counterparts (Tehranifar et al., 2010). These differences are influenced by genetic factors and environmental conditions, which affect the fruits' nutritional and medicinal value (Mousavinejad et al., 2009; Viuda-Martos et al., 2010).

Pomegranates are rich in polyphenol compounds. Ellagitannins, a class of hydrolyzable tannins abundantly found in pomegranates, are well known for their significant health benefits. Compounds such as punicalagin, punicalin, and pedunculagin exhibit strong antioxidant activities and help prevent diseases associated with oxidative stress. These ellagitannins have also been linked to anticancer effects, including the inhibition of prostate cancer cell proliferation (Ranjha et al., 2019).

High-performance liquid chromatography coupled with diode-array detection and electrospray ionization mass spectrometry (HPLC-DAD-ESI-MS) is an advanced analytical method used to identify and quantify phenolic compounds in pomegranate. This technique enables precise profiling of complex mixtures and facilitates the detection of various bioactive substances, including ellagitannins, across different pomegranate varieties (Fischer et al., 2011; Mena et al., 2012).

Pomegranate juice and paste are widely utilized in the food and nutraceutical industries due to their unique flavor and valuable nutritional and medicinal properties. Pomegranate juice is rich in bioactive compounds such as anthocyanins, tannins, phenolic acids, and vitamin C, which play a crucial role in reducing oxidative stress, promoting cardiovascular

health, and alleviating inflammation (Gil et al., 2000; Viuda-Martos et al., 2010). Pomegranate paste, a concentrated form of the juice, contains a higher concentration of phenolic compounds and exhibits greater antioxidant capacity. It is used both as a flavoring agent and a functional ingredient in traditional and modern cuisine (Kamal et al., 2018). Studies have shown that these products may help regulate blood pressure, reduce lipid levels, and even inhibit the growth of cancer cells (Aviram et al., 2004). Additionally, pomegranate-derived products such as juice and paste are rich in specific phytochemicals like punicalagins, ellagic acid, and gallotannins, which have protective effects on body cells and demonstrate potential therapeutic applications (Lansky & Newman, 2007).

Wild edible plants, including wild pomegranates, play a vital role in enhancing food security and supporting local economies, particularly in rural areas. These plants offer nutritional benefits and serve as alternative food sources during scarcity. Their sustainable harvesting and utilization can contribute to biodiversity conservation and create economic opportunities for local communities (Asfaw et al., 2023). We aimed to study the polyphenol compounds of Juice and paste from wild and cultivated pomegranates in the north of Iran, which is the key region of wild and cultivated pomegranates in Iran.

2. MATERIAL AND METHOD

To compare the phytochemical composition of wild and cultivated pomegranates in the Behshahr region, two varieties—wild (sour) and cultivated (sweet) pomegranates—were selected, and their phytochemical parameters were analyzed. Three replications examined Phytochemical compounds in different parts of the fruit, including pomegranate juice and pastes. Polyphenolic compounds were identified using HPLC-DAD-ESI-MS and quantified using HPLC-DAD at the laboratory of the CEBAS-CSIC Institute (for more details in methodology: Mena et al., 2012).

3. RESULTS

In the comparative analysis of the mean levels of various ellagitannins, three compounds—Punicalagin I, Punicalagin III, and Punicalin—showed a similar pattern, with all three reaching their highest concentrations only in the sweet (cultivated) variety and specifically in the pomegranate juice. The paste from the sour (wild) variety contained the highest level of Galloyl-HHDP-glc, followed by the cultivated paste variety. Trace amounts of this compound were also detected in the juice of the sweet variety. Pedunculagin was found exclusively in the paste, with higher concentrations in the wild variety compared to the cultivated variety. The levels of HHDP-diglc differed significantly between the two varieties across different parts (juice and paste), with higher concentrations found in the juice for both varieties. However, no significant differences were observed between the different parts within each variety (Figure 1).

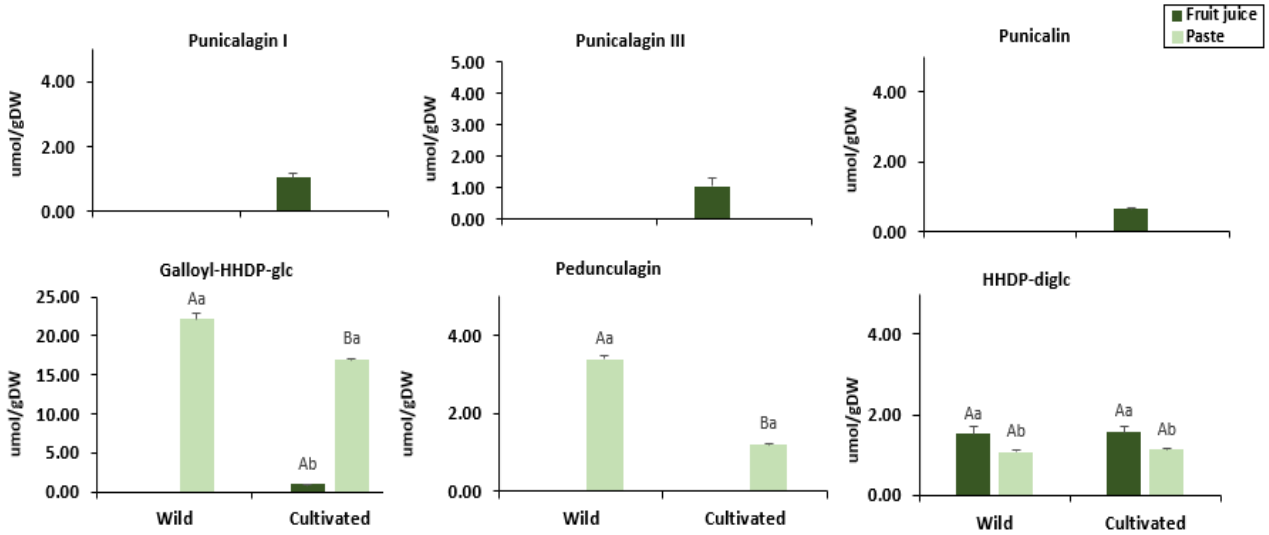

Figure 1 – Comparison of the mean levels of ellagitannin compounds in the juice and paste of wild and cultivated pomegranate varieties. Uppercase letters indicate significant differences between the fruit parts within each variety, while lowercase letters indicate significant differences between the varieties within each fruit part. The absence of uppercase or lowercase letters denotes no significant difference.

According to the results of the analysis of variance (ANOVA) table, the effects of variety, different fruit parts, and the interaction between variety and fruit parts were significant for all compounds except for the impact of variety and the interaction effect in the compound HHDP-diglc (Table 1). Additionally, the fruit parts had the most significant influence on the compounds Galloyl-HHDP-glc and Pedunculagin, followed by the effects of varieties and their interaction, which were significant for the compounds Pedunculagin and Punicalin.

Table 1: Results of Analysis of Variance (ANOVA) for the effects of variety, fruit parts, and their interaction on phytochemical compounds.

	Df	Cultivar	Part	Cultivar * Part
Punicalagin. I	1	-	-	-
Punicalagin. III	1	-	-	-
Punicalin	1	139.07 ***	88.19 ***	101.59 ***
Galloyl.HHDP.glc	1	29.13 **	2357.59 ***	60.94 ***
Peduncalagin	1	780.48 ***	3519.41 ***	780.4 ***
HHDP.diglc	1	0.14	12.37 **	0.00

The symbol *** indicates significance at the 0.001 level and **at the 0.001 level.

The correlation between parameters of different fruit parts and polyphenolic compounds in two pomegranate varieties was analyzed using Principal Component Analysis (PCA). The first principal component explained 59.9% of the variance, while the second component accounted for 31% of the variance (Figure 2). According to the results, Punicalagin I, Punicalagin III, Punicalin, and HHDP-diglc contributed most significantly to the first component. In contrast, Galloyl-HHDP-glc and Pedunculagin had the highest contributions to the second component.

Based on the results of this analysis, the juice of the sweet (cultivated) variety is positioned in the upper right quadrant, showing the strongest association with the parameters Punicalagin I and Punicalagin III. The second group, which includes the juice of the sour (wild) variety, is located in the lower right quadrant and is most closely related to Punicalin and HHDP-diglc. The third group, comprising a paste of both sour and sweet varieties, is located in the upper left quadrant and exhibits the highest correlation with Galloyl-HHDP-glc and Pedunculagin.

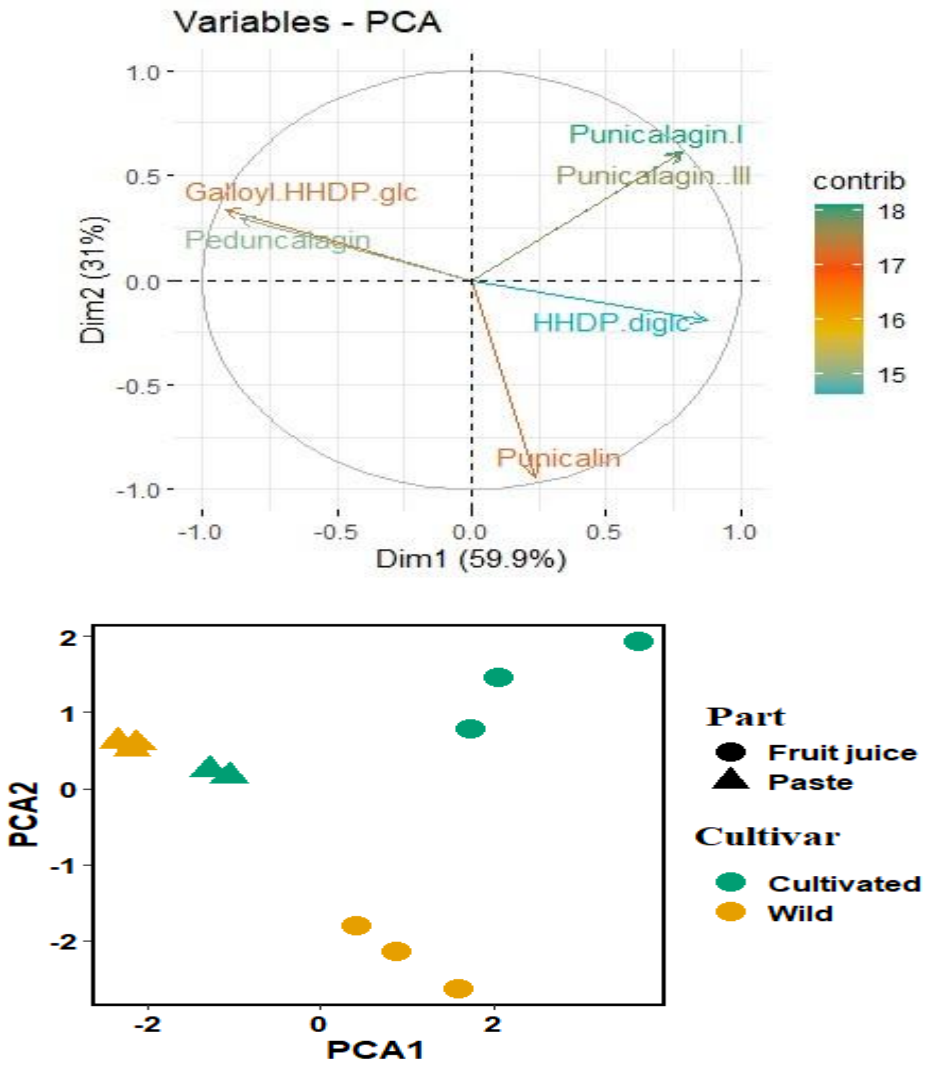

Figure 2 – PCA results (Components 1 and 2) of phytochemical compounds in different fruit parts of two pomegranate varieties.

Heat map results also confirmed the PCA results. The chemical parameters were grouped into separate clusters, with Galloyl-HHDP-glc and Pedunculagin forming the first group, and the other parameters belonging to the second group. Likewise, different fruit parts were clustered separately, with one cluster containing only the juice from both varieties, and the second cluster including the paste from both varieties in the studied sites (Figure 3).

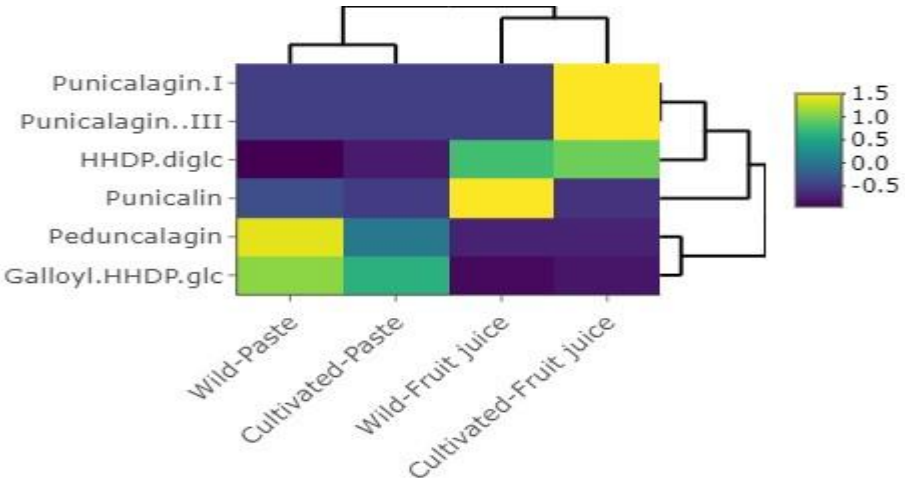

Figure 3 – Heatmap of the two fruit parts studied at the research site based on biochemical traits. Yellow colors indicate the highest mean values, while blue colors represent the lowest mean values.

4. DISCUSSION AND CONCLUSIONS

The examination of various ellagitannin compounds in pomegranate indicated that Punicalagin I, Punicalagin III, and Punicalin had the highest concentrations in the juice of the sweet (cultivated) variety. Prior research has shown that Punicalagin and Punicalin rank among the main ellagitannins in pomegranate, particularly abundant in the juice and other edible parts of the fruit. These compounds tend to be more prevalent in cultivated varieties, which can be attributed to genetic differences and agronomic conditions (Feng et al., 2019). In comparison, Galloyl-HHDP-glc exhibited the highest levels in the pomegranate paste (concentrate) from the sour (wild) cultivar. This phenomenon may arise from the elevated phenolic content found in wild pomegranates, often linked to environmental stress and genetic diversity (Tehranifar et al., 2010). Galloyl-HHDP-glc, a form of ellagitannins, is typically found in higher amounts in fruit parts rich in fiber and polyphenols, such as the peel and inner membranes, while appearing in lesser quantities in the juice, which predominantly contains aril extracts. Consequently, its limited presence in the juice of the sweet cultivar is understandable (Seeram et al., 2005). Numerous studies have established that Pedunculagin is a major ellagitannin mainly located in the peel and tougher parts of pomegranate fruits, especially in wild varieties, which accumulate more of this compound due to greater exposure to biotic and abiotic stressors. Additionally, the concentration process used in paste production, which often involves whole fruit or concentrated extracts, typically maintains or even enhances these more stable compounds (Fischer et al., 2011). The noted reduction in HHDP-diglc levels in paste compared to juice can be explained by the compound's thermal instability and the decrease in water content during the concentration process, a finding that aligns with earlier research (Fischer et al., 2011). The ANOVA results demonstrated that fruit parts have a more significant impact on polyphenol content than cultivar differences or their interaction, consistent with findings from other studies. Various fruit tissues such as the peel, internal membranes, seeds, and juice—exhibit unique distributions of ellagitannins. Specifically, compounds like Galloyl-HHDP-glc and Pedunculagin are more concentrated in the peel and internal membranes, where they serve protective functions against biotic stress and UV radiation. In contrast, HHDP-diglc, which showed no significant effects from cultivar or interaction, appears to be more closely related to specific tissue structures and molecular stability, exhibiting less dependence on genotype (Mena et al., 2012).

Principal Component Analysis (PCA) reveals that the first principal component (PC1) explains about 59.9% of the variance, largely connected to Punicalagin I, Punicalagin III, Punicalin, and HHDP-diglc. This suggests a strong association with genotypic variations among cultivars. Meanwhile, the second component (PC2), accounting for 31% of the variance, corresponds to Galloyl-HHDP-glc and Pedunculagin, which are more prevalent in fibrous and inedible parts like the peel and membranes, highlighting structural differences in the fruit's anatomy (Viuda-Martos et al., 2010; Mphahlele et al., 2016). Additionally, cluster analysis indicated that Galloyl-HHDP-glc and Pedunculagin, owing to their intricate chemical structures and stability, formed a distinct cluster. These compounds are generally found in protective, non-edible tissues, such as the pericarp, exhibiting unique biochemical properties and enhanced resistance to degradation during thermal treatments, like those used in paste production (Seeram et al., 2005).

Our results demonstrate that pomegranate paste and juice are abundant in polyphenols and contain bioactive compounds, emphasizing their significance in food science. Additionally, wild pomegranate also showed high potential for food security and industry.

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Peer-review / Akran Değerlendirmesi
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Author Contributions / Yazar Katkıları
Conceptualization: M.A. and D.M.; Investigation: E.Z.; Material and Methodology: D.M.; Supervision: D.M. and M.A.; Visualization: E.Z.; Writing-Original Draft: E.Z.; Writing-review & Editing: M.A. and D.M.; Other: All authors have read and agreed to the published version of the manuscript.

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

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Taxonomic And Functional Diversity of Coastal Wetland Vegetation Zones In Minakaleh Peninsula

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Abstract: The Miankaleh Peninsula is an important international wetland located in the southern part of the Caspian Sea. However, the wetland vegetation in Miankaleh has been rarely investigated. To address this, we evaluated the taxonomic diversity of three wetland zones, each dominated by a dominant species: Salicornia, Juncus, and Tamarix. These zones were selected due to their proximity to the wetland and their distinct dominant vegetation types. We sampled five macroplots in each zone and calculated several commonly used indices of biodiversity and evenness, including the Shannon, Simpson, and Margalef diversity indices, as well as the McIntosh and Smith-Wilson evenness indices. Functional diversity was also assessed using multiple indices, including functional richness, evenness, divergence, dispersion, and Rao's quadratic entropy to complement the taxonomic analysis. The data revealed significant ecological differences between these zones, with the Juncus zone exhibiting the highest species richness, while the Salicornia zone had the lowest. NMDS results also showed differences in vegetation composition. The diversity and evenness indices, as well as the functional diversity indices, were highest in the Juncus and Tamarix zones and lowest in the Salicornia zone. These findings suggest that dominant vegetation types can significantly influence species composition and patterns of community-level diversity even within a geographically limited area. These results highlight that, similar to taxonomic patterns, functional trait diversity is strongly influenced by the dominant species in each zone, resulting in distinct ecological structures even in closely situated wetland habitats.

Keywords: diversity, evenness, Caspian Sea, vegetation ecology.

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1. INTRODUCTION

Wetlands are critical ecosystems that provide a multitude of essential environmental services. They play a significant role in flood control, climate regulation, carbon storage, and aquifer recharge, making them indispensable for maintaining ecological balance (Costa, Silva, & Evans, 2013; Whigham, 2018). Wetlands are highly diverse, serving as breeding grounds and habitats for numerous species of plants, invertebrates, fish, and wildlife, thus contributing significantly to global biodiversity (Costa, Silva & Evans, 2013; Gaiser & Rühland, 2010). Despite the recognized ecological importance of wetland vegetation, many wetlands, like Miankaleh, remain poorly investigated in terms of diversity patterns (Shokri, Safaian, Ahmadi, & Amiri, 2004; Sharifnia, Asri, & Gholami-Te, 2007; Amirnezhad, Rafiei, & Atghaei, 2010).

To address this gap, the present study examines the taxonomic and functional diversity of wetland vegetation across three ecologically distinct zones within the Miankaleh Peninsula, each characterized by a different dominant plant genus: Salicornia, Juncus, and Tamarix. These zones were selected for their proximity to the wetland and their contrasting dominant vegetation, which are hypothesized to influence species composition and ecological functioning. This study seeks to quantify and compare community-level diversity and trait composition across zones by employing a variety of commonly used taxonomic diversity indices (such as Shannon, Simpson, Margalef) and evenness metrics (like McIntosh, Smith-Wilson) (Spellerberg & Fedor, 2003), as well as functional diversity indices (including FRic, FEve, FDiv, FDis, and RaoQ) (Mendes, Evangelista, Thomaz, Agostinho, & Gomes, 2008; Villéger, Mason, & Mouillot, 2008).

2. MATERIAL AND METHOD

Study Area

Miankaleh is a peninsula located in Mazandaran Province, Iran, covering an area of 68,800 hectares. This peninsula, located in the southeastern part of the Caspian Sea, is renowned for its unique flora, fauna, and high conservation value (Fig. 1). It is one of Iran's thirteen biosphere reserves under the Man and the Biosphere (MAB) Programme. It is considered a suitable habitat for terrestrial and aquatic plants, animals, and migratory birds (Ejtehadi et al., 2003). The region receives an average annual precipitation of 675 mm and has a mean annual temperature of 17.6°C. The area's climate is classified as semi-humid (Ghahroudi Tali et al., 2012).

Miankaleh hosts over 261 plant species from 60 families and 177 genera. The most represented families include Asteraceae, Poaceae, Papilionaceae, Cyperaceae, and Brassicaceae. The most frequent life forms in Miankaleh are

therophytes (annual plants) and hemicryptophytes (perennial plants), accounting for 52.5% and 29% of the species, respectively (Sharifnia, Asri, & Gholami-Te, 2007).

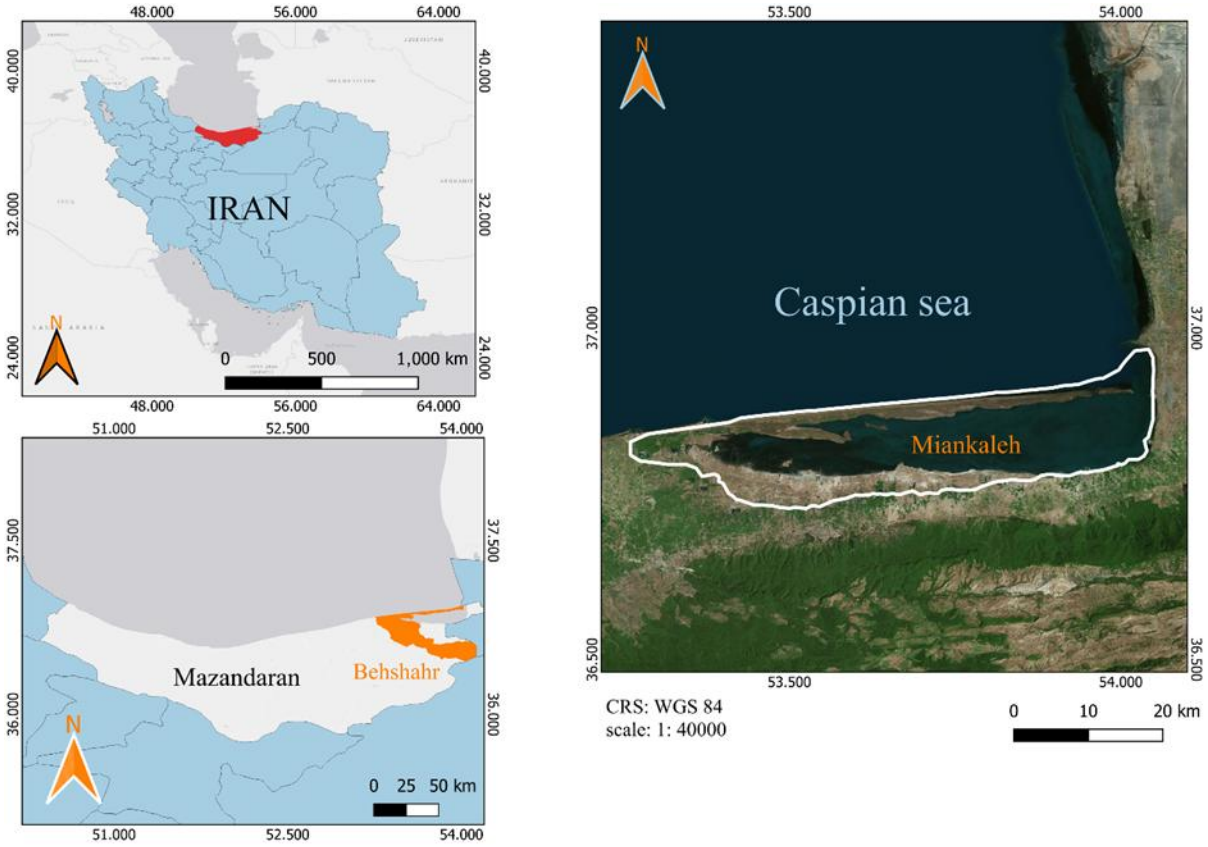

Fig 1. Study area location

Sampling Method

Three vegetation zones, including Tamarix, Salicornia, and Juncus, were selected (Fig. 2). Vegetation sampling was conducted during the appropriate growing season for these plants in late spring 2023. Fifteen 30 m × 30m plots were established near the Miankaleh wetland, with five plots located in each vegetation zone. Within each plot, the percentage cover of the dominant species (excluding annuals), ground cover, and the geographical coordinates of the plot were recorded (Arzani & Abedi, 2015). To assess taxonomic diversity indices, the *adiv* package and for functional indices FD package in R software were used (Pavoine, 2020; Laliberté & Legendre, 2010; Laliberté, Legendre, & Shipley, 2014). Various indices were calculated to analyze biodiversity, species richness, and evenness. GLMM for comparisons, PCA for diversity indices, and NMDS for vegetation compositions were applied for statistical analysis.

Traits

To assess plant functional diversity across the study zones, a set of key functional traits was measured for the dominant species in each plot. The selected traits included: chlorophyll content, fresh leaf mass (g), dry leaf mass (g), leaf thickness (cm), leaf area (cm^2), specific leaf area (SLA, cm^2/g), leaf dry matter content (LDMC), plant height (m), and leaf length (cm).

Fresh leaves were collected from representative individuals and processed according to standard protocols for functional traits (Cornelissen et al., 2003). Chlorophyll content was measured using a portable SPAD 504. Fresh mass was recorded immediately after collection. Leaves were then scanned to determine leaf area, measured for thickness using a digital caliper, and dried at 60°C for 48 hours to obtain dry mass. SLA was calculated as the ratio of leaf area to dry mass, while LDMC was calculated as the ratio of dry mass to fresh mass. Plant height was measured from the base to the apex of each individual, and leaf length was taken as the longest dimension of the leaf blade. These traits were used to calculate taxonomic and functional diversity indices to compare vegetation zones.

Fig 2. Landscape of study wetland zones

3. RESULTS

According to Table 1, only Margalef diversity and species richness were statistically significant among all taxonomic indices. The Juncus zone (4) showed higher values compared to both the Tamarix (3) and Salicornia (2.8) zones, with significant differences observed. In terms of Margalef diversity, the Juncus zone again exhibited the highest value (0.71), compared to the Tamarix (0.52) and Salicornia (0.49) zones. none of the functional diversity indices showed any significant differences among the three vegetation zones.

Indices	Salicornia	Tamarix	Juncus	F Value	P Value
Richness	2.80 ± 0.20b	3.00 ± 0.32b	4.00 ± 0.00a	8.86	0.00
Simpson diversity	1.64 ± 0.21	1.93 ± 0.18	1.85 ± 0.23	0.50	0.62
Shannon diversity	0.61 ± 0.11	0.76 ± 0.10	0.74 ± 0.16	0.42	0.67
Margalef diversity	0.49 ± 0.06b	0.52 ± 0.06b	0.71 ± 0.02a	6.31	0.01
McIntosh evenness	0.49 ± 0.11	0.63 ± 0.07	0.48 ± 0.12	0.72	0.51
SmithWilson evenness	0.43 ± 0.07	0.47 ± 0.08	0.30 ± 0.08	1.22	0.33
FRic	4.78 ± 0.60	3.57 ± 1.46	1.98 ± 0.42	2.65	0.12
FEve	0.60 ± 0.13	0.61 ± 0.14	0.65 ± 0.12	0.04	0.96
FDiv	0.74 ± 0.08	0.86 ± 0.05	0.87 ± 0.05	1.40	0.29
FDis	1.57 ± 0.35	2.24 ± 0.30	1.61 ± 0.44	1.04	0.38
RaoQ	4.08 ± 1.02	6.35 ± 0.92	4.39 ± 1.32	1.25	0.32

Table 1. Analysis of variance (ANOVA) of Taxonomic indices across vegetation zones

The NMDS (Non-metric Multidimensional Scaling) plot (Fig. 3) illustrates the variation in plant community composition across the Juncus, Salicornia, and Tamarix zones (Stress=0.1). All three zones showed differences in plant compositions ($F=4.45$; $P< 0.01$ according to PERMANOVA results).

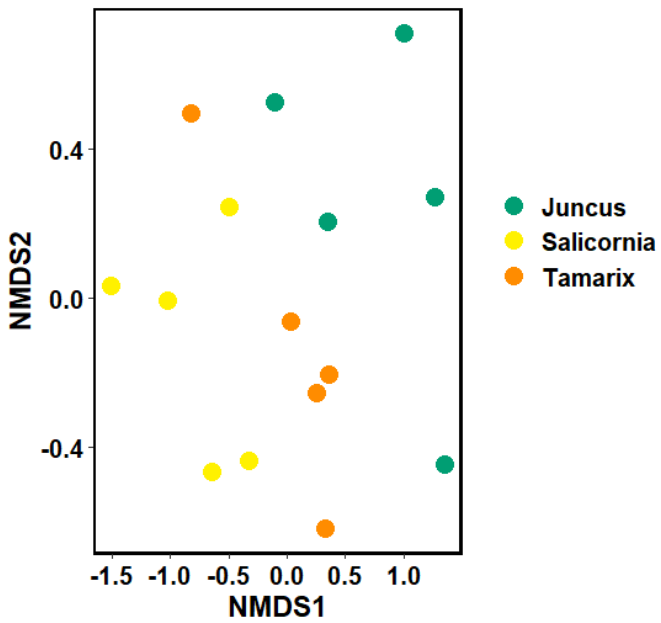

Fig 3. Compositional differentiation of shrub communities based on NMDS analysis

In the principal component analysis (PCA), the indices FRic, FEve, FDiv, Margalef diversity, and Richness clustered together on the positive side of the first dimension (Dim1) and on the negative side of the second dimension (Dim2), which is associated with the Juncus zone. The indices Smith-Wilson evenness, McIntosh evenness, Functional Dispersion (FDis), Rao's Quadratic Entropy (RaoQ), Shannon diversity, and Simpson diversity were positioned on the positive side of both the first dimension (Dim1) and the second dimension (Dim2) in the PCA, associated with the Tamariz zone. The Salicornia zone is also mainly located in the negative part of Axis 1, which is negatively associated with most taxonomic and functional indices. The first axis explains 53.6% of the variation, while the second axis accounts for 22.8% of the variation. Results of heatmaps also confirm the PCA patterns(Fig.4).

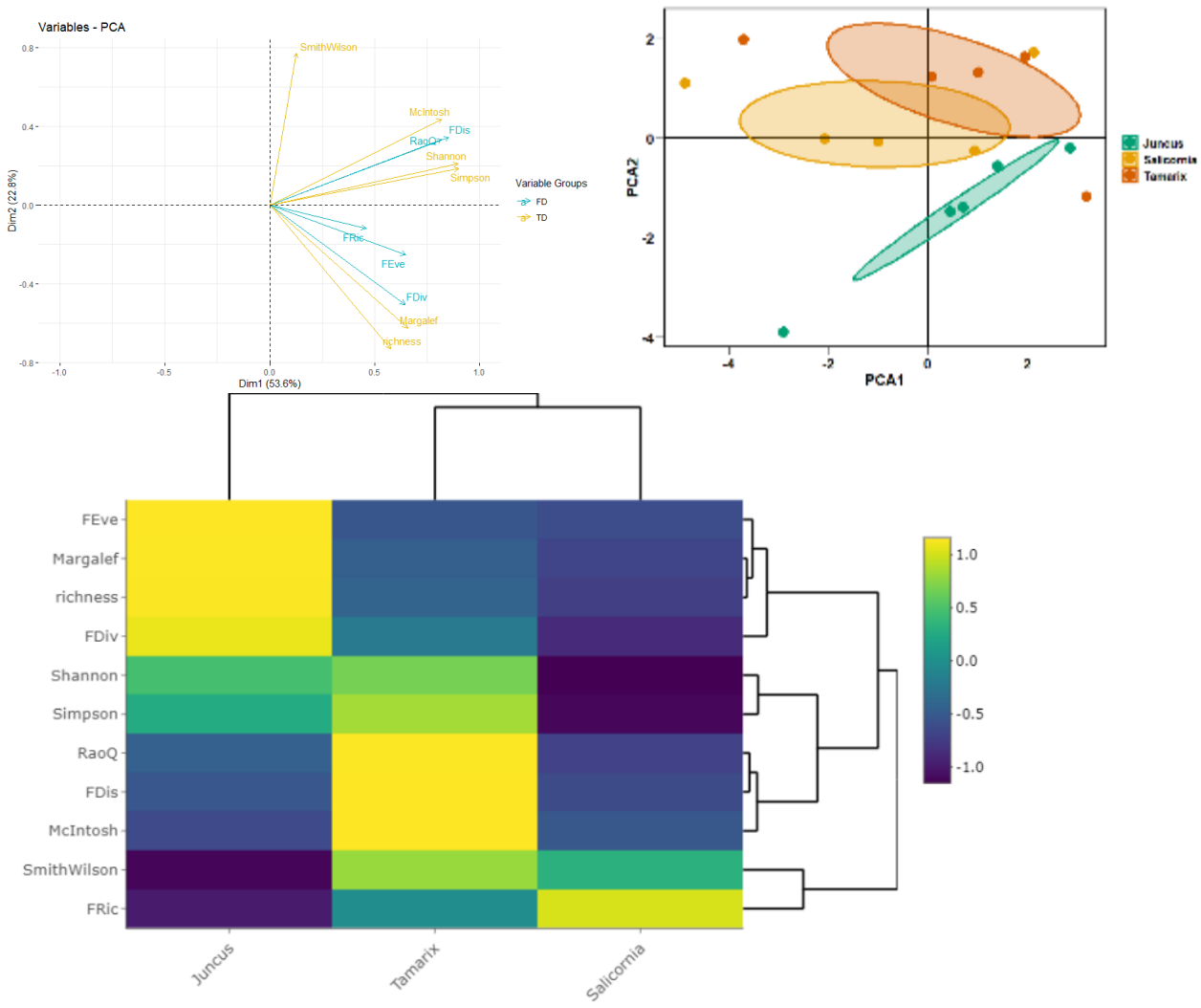

Fig4. PCA analysis and Heatmap on taxonomic and functional indices in 3 zones

4. DISCUSSION AND CONCLUSIONS

This study revealed distinct taxonomic and functional diversity patterns among the three wetland zones. The Juncus and Tamarix zones generally exhibited greater diversity and functional complexity, while the Salicornia zone consistently showed the lowest values, likely due to stronger environmental filters such as salinity and waterlogging (Dembicz et al., 2020; Matthews & Endress, 2010).

The Salicornia zone showed the lowest levels of both taxonomic and functional diversity, indicating a limited and simplified community composition. This pattern arises from extreme salinity, anoxia, and high abiotic stress, leading to the dominance of one or a few highly stress-tolerant species. Such dominance results in strong environmental filtering, as the species possess similar traits, such as succulence and salt tolerance, ultimately reflecting minimal ecological multifunctionality and low niche differentiation (Nathan, Osem, Shachak, & Meron, 2016). This phenomenon may be attributed to convergence in functional traits due to similar adaptive strategies across all zones or to limitations in trait variability at the community level (Pakeman, 2011; Ricotta, 2005; Maire et al., 2012). The Tamarix zone, on the other hand, was associated with several indices such as Shannon, Simpson, RaoQ, FDis, and both evenness indices, indicating a balanced community structure and trait distribution in a moderately stressful environment. The consistent abundance along with moderate richness suggests a balance between filtering processes and the coexistence of stress-tolerant strategies. High functional dispersion indices (FDis) and RaoQ reveal the presence of functionally dissimilar species that thrive despite taxonomic constraints. This indicates functional divergence in response to stress rather than simply an increase in species richness. The Juncus zone is characterized by various indices, including Richness, Margalef, Functional Richness (FRic), Evenness (FEve), and Functional Diversity (FDiv), suggesting a highly functional and niche-structured community. The high levels of taxonomic and functional richness indicate niche complementarity and minimal environmental filtering. Elevated FRic and FDiv demonstrate a broad range of traits and their divergence, facilitating efficient resource use and coexistence among species. As a result, these zones contribute to enhanced multifunctionality, resilience. The low functional redundancy in Salicornia suggests reduced ecological stability and productivity (da Silva Camilo et al., 2018), while higher functional diversity in Juncus and Tamarix zones points to more resilient ecosystems.

Despite the lack of statistically significant differences in univariate functional diversity indices among the three vegetation zones, multivariate analyses (PCA and NMDS) revealed clear patterns in trait and species composition (Legendre & Legendre, 2012). This highlights the value of ordination techniques in uncovering subtle ecological gradients and community-level functional structure that may not be evident through univariate statistics alone.
Overall, our findings suggest that wetland ecosystems are vulnerable due to their limited support for a small number of species, which are threatened by declining water levels in the Caspian Sea. Therefore, enhanced conservation plans are crucial for protecting these habitats.

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Conflict of Interest
The authors declare no conflicts of interest.

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Total phenolic content and antioxidant capacity of *Verbascum thapsus* L. variations along the altitude gradient

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Abstract: This study aimed to investigate the effects of altitude on the biochemical parameters of *Verbascum thapsus* L. leaves in the rangelands of Chahardangeh, Sari. Specifically, we measured the total phenolic content and antioxidant capacity using DPPH in leaf samples collected from three distinct elevation zones (1300-1400, 1400-1500, 1500-1600). The results indicated that as altitude increased, the mean total phenolic content rose from 6,801.1 mg GAE/Kg FW at an altitude of 1,300–1,400 meters to 11759.8 mg GAE/Kg FW at 1,500–1,600 meters. Similarly, the mean DPPH radical scavenging activity increased from 72.9% at 1,300–1,400 meters to 87.9% at 1,500–1,600 meters. Understanding how altitude affects the production of secondary metabolites in medicinal plants is crucial, both scientifically and practically, in the context of climate change scenarios. This research provides valuable insights into the relationship between altitude and secondary metabolite accumulation in *V. thapsus*, highlighting the plant's biochemical response to this crucial ecological factor.

Keywords: Phytochemistry, Mountain grasslands, Bioactive compounds

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1. INTRODUCTION

Iran's varied climate and geography support a rich array of flora, featuring numerous plant species known for their notable medicinal properties. Recent scientific advancements have sparked increased interest in substituting synthetic components in pharmaceuticals with natural, plant-based alternatives. In various nations, these natural compounds have become vital to contemporary healthcare and therapeutic practices, indicating a worldwide transition towards more sustainable and biocompatible treatment methods (Ji et al., 2009). Iran boasts a variety of ecological conditions that support the growth of more than 2,500 species of medicinal plants. Numerous studies have shown that many of these plants harbor valuable bioactive compounds, offering potential uses in the pharmaceutical, food, and cosmetic sectors. By enhancing research infrastructure and combining traditional knowledge with modern scientific methods, we can discover and commercialize new compounds that hold significant value (Azarnivand & Zare Chahooki, 2010). Multiple factors can affect both the quantity and quality of secondary metabolites in medicinal plants. These factors include the specific plant part utilized, the species of the plant, ecological conditions, phenological stages, biotic stresses like grazing, harvesting time, post-harvest management, and the method of extracting active compounds (Feduraev et al., 2019). *Verbascum thapsus L.,* a biennial member of the Scrophulariaceae family, can reach heights exceeding 2 meters. Its small yellow flowers are closely grouped along a tall flowering stem that emerges from a substantial rosette of basal leaves. This plant thrives in diverse habitats but favors sunny locations. It can germinate from long-lived seeds, which remain viable in the soil for many years. As a herbaceous species, *V. thapsus* disperses quickly by producing a large number of seeds (Badad et al., 2023). From a phytochemical standpoint, *V. thapsus* yields various secondary metabolites, such as phenylethanoid glycosides, iridoid glycosides, tetraglycosidic triterpenes, saponins, terpenes, flavonoids, carotenoids, and carbohydrates (Jan et al., 2022). This plant is known for a multitude of biological activities, including antiviral, antioxidant, analgesic, sedative, anti-inflammatory, hypnotic, antibacterial, antifungal, and anticancer effects (Gupta et al., 2022). Considering this species is found in mountainous habitats, we aimed to study the variations in phenolic compounds and antioxidants along an altitude gradient.

2. MATERIAL AND METHOD

Mazandaran Province, with an area of approximately 23,756 square kilometers, is bordered by Golestan Province to the east, Gilan Province to the west, and Semnan, Tehran, Alborz, and Qazvin to the south. This study was conducted in the rangelands of the Chahardangeh region, Sari County, Mazandaran Province, located at a longitude of 53°43′36″E and a latitude of 36°26′55″N. The average annual precipitation in this area is approximately 300 mm, with a yearly mean temperature of 11°C. Field surveys were carried out to identify the distribution of *Verbascum thapsus* across different elevation ranges (1300–1400 m, 1400–1500 m, and 1500–1600 m above sea level). To investigate the effect of elevation on the antioxidant activity of the studied species, 12 individual plants were randomly selected from their growing areas within each elevation range. Sufficient leaf samples were collected from each plant in triplicate during the full flowering stage. Notably, after harvesting the leaves from the 12 plants, the samples were mixed in three sets of four replicates each,

resulting in 3 samples per elevation range and 9 samples across all three elevation ranges. The collected samples were then transported to the laboratory under appropriate **conditions.** Total phenolic content was determined using the Folin-Ciocalteu colorimetric method with gallic acid as the standard(**Singleton et al., 1999)**, and antioxidant activity was assessed via the DPPH radical scavenging assay(**Brand-Williams et al., 1995)**. Principal Component Analysis (PCA) was employed to examine the relationship between the total phenolic content, antioxidant capacity of the target plant's leaves, and the specified altitudes. A heatmap was also generated to visualize this relationship better.

3. RESULTS

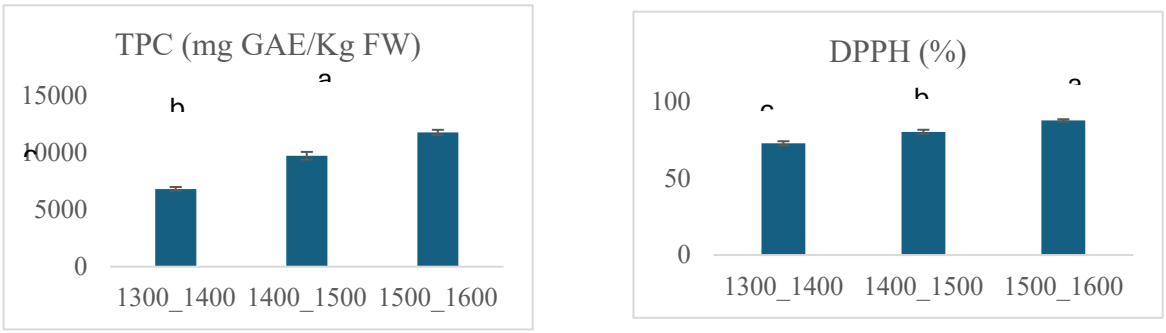

Figure1. Mean±SE (n=3) of TPC and DPPH in three altitudes.

In the analysis of variance for the evaluated parameters (Table 1), the lowest F-value was observed for DPPH, while the highest belonged to TPC. It is noteworthy that both TPC and DPPH parameters were statistically significant. According to the mean comparison chart (Figure 1), the values of the parameters increased with elevation; therefore, the highest levels of TPC and DPPH were recorded at the 1500–1600 m elevation range.

Table 1. Analysis of variance (ANOVA) results for TPC and DPPH parameters

Factor	Df	F-value	Significance level
TPC (mg GAE/Kg FW)	**3**	**93.33**	**P < 0.001**
DPPH (%)	**3**	**30.35**	**P < 0.001**

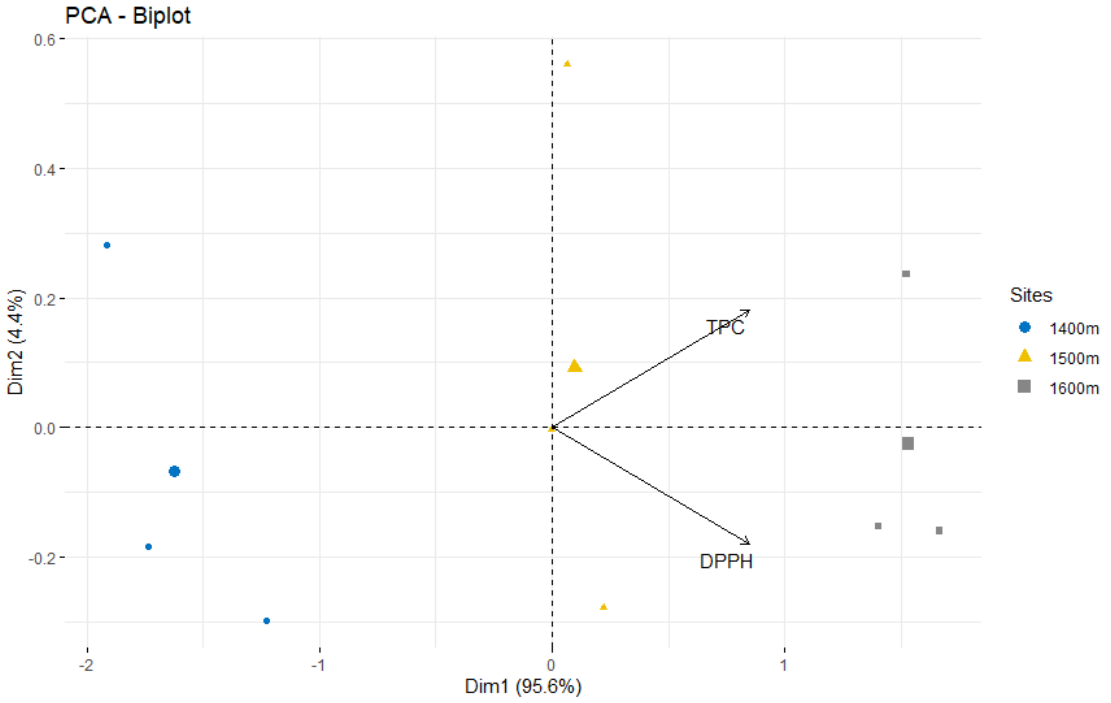

Figure2. Biplot of Principal Component Analysis (PCA) based on total phenolic content (TPC) and antioxidant capacity (DPPH) in samples from different altitudes (1400, 1500, and 1600 m).

Principal Component Analysis (PCA) in Figure 2, showed that the first component (Dim1), accounting for 95.6% of the variance, provided the most significant separation among the samples based on altitude. Samples from the 1600 m

elevation exhibited the highest total phenolic content (TPC) and antioxidant capacity (DPPH) levels. Higher altitude is at the positive end of PCA1, while 1400m is at the negative end of PCA1.

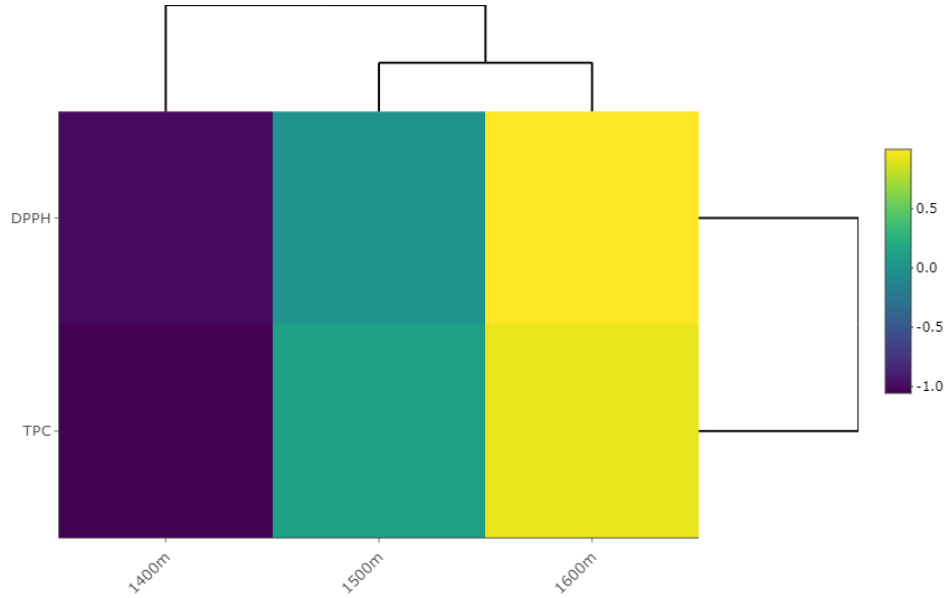

Figure3. The heat map shows TPC and DPPH values at different altitudes.

The heatmap clustering(Figure 3) indicates that both TPC and DPPH levels are substantially higher at 1600 m than at lower altitudes. Also, parameters at 1400 m are located in separate clusters.

4. DISCUSSION AND CONCLUSIONS

Research indicates that temperature, relative humidity, wind speed, and light intensity variations at higher altitudes can change how plants respond physiologically, influencing their secondary metabolite composition (Hashim et al., 2020). In this study, the measured parameters were found to increase with elevation, reaching the highest total phenolic content (TPC) and DPPH levels at an altitude of 1500–1600 m. Likewise, a study of Tartary buckwheat seeds and hulls reported a notable rise in total phenol content and antioxidant potential with increasing altitude (Kishore et al., 2010). A similar increase in total phenol content and DPPH antioxidant activity with altitude was observed in Paeonia emodi, an important plant found in the Himalayas (Joshi et al., 2022). This trend was also noted in Taxus wallichiana (Adhikari et al., 2022). The elevation-related rise in TPC and DPPH is thought to result from enhanced biosynthetic pathways for phenolic compounds in Verbascum thapsus, leading to greater total phenol content and antioxidant activity (Rana et al., 2020). Higher altitudes correlate with increased oxidative stress due to reduced oxygen pressure and heightened UV radiation. This stress drives plants to bolster their antioxidant defenses, resulting in increased production of phenolic compounds and antioxidant enzymes (Cui et al., 2016). Additionally, plants at higher elevations face more extreme temperatures and elevated UV radiation, which can harm cellular components. To counteract this damage, they produce more phenolic compounds that serve as UV shields and antioxidants (Agrawal et al., 2024). Furthermore, at elevated altitudes, plants often increase the synthesis of secondary metabolites like phenols, flavonoids, and tannins. These compounds exhibit potent antioxidant properties and are crucial for safeguarding the plant from environmental stresses. While further research is necessary to fully comprehend how altitude affects the total phenol content and antioxidant capacity of Verbascum thapsus, existing evidence highlights altitude's vital role in regulating secondary metabolite production in this species. More in-depth studies could provide deeper insights into this phenomenon, aiding in the enhancement of yield and quality in medicinal plants. Thus, altitude significantly influences both the composition and concentration of secondary metabolites in plants.

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Author Contributions / Yazar Katkıları

Conceptualization: H.S.; Investigation: R.B.; Material and Methodology: M.A., GA.DT.; Supervision: M.A., GA.DT; Visualization: R.B; Writing-Original Draft: R.B.; Writing-review & Editing: M.A., GA.DT.; Other: All authors have read and agreed to the published version of the manuscript.

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

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Cytotoxic and Apoptotic Activity of Usnic acid on HS578T Cell Line

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Abstract: Cancer is one of the leading causes of death worldwide, responsible for approximately one in every six deaths. Despite the availability of conventional treatment options such as chemotherapy, radiotherapy, and surgery, these approaches often fail to provide a definitive cure, underscoring the need for new therapeutic strategies. One such potential approach is the use of natural compounds with anticancer properties. Usnic acid (UA), a secondary metabolite derived from lichens, has garnered attention for its broad range of biological activities, including antimicrobial, anti-inflammatory, and antioxidant effects. However, its potential as an anticancer agent, particularly its cytotoxic and apoptotic effects, has not been fully explored. This study aimed to investigate the cytotoxic and apoptotic effects of usnic acid on the HS578T human breast cancer cell line. The cytotoxicity of usnic acid was assessed using the MTT assay, where cells were treated with various concentrations of UA for 24 hours. The results showed that usnic acid exhibited significant dose-dependent cytotoxicity against HS578T breast cancer cells, with a calculated half-maximal effective concentration (EC_{50}) of 15.97 µM. Interestingly, usnic acid did not exhibit any cytotoxic effects on the non-tumorigenic HEK293 cell line, indicating its selective action against cancer cells. To assess the apoptotic effects of usnic acid, flow cytometry was performed using Annexin V/PI fluorescent staining. The percentage of apoptotic cells was determined in both the control and UA-treated groups. In the control group, 6.86% of cells were apoptotic, while the positive control (paclitaxel) induced apoptosis in 10.32% of cells. In the usnic acid-treated group, the apoptosis rate was 8.27%, indicating that usnic acid can induce apoptosis in a significant proportion of cancer cells. These results suggest that usnic acid exhibits selective cytotoxic and apoptotic effects on breast cancer cells, making it a promising candidate for further research and development as a potential therapeutic agent in cancer treatment. Given its potential for targeting cancer cells without affecting non-tumorigenic cells, usnic acid could provide a novel avenue for the development of more effective and less toxic cancer therapies.

Keywords: Usnic Acid, HS578T Cell Line, Cytotoxicity, Apoptotic activity.

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1. INTRODUCTION

Responsible for one out of every six deaths worldwide, cancer is a global health problem as a very complex disease that progresses gradually with a generalized loss of growth control (Debela et al., 2021). Although methods such as chemotherapy, surgery and radiotherapy are used for the treatment of cancer, they do not offer a definitive solution. For this reason, new approaches such as immune-mediated therapies, drugs and biological molecules, newly synthesized chemicals, herbal extracts and hybrid molecules are also promising in preventing the proliferation and metastasis of cancer cells, and research and studies on this are very important for the future of cancer treatment (Roy & Li, 2016; Sahin et al., 2021).

Usnic acid [2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzo-furandion], a lichen metabolite, the product of secondary metabolism and first isolated in 1844, is a yellowish pigment (Araújo et al., 2015; Cocchietto et al., 2002). It has been studied extensively and has become commercially widespread with potential activities such as anti-inflammatory, antiviral, analgesic, UV protection, healing, antimicrobial, antiprotozoal, antioxidant and larvicidal. However, some studies have reported liver toxicity and contact allergy. Therefore, more studies are needed to determine the efficacy and safety of usnic acid (Araújo et al., 2015).

Usnic acid (UA) exhibits a high affinity for lipids, resulting in high membrane permeability and ease of diffusion through biological membranes. UA is thought to play an important role in cytotoxicity, as this diffusion leads to the breakdown of proton gradients, resulting in a reduction in the connectivity between electron transport and ATP synthesis (Abo-Khatwa et al., 1996). In vivo and in vitro models, including cultured hepatocytes and cancer cells, as well as isolated mitochondria, have shown the occurrence of mitochondrial disorder such as decreased mitochondrial membrane potential, reduction in ATP levels, inhibition of oxidative phosphorylation, swelling, increased ROS production and adaptive overexpression of genes associated with the electron transport chain, the Krebs cycle, and lipid metabolism (Bessadottir et al., 2012; Einarsdóttir et al., 2010). These findings support the hypothesis that UA cytotoxicity is mediated by mitochondrial dysfunction (Guzow-Krzemińska et al., 2019).

Such events trigger apoptosis, autophagy and necrosis the mitochondrial pathway (Chen et al., 2014). In a study, it was shown that the proton shuttle properties of UA affect lysosomes in T47D, MCF-7 breast cancer cell lines, but not normal skin fibroblasts (Bessadottir et al., 2012). In another study, UA was shown to disrupt calcium homeostasis in HepG2 and primary rat hepatocytes and cause ER stress with an increase in cytosolic Ca2+ concentration followed by activation of caspases in HepG2 cells (Chen et al., 2015).

Usnic acid is a natural compound known to exhibit cytotoxic effects on various cancer cell lines. Studies have shown that usnic acid can inhibit cell proliferation and trigger apoptotic mechanisms. In this study, the cytotoxic and apoptotic effects of usnic acid were evaluated on the HS578T human breast cancer cell line.

2. MATERIAL AND METHOD

This study outlines the experimental strategies employed to investigate the effects of usnic acid on cell viability and programmed cell death with each method comprehensively described under dedicated subheadings.

2.1. Cell culture

In this study, HS578T (human breast cancer) and HEK293 (human embryonic kidney) cell lines were used. The cells were incubated in DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin under conditions of 5% CO_2, 95% humidity, and 37 °C. When the cell confluence reached 90%, they were subcultured for further experiments (Arslan et al., 2020).

2.2. Determination of cytotoxic activity

The cytotoxic activity of usnic acid on HS578T and the control cell line HEK293 was determined using the MTT assay. Cells were counted using a Thoma chamber and trypan blue, and then seeded into 96-well plates at a density of 2×10^3 cells per well. Serially diluted concentrations of usnic acid (5–50 µM) were applied to the cells. Dimethyl sulfoxide (DMSO, >1%) was used as the control group. After 24 hours of exposure, the medium in each well was replaced with 100 µL of fresh medium and 10 µL of MTT, followed by incubation at 37 °C for approximately 2 hours. At the end of the incubation, the resulting formazan crystals were dissolved by adding 50 µL of DMSO. The absorbance of the resulting color was measured at 590 nm using a microplate spectrophotometer. The half-maximal effective concentration (EC_{50}) of usnic acid on the cells was determined from cell viability data according to previously established methods (Tüfekçi et al., 2024).

2.3. Determination of apoptotic activity

To determine the apoptotic activity of usnic acid on the HS578T cell line, 30×10^3 cells were seeded into 6-well plates. The cells were exposed to the EC_{50} concentration of usnic acid for 24 hours. Following incubation, the cells were harvested using 500 µL of trypsin, centrifuged at 2000 RPM for 5 minutes, and the resulting pellet was washed twice with phosphate-buffered saline (PBS). The apoptotic effect on the cells was assessed using the Annexin V/PI Apoptosis Detection Kit (Elabscience, USA) with fluorescent dyes. Paclitaxel was used as a positive control. The percentages of viable and apoptotic cells were analyzed by flow cytometry (Sahin et al., 2024).

2.4. Statistical analysis

All experiments were conducted in triplicate to ensure the reliability and reproducibility of the results. Data obtained from each experimental group are expressed as the mean ± standard deviation (SD). Statistical analyses, including comparisons between treated and control groups as well as within group variations, were performed using GraphPad Prism version 9. This software was used to assess the significance of observed differences and to evaluate overall trends in the data.

3. RESULTS

3.1. Cytotoxic activity of usnic acid on HS578T cells

The cytotoxic activity of usnic acid on HS578T breast cancer cells and non-tumorigenic HEK293 cell lines was determined using the MTT assay. In the study, various concentrations of usnic acid were applied to the cells for 24 hours. The results indicate that usnic acid exhibited cytotoxic activity against the HS578T breast cancer cell line. However, it did not show any cytotoxic effect on the non-tumorigenic HEK293 cell line. Based on the data obtained, the half-maximal effective concentration (EC_{50}) of usnic acid for the HS578T cell line was calculated to be 15.97 µM. The dose-dependent cytotoxic effects of usnic acid on the cell lines are presented in Figure 1.

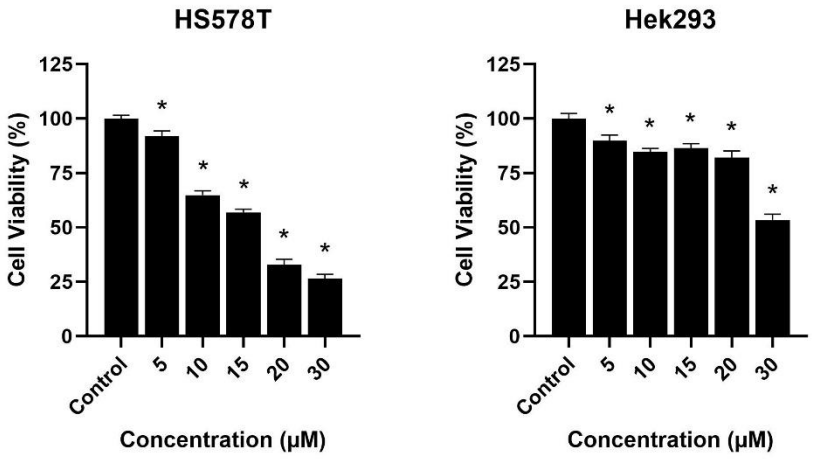

Figure 11. The cytotoxic activity of usnic acid on HS578T and Hek293 cells. A statistically significant difference compared to the negative control is indicated (*P < 0.05).

3.2. Determination of apoptotic activity

The apoptotic activity of usnic acid on the HS578T breast cancer cell line was determined by flow cytometry using Annexin V/PI fluorescent staining. Cells were treated with the EC_{50} concentration of usnic acid in 6-well plates, and paclitaxel was used as a positive control due to its known apoptotic effects. Upon analysis of the results, the percentage of apoptotic cells was found to be 6.86% in the control group, 10.32% in the positive control group, and 8.27% in the usnic acid-treated group. These findings indicate that usnic acid induces a moderate level of apoptosis compared to the control. The flow cytometry results are presented in Figure 2, clearly demonstrating the distribution of apoptotic and viable cells among the groups.

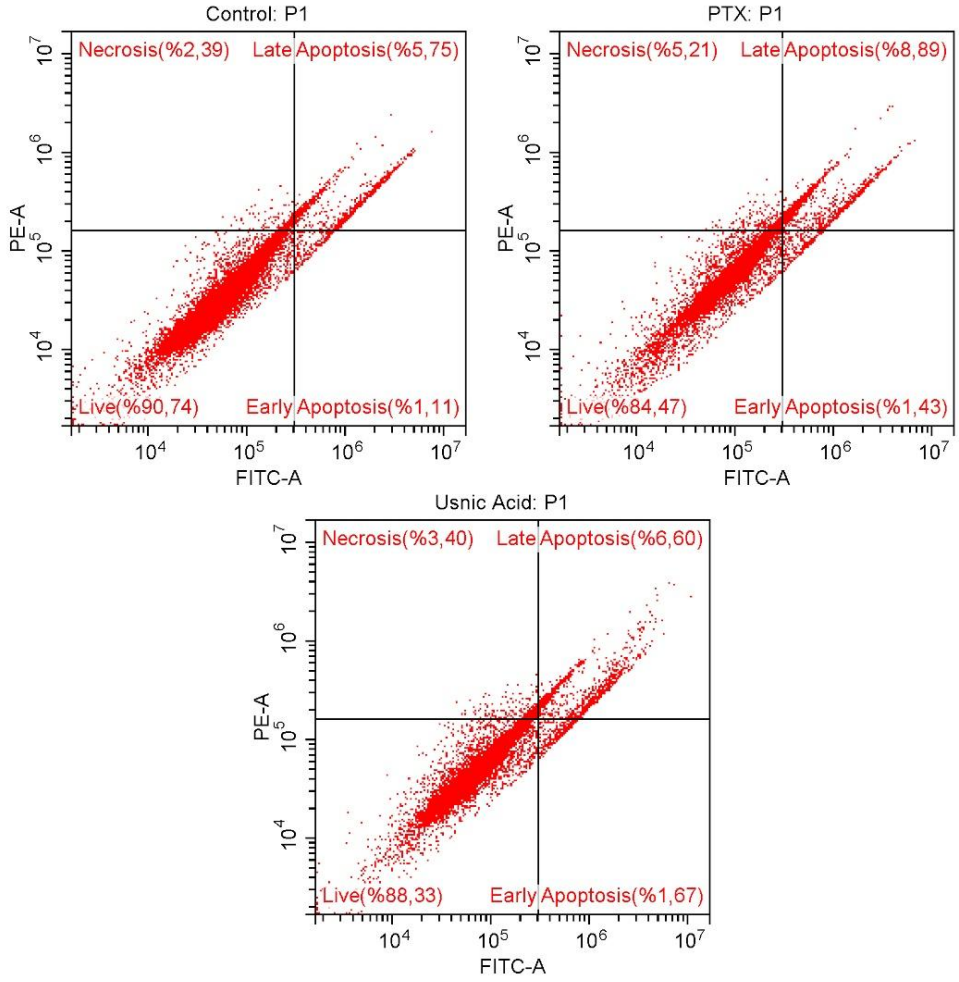

Figure 12 Flow cytometry images showing the percentage of apoptotic HS578T cells following treatment with usnic acid. Paclitaxel (PTX, 11.4 µM) was used as a positive control. A statistically significant difference compared to the negative control is indicated (*P < 0.05).

4. DISCUSSION

In this study, the cytotoxic and apoptotic effects of usnic acid on the HS578T human breast cancer cell line were systematically evaluated. The findings demonstrated that usnic acid exerted a dose-dependent cytotoxic effect on HS578T cells, with a calculated half-maximal effective concentration (EC_{50}) of 15.97 µM. Notably, no EC_{50} value could be determined for the non-tumorigenic HEK293 cell line, although a slight decrease in cell viability was observed at the highest concentration tested, suggesting that usnic acid may have limited cytotoxicity toward healthy cells while exhibiting selectivity for malignant ones.

The results obtained from the MTT assay support the hypothesis that the lipophilic nature of usnic acid facilitates its penetration through cellular membranes, leading to mitochondrial disruption. Previous studies have shown that usnic acid reduces mitochondrial membrane potential, diminishes ATP production, and increases the generation of reactive oxygen species (ROS), ultimately initiating cell death via apoptosis or necrosis (Singh et al., 2013). The present study aligns with this mechanistic pathway, as an increased rate of apoptosis was observed in cells exposed to usnic acid.

Flow cytometric analysis using Annexin V/PI staining confirmed that usnic acid induces apoptosis in HS578T cells. The apoptotic cell percentage in the usnic acid-treated group was 8.27%, compared to 6.86% in the control and 10.32% in the paclitaxel-treated positive control group. Although the apoptotic induction by usnic acid was slightly lower than that of paclitaxel, it still represents a significant increase relative to the untreated group. The selective cytotoxicity of usnic acid toward cancer cells may offer a therapeutic advantage by minimizing adverse effects on normal tissues.

The absence of cytotoxicity in HEK293 cells further supports the selectivity of usnic acid. This differential response may be attributed to variations in mitochondrial dynamics or oxidative stress susceptibility between healthy and malignant cells. The observed apoptotic effects are likely linked to the disruption of mitochondrial pathways, as reported in previous

studies involving other cancer models. For instance, a study involving LNCaP prostate cancer and RWPE-1 normal prostate epithelial cells showed that usnic acid induced apoptosis and cytotoxicity selectively in cancerous cells, without significant toxicity in normal counterparts (Eryılmaz et al., 2017).

Similarly, research on AGS and SNU-1 gastric cancer cells demonstrated that usnic acid elevates ROS levels, leading to DNA damage and apoptosis (Kumar et al., 2020). In A549 lung carcinoma cells, the compound was shown to arrest the cell cycle at the G0/G1 phase and activate apoptotic pathways (Singh et al., 2013). These mechanisms are consistent with those observed in the present study, indicating that usnic acid may exert its effects through conserved mitochondrial and oxidative stress-mediated pathways.

In the context of breast cancer, usnic acid has also been reported to enhance the expression of apoptosis-related genes in SK-BR-3 cells, further supporting its role in promoting programmed cell death (Özben & Cansaran-Duman, 2020)). Although the current study focused on a different breast cancer cell line (HS578T), the increase in apoptotic markers observed reinforces the broader applicability of usnic acid's pro-apoptotic potential across breast cancer subtypes.

5. CONCLUSION

The results of this study indicate that usnic acid exerts selective cytotoxic and apoptotic effects on HS578T breast cancer cells. These effects are in line with those reported in previous studies using different cancer cell lines and support the compound's potential as a natural anticancer agent. Given its low toxicity to non-cancerous cells and its efficacy in inducing apoptosis in malignant cells, usnic acid holds promise as a candidate for future anticancer drug development. Nevertheless, further in vivo studies and detailed mechanistic investigations are warranted to fully establish its clinical applicability.

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

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Cytotoxicity and Colony Formation Capacity of Thymol on HS578T Breast Cancer Cell Line

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Abstract: Cancer remains one of the most prevalent and critical public health concerns worldwide, ranking among the leading causes of death alongside cardiovascular diseases. Despite advancements in treatment approaches such as surgery, radiotherapy, and chemotherapy, the side effects and resistance mechanisms associated with these therapies continue to prompt the search for alternative therapeutic agents. Colony formation capacity is a critical parameter for evaluating the long-term proliferative ability and tumorigenic potential of cancer cells. It plays an important role in identifying treatment-resistant cells and assessing the efficacy of anticancer agents. In this context, natural compounds derived from medicinal plants have gained significant interest due to their potential anticancer properties and relatively low toxicity. Thymol, a naturally occurring monoterpene found in plants such as *Thymus vulgaris* and *Trachyspermum ammi*, is known for its antioxidant, antimicrobial, and anticancer activities. This study aimed to evaluate the cytotoxic and colony forming effects of thymol on the HS578T human breast cancer cell line and the non-cancerous HEK293 cell line. Cytotoxicity was assessed using the MTT assay following a 24-hour exposure to various concentrations of thymol. The results demonstrated that thymol reduced cell viability in a dose-dependent manner, with EC_{50} values calculated as 62.98 µM for HS578T cells and 108.87 µM for HEK293 cells, indicating selective cytotoxicity toward cancer cells. Additionally, the impact of thymol on colony formation was investigated to assess its long-term inhibitory effects on cancer cell proliferation. HS578T cells treated with the EC_{50} concentration of thymol for 24 hours were incubated for 10–12 days, after which colonies were stained with crystal violet and analyzed using ImageJ software. The results revealed a significant inhibition of colony formation in thymol-treated cells, with approximately 81% suppression compared to the untreated control group. These findings suggest that thymol possesses promising cytotoxic and anti-proliferative properties against breast cancer cells and may serve as a potential candidate for further development as an anticancer agent. Continued exploration of thymol and its derivatives could contribute to more effective and safer therapeutic strategies in breast cancer treatment.

Keywords: Cytotoxicity, Thymol, Colony formation assay, HS578T

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1. INTRODUCTION

Cancer remains a major global public health concern and is one of the most prevalent diseases of modern times. Fundamentally, it arises from cells that have lost their physiological characteristics and have undergone genetic mutations, resulting in uncontrolled proliferation. Many types of cancer develop resistance or exhibit potential resistance to conventional treatment methods (Zugazagoitia et al., 2016). In developed countries, cancer affects approximately one in every three individuals and, alongside cardiovascular diseases, is one of the two leading causes of death. Globally, cancer accounts for approximately one in every six deaths (Bray et al., 2024).

Currently available cancer treatments include surgery, radiotherapy, immunotherapy, and chemotherapy with potent anticancer (antitumor) drugs. While these therapies are effective, they often significantly reduce the patient's quality of life and can cause severe side effects. Consequently, the search for alternative anticancer agents with fewer side effects has become a major focus in cancer research (Babaç et al., 2023).

Breast cancer is among the most commonly diagnosed cancers worldwide. According to data published by the World Health Organization in 2022, it is the most frequently diagnosed cancer in women, accounting for 23.8% of cases, and the second most common cancer overall after lung cancer, with a global incidence rate of 11.5% (Nicoletto & Ofner, 2022). Breast cancer is categorized into different clinical subtypes based on three molecular markers: estrogen receptor (ER), progesterone receptor (PR), and HER2/Neu amplification. In this study, the triple-negative breast cancer (TNBC) cell line HS578T was used. TNBC lacks all three markers, which makes it more aggressive and difficult to treat. This subtype accounts for approximately 10–15% of all breast cancer cases and is associated with poorer prognoses (Brazell, 2017).

Advances in molecular biology and pharmaceutical chemistry play a crucial role in the development of targeted therapies, ultimately improving patients' quality of life. For this reason, researchers continue to explore natural compounds, chemical agents, and hybrid molecules as potential cancer treatments (Küçükbağrıaçık et al., 2024).

Thymol, a natural monoterpene found in the essential oils of various medicinal plants such as *Trachyspermum ammi* and *Thymus vulgaris*, has gained attention for its pharmacological properties. It is known to exhibit antimicrobial, anti-inflammatory, antioxidant, and antimalarial activities in the human body (Raghuvanshi et al., 2019). Thymol is also widely used in the food industry due to its essential oil content. Despite its natural origin, thymol possesses strong chemical activity and high potential for hybrid molecule derivatization. One of the key features contributing to its potency is its phenol group, which enhances the body's endogenous antioxidant capacity by neutralizing free radicals (Zeybek, 2022).

Thymol is a naturally occurring monoterpene found in plants, known for its antioxidant, antimicrobial, and anticancer properties. Studies have shown that thymol exhibits cytotoxic effects on various cancer cell lines by inhibiting cell proliferation and promoting apoptosis. In this study, the cytotoxic effect of thymol and its impact on colony formation were investigated in the HS578T breast cancer cell line.

2. MATERIAL AND METHOD

The experimental procedures employed in this study have been systematically presented under specific subheadings, each detailing the methodology used to evaluate the cytotoxic and migratory effects of thymol on the selected cell lines.

2.1. Cell culture

In this research, HS578T (human breast cancer cell line) and Hek293 (human embryonic kidney cell line) were utilized. The cells were maintained in DMEM containing 10% fetal bovine serum and 1% penicillin-streptomycin, and incubated at 37 °C in a humidified atmosphere with 5% CO_2. Once cell confluency reached approximately 90%, they were passaged for subsequent experimental procedures (Arslan et al., 2020).

2.2. Determination of cytotoxic activity via MTT test

To evaluate the cytotoxic effects of thymol on HS578T and Hek293 cell lines, an MTT assay was performed. Cells were counted using a Thoma counting chamber with trypan blue staining and seeded into 96-well plates at a density of 2×10^3 cells per well. Various concentrations of thymol (ranging from 20 to 200 µM) were applied through serial dilution. A solution of dimethyl sulfoxide (DMSO, >1%) served as the control. Following a 24-hour treatment period, the medium was replaced with 100 µL of fresh culture medium and 10 µL of MTT solution, and the cells were incubated at 37 °C for approximately 2 hours. After incubation, 50 µL of DMSO was added to dissolve the formazan crystals formed. The absorbance was then recorded at 590 nm using a microplate reader. The EC_{50} value for thymol was calculated based on cell viability data, following the methodology described before (Tüfekçi et al., 2024).

2.3. Colony forming assay

A total of 1×10^3 cells were seeded into 6-well culture plates and incubated at 37 °C in an atmosphere containing 5% CO_2 for 24 hours. After this initial incubation period, cells were treated with the EC_{50} concentration of thymol. Following 24 hours of treatment, the drug-containing medium was replaced with fresh growth medium, which was subsequently renewed every 2–3 days. The incubation was continued for about 10 to 12 days. At the end of this period, the medium was discarded, and the colonies were fixed using cold methanol. Fixed cells were stained with 0.4 mg/mL crystal violet solution. The stained colonies were then visualized and quantified using ImageJ software, and results were compared to the untreated control group (Mutlu et al., 2022).

2.4. Statistical analysis

To ensure the consistency and validity of the findings, all experiments were independently repeated three times. The results for each group are presented as mean values accompanied by standard deviations (± SD). Statistical evaluations, including comparisons between control and treatment groups and intra-group variability, were carried out using GraphPad Prism (version 9). This program facilitated the analysis of data significance and helped identify general patterns and trends across experimental conditions.

3. RESULTS

The cytotoxic effects of thymol on HS578T breast cancer cells and HEK293 human embryonic kidney cells were evaluated using the MTT assay, a standard method for assessing cell viability. Cells were treated with a range of thymol concentrations for 24 hours, allowing for the determination of a concentration-response relationship. Following treatment, MTT reagent was added, and viable cells reduced the tetrazolium dye into purple formazan crystals, which were then quantified by measuring absorbance at 570 nm. A dose-dependent decrease in cell viability was observed in HS578T cells, with an EC_{50} of 62.98 µM, indicating strong cytotoxicity. In contrast, HEK293 cells were more resistant, with a higher EC_{50} value of 108.87 µM. These results demonstrate that thymol selectively induces cytotoxicity in cancer cells while sparing normal cells, which is a promising feature for potential therapeutic applications. (Fıgure 1).

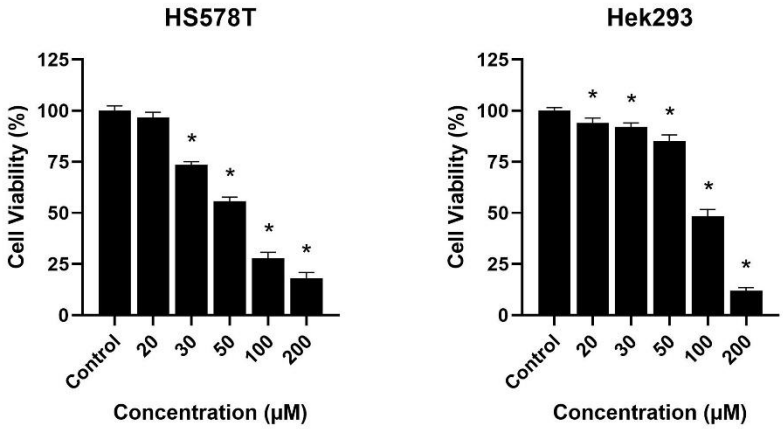

Figure 13: Cytotoxic activity of thymol on HS578T and HEK293 cell lines. Error bars represent standard deviation. * = $P < 0.05$

To evaluate the effect of thymol on the colony-forming ability of breast cancer cells, HS578T cells were treated with the EC_{50} concentration of thymol (62.98 µM) for 24 hours. Following this initial exposure, the treated and control cells were allowed to grow for an extended incubation period of 10 to 12 days under standard culture conditions to enable colony development. Throughout this period, the medium was refreshed regularly to maintain optimal growth conditions. At the conclusion of the incubation, when colonies in the control group reached a confluent appearance, all samples were fixed using methanol and stained with crystal violet to visualize the colonies. Photographs of the stained plates were captured, and quantitative analysis of colony number and area was performed using ImageJ image analysis software. The results clearly indicated that thymol markedly inhibited colony formation in HS578T cells. Specifically, treatment with the EC_{50} dose of thymol led to an approximately 81% reduction in the number of colonies compared to the untreated control group. (Figure 2).

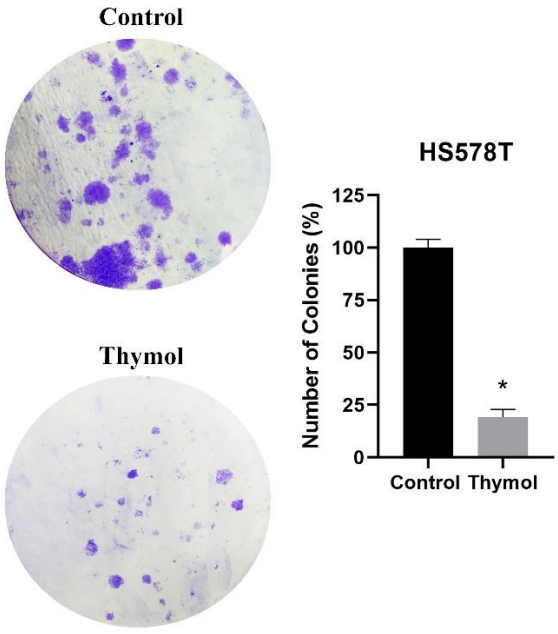

Figure 14: Representative images showing the effect of thymol on colony formation in the HS578T cell line. The bar graph represents the relative change between the control group and thymol-treated wells. * = $P < 0.05$

4. DISCUSSION

Thymol is a natural monoterpene phenol compound found in various plants, particularly in high concentrations in thyme (*Thymus vulgaris*), and has recently emerged as a phytochemical of growing interest due to its anticancer potential. Numerous in vitro studies have demonstrated that thymol exhibits notable cytotoxic effects against various cancer cell

lines, including breast, lung, colon, and prostate cancers. This cytotoxicity is associated with thymol's dose-dependent reduction in cancer cell viability and its ability to activate apoptotic pathways. Moreover, colony formation assays have shown that thymol suppresses the long-term tumorigenic potential of cancer cells, as evidenced by a significant reduction in their proliferation and colony-forming capabilities. Overall, thymol impairs cancer cell survival and inhibits the expansion of tumor cell populations, making it a promising candidate as an anticancer agent.

In this study, the cytotoxic effect of thymol and its influence on colony formation capacity were investigated in the HS578T human breast cancer cell line. As a control, the non-cancerous human embryonic kidney cell line HEK293 was used. The cytotoxic activity of thymol was assessed using the MTT assay. The results demonstrated that thymol exerted a dose-dependent cytotoxic effect on HS578T cells. Treatment with five different concentrations revealed that increasing thymol doses led to a significant inhibition of cell proliferation. Based on the MTT viability data, the EC_{50} values were calculated as 62.98 µM for HS578T and 108.87 µM for HEK293 cells. These findings indicate that thymol exhibits higher cytotoxic activity in breast cancer cells at lower concentrations compared to non-cancerous cells.

The colony formation assay is a widely used *in vitro* method for evaluating the long-term proliferative capacity and tumor-forming potential of cells. To evaluate the effect of thymol on colony formation, HS578T cells were treated with the EC_{50} dose and incubated for 10–12 days. Once the control group reached a confluent appearance, cells were fixed with methanol and stained using crystal violet. Colonies formed were analyzed using ImageJ software. Compared to the control group, thymol treatment led to approximately an 81% reduction in colony formation. This significant inhibition suggests that thymol impairs long-term proliferation and tumorigenic potential in HS578T breast cancer cells.

Llana-Ruiz-Cabello et al. (Llana-Ruiz-Cabello et al., 2014) reported that carvacrol and thymol exerted noticeable cytotoxic influences on Caco-2 cells, a human intestinal epithelial cell line. Their research highlighted a reduction in cell viability that was dependent on both dosage and exposure duration, indicating the potential toxicity of these natural compounds. Morphological assessments revealed significant structural damage, including membrane disintegration, cellular shrinkage, and overall loss of integrity. Notably, thymol induced more severe morphological deterioration compared to carvacrol. These findings underline the importance of considering the cytotoxic potential of these substances when used as food additives or in packaging applications.

In another investigation, a novel compound synthesized by hybridizing artemisinin and thymol (ART-Thymol) was evaluated for its cytotoxic properties across various cancer cell lines (Kavak et al., 2022). The results indicated that ART-Thymol demonstrated a strong cytotoxic effect, particularly in Caco-2 colorectal cancer cells, with an EC_{50} value of 6.03 µM. This enhanced activity appears to stem from the combined cytotoxic characteristics of both artemisinin and thymol. When compared to their individual effects—artemisinin ($EC_{50} \approx 204–156$ µM) and thymol ($EC_{50} \approx 250–293$ µM)—ART-Thymol exhibited significantly higher potency. These findings suggest that ART-Thymol may serve as a promising candidate for future anticancer drug development due to its ability to suppress cancer cell proliferation effectively.

Taibi et al. (Taibi et al., 2024) also examined the cytotoxicity of carvacrol and thymol on various breast cancer cell lines, including MCF-7, MDA-MB-231, and MDA-MB-468. According to MTT assay results, the 1:1 combination of carvacrol and thymol showed stronger cytotoxic effects than either compound alone. The observed IC_{50} values (0.92–1.70 µg/mL) demonstrated a significant decrease in cell viability upon treatment with the mixture. Furthermore, the combination exhibited a high selectivity index (SI: 144.88–267.71), indicating preferential toxicity towards cancer cells over normal cells. These results support the potential of a synergistic interaction between carvacrol and thymol, enhancing their anticancer activity and reinforcing their relevance as natural agents in cancer therapy research.

In a separate study, thymol alone was shown to inhibit cell proliferation in MDA-MB-231 (breast cancer) and HCT-8 (colon cancer) cell lines (Anvarbatcha et al., 2023). Thymol treatment caused cell cycle arrest at the G0/G1 phase and triggered apoptosis. Dose-dependent decreases in viability were observed, along with characteristic apoptotic morphological changes. The compound was also found to elevate intracellular reactive oxygen species (ROS) levels, inducing oxidative stress and initiating apoptotic signaling pathways. Mechanistically, thymol enhanced the activity of the tumor suppressor protein p53 while reducing the expression of anti-apoptotic proteins such as Bcl-xL. This was accompanied by the activation of caspase-9 and caspase-3, suggesting that thymol induces cell death through the intrinsic (mitochondrial) apoptotic pathway. Collectively, these data indicate that thymol selectively targets cancer cells and may hold promise as a natural anticancer drug candidate due to its dual capacity to halt cell division and induce programmed cell death.

5. CONCLUSIONS

In summary, the findings of this study support the potential of thymol as an effective anticancer agent, particularly against human breast cancer cells. Thymol demonstrated a clear dose-dependent cytotoxic effect on HS578T cells, with greater selectivity toward cancer cells compared to non-cancerous HEK293 cells. Furthermore, the substantial reduction in colony formation highlights thymol's ability to suppress the long-term proliferative and tumorigenic capacity of breast cancer cells. These results contribute to the growing body of evidence that thymol, as a natural phytochemical, holds promise for further investigation and development as a complementary or alternative therapeutic option in cancer treatment.

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Conceptualization: K.K.; Investigation: K.K, C.K.; Material and Methodology: K.K, S.A..; Supervision: S.A.; Visualization: K.K, C.K.; Writing-Original Draft: K.K, C.K.; Writing-review & Editing: K.K, S.A.; 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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Cultivation of *Beauveria bassiana* on Rice and Pathogenicity Evaluation Against *Galleria mellonella*

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Abstract: *Galleria mellonella (L.)* is one of the most widespread and economically significant pests causing damage to beekeeping and the storage of apicultural products. The larvae of this species damage wax, cappings, and honeycombs, leading to the weakening of bee colonies and a reduction in apiary productivity. Under ecological constraints and the need to reduce the use of chemical insecticides, the application of entomopathogenic fungi emerges as a promising alternative. In the present study, the pathogenicity of three *Beauveria bassiana* strains was evaluated. The strains were cultivated on rice substrate using solid-state fermentation. The mean conidial yield ranged from 2.3×10^9 to 4.1×10^9 spores/g dry substrate, with viability ranging from 85–95%. For laboratory bioassays, an immersion method was used, in which larvae were submerged in a conidial suspension at a concentration of 1×10^7 spores/mL. The highest virulence was exhibited by strain BAP_2-10**,** causing up to 95% mortality by day 15 after treatment. The other strains also demonstrated significant activity, but with slightly lower lethality. The results confirm the high bioinsecticidal potential of the tested isolates and justify their use as a basis for the development of biological formulations. Future work will focus on large-scale field trials and the development of stable formulations, which will enable the integration of fungal insecticides into comprehensive plant protection and apicultural product preservation strategies in various agroecosystems.

Keywords: *Beauveria bassiana*, *Galleria mellonella*, biocontrol, solid-state fermentation, pathogenicity, entomopathogenic fungus.

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INTRODUCTION

The intensive use of chemical insecticides in agriculture and apiculture has led to a number of serious consequences, including the development of insect resistance, environmental pollution, and risks to human health [1]. In this context, increasing attention is being paid to biological methods of protection, in particular the use of entomopathogenic fungi as microbial insecticides [2].

One of the most studied and widely applied species in biocontrol is *Beauveria bassiana* (Bals.-Criv.) Vuill., which possesses a broad spectrum of pathogenicity against various insects from the orders *Coleoptera*, *Lepidoptera*, *Hemiptera*, and *Orthoptera* [3]. Due to its ability to penetrate the host body through the cuticle, multiply rapidly, and cause death, this fungus is considered a promising agent against pests such as *Galleria mellonella (L.)***,** which causes significant damage to beekeeping, apicultural products, and wax storage facilities [4].

One of the key steps in developing effective *B. bassiana*-based biopesticides is the creation of cost-effective and reproducible cultivation methods with high yields of viable spores [5]. Among various solid-phase substrates, rice is traditionally used as an accessible and efficient nutrient medium, enabling high conidial production at minimal cost.

The present study aims to cultivate *Beauveria bassiana* strains on rice under solid-state fermentation conditions, as well as to evaluate sporulation and the viability of the resulting fungal material. In addition, pathogenicity tests of these strains were conducted against *Galleria mellonella* larvae under laboratory conditions using the immersion inoculation method. The results will help identify the most effective strains and assess their potential for further application in biological control systems for pests in apiculture.

MATERIALS AND METHODS

*Fungal isolates and cultivation***.** Three strains of the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill., isolated from different regions of Southern Kazakhstan — BAP_2-10 BSc_7-16, and $BVes_3$-06— were used in this study. Cultivation was carried out on Sabouraud Dextrose Agar (SDA) at a temperature of 25 ± 0.5 °C in complete darkness for 10 days until abundant sporulation was achieved.

For large-scale spore production, solid-state fermentation on rice substrate was employed: 200 g of rice were soaked in distilled water for 12 h, after which 0.5 % $CaSO_4$ and 0.1 % $CaCO_3$ (w/w) were added. The substrate was sterilized by autoclaving at 121 °C for 20 min. Inoculation was performed with a spore suspension at a rate of 2×10^8 conidia per 100 g of rice. Incubation was conducted at 25 ± 2 °C with a 12/12 h light/dark photoperiod for 14 days [6].

Preparation of inoculum. After completion of fermentation, the biomass was dried to a moisture content of 10–12 %, and spores were separated from the substrate by sieving through a 500 µm mesh stainless steel screen. Spore concentration was determined by direct microscopic counting using a Goryaev hemocytometer at ×400 magnification. The working suspension was prepared in sterile distilled water containing 0.1 % Tween 80, adjusting the final concentration to 1×10^7 conidia/mL [7].

*Assessment of spore viability***.** Spore viability was determined using a germination test. One gram of dry fungal material was resuspended in 10 mL of 0.05 % Tween 80 solution and homogenized on a vortex mixer for 5 min. Tenfold serial dilutions (10^{-2} and 10^{-4}) were prepared from the suspension, and 100 µL aliquots were plated onto SDA Petri dishes in duplicate [8]. Incubation was performed at 26 ± 1 °C for 24 h. After incubation, the percentage of germinated spores was determined by microscopic examination.

Insect rearing. In the experiments, third-instar (L3) larvae of *Galleria mellonella (L.)* were used. The larvae were maintained on a diet of natural beeswax and honeycomb under controlled laboratory conditions: temperature 28 ± 1 °C, relative humidity 50 ± 10 %, and a 16 h light / 8 h dark photoperiod. Prior to the start of the experiment, insects were starved for 24 h to standardize their physiological state [9].

Pathogenicity assessment. The immersion method was used for bioassays, in which larvae were submerged for 5 seconds in prepared conidial suspensions. The control group was treated with an aqueous solution of 0.1 % Tween 80. The experiment was conducted in four biological replicates, with 10 larvae in each replicate. After treatment, the larvae were placed in plastic containers with pieces of honeycomb and maintained under standard rearing conditions. The number of live and dead individuals was recorded on days 3, 5, 7, 10, and 15 after treatment.

Dead *G. mellonella* larvae were transferred to Petri dishes containing moistened filter paper and held for 14 days to observe fungal sporulation. To confirm the fungal etiology of mortality, preparations were made from infected cadavers and examined microscopically [10].

*Statistical analysis***.** Mortality data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

After 14 days of incubation of the strains on rice at 25 ± 2 °C with a 12/12 h light/dark photoperiod, the mean conidial yield ranged from 2.3×10^9 to 4.1×10^9 spores per gram of dry substrate. The highest sporulation was observed in strain $BVes_3$-06 , whereas BAP_2-10 exhibited the lowest yield. Differences in spore production among the strains were statistically significant ($p < 0.05$)

After separation of conidia from the substrate and preparation of suspensions, the spore concentration in the working solutions reached 1×10^7 conidia/mL. Spore viability, determined by germination after 24 h on SDA, ranged from 85 to 95 %. The addition of 0.1 % Tween 80 ensured uniform dispersion of conidia in the suspension (Figure 1).

Figure 1 – Spore germination test of strain BAP_2-10

According to the obtained data, all strains caused a progressive increase in mortality over time; however, statistically significant differences in virulence were found among them (ANOVA, $p < 0.05$) (Table 1).

Table 1 – Mortality rates caused by the tested entomopathogenic strains of *B. bassiana* in *Galleria mellonella*

№	Strains	Concentration	Mortality, days, %				
			3	5	7	10	15
1	BAP_2-10	10^7	0,0	2,5±2,5	15,0±6,4	55,0±2,8	92,5±4,7
2	BSc_7-16	10^7	0,0	25,0±5,0	42,5±6,2	65,0±8,6	82,5±6,9
3	BVes3-06	10^7	0,0	12,5±2,5	32,0±11,0	60,0±8,1	65,0±9,5
Control			0,0	0,0	0,0	0,0	0,0

As shown in the table, the highest pathogenicity was demonstrated by strain BAP_2-10 causing 92,5% mortality by day 15. A comparable level of activity was observed for strain BSc_7-16 with 82,5% mortality. Strain $BVes_3$-06 exhibited moderate virulence, with 65 % mortality. Control individuals remained viable throughout the experiment, indicating the absence of any adverse effect from the surfactant itself.

Mortality in insects generally began on days 3–5 after treatment, with peak mortality levels recorded on days 10–15 (Figure 2).

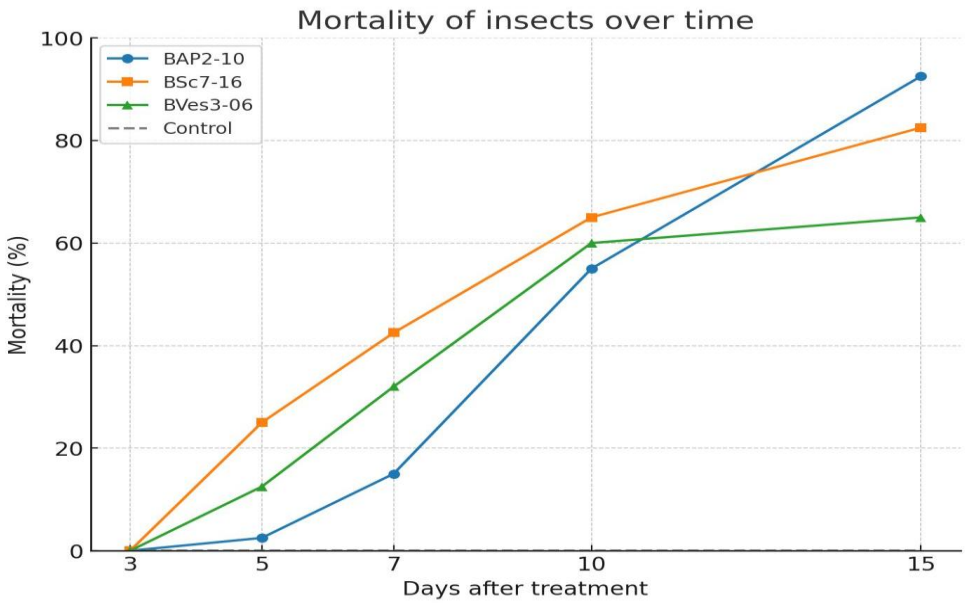

Figure 2 – Mortality rates for different fungal strains

To confirm the fungal etiology, infected individuals were incubated in Petri dishes lined with moistened filter paper for 14 days, during which active sporulation was observed. The presence of *B. bassiana* conidia on the surface of cadavers was also confirmed by microscopic examination.

The results of the present study showed that all three strains of *Beauveria bassiana*, cultivated on rice using solid-state fermentation, demonstrated pathogenicity against *Galleria mellonella* (L.). However, significant differences were observed among them in both conidial yield and virulence.

The highest sporulation was recorded for strain $BVes_3$-06 (4.1×10^9 conidia/g), confirming the effectiveness of rice as a substrate for solid-state fermentation. Nevertheless, despite the high conidial yield, this strain exhibited only moderate pathogenicity, with 75 % mortality by day 15. In contrast, strain BAP_2-10 with a lower sporulation rate (2.3×10^9 conidia/g), showed the highest virulence — 95 % mortality.

This phenomenon highlights that high sporulation does not necessarily correlate with pathogenic activity. Such discrepancies may be attributed to physiological characteristics of the strains, including the degree of adhesion to the insect cuticle, the rate of mycelial germination, the level of toxin and enzyme expression (e.g., proteases and lipases), and the resilience of conidia to environmental conditions.

The immersion method ensured uniform exposure of on the insect body, making it suitable for standardized laboratory bioassays. The addition of Tween 80 provided homogeneous conidial distribution and had no adverse effects, as evidenced by the absence of mortality in the control group.

CONCLUSIONS

The present study demonstrated that all three strains of *Beauveria bassiana*, cultivated on rice substrate, exhibited pathogenic activity against *Galleria mellonella (L.)* larvae. The highest virulence was recorded for strain BAP_2-10, which caused 95 % mortality by day 15 despite having a lower sporulation level. In contrast, strain $BVes_3$-06 achieved the highest conidial yield but displayed only moderate pathogenicity, confirming the absence of a direct correlation between sporulation and infection efficacy. Rice proved to be a suitable, cost-effective, and productive substrate for the cultivation of viable conidia. The immersion inoculation method used was reliable and reproducible, while the addition of Tween 80 had no effect on the survival of the control group. These findings highlight the importance of a comprehensive evaluation of *B. bassiana* strains, considering not only their sporulation capacity but also their virulence characteristics.
However, for successful implementation in practical biological control programs, further research is required to assess efficacy under field conditions, optimize formulation, and refine application methods. Integrating the natural pathogenicity of *B. bassiana* with other biological control strategies within an Integrated Pest Management (IPM) framework may provide sustainable and environmentally safe solutions for managing *Galleria mellonella* and other economically significant insect pests.

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Development of a Biopesticide Based on *Bacillus thuringiensis* for Lepidopteran Pest Control

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Abstract: The increasing resistance of agricultural pests to chemical pesticides and the associated environmental risks highlights the urgent need for sustainable pest management strategies. This study focused on the development of a domestic biopesticide based on the entomopathogenic bacterium Bacillus thuringiensis (Bt) for the control of lepidopteran pests in Kazakhstan. A total of 203 natural substrate samples and 334 insect cadavers were collected from forest ecosystems across several regions, including Ile-Alatau National Nature Park. Thirty bacterial isolates were obtained and identified, and their insecticidal activity was tested against larvae of the apple ermine moth (Hyponomeuta malinella L.) and leafrollers (Tortricidae). Laboratory trials demonstrated that higher spore concentrations (1×10^8 CFU/mL) resulted in significantly greater larval mortality (79–98%) compared to lower concentrations (54–87%). Two isolates, DenP16-29 and OZSh-07, showed the highest efficacy and were selected for field trials. Field applications on wild Malus sieversii trees confirmed high virulence, with DenP16-29 achieving up to 92% mortality of lepidopteran larvae. These findings demonstrate the strong potential of local B. thuringiensis isolates as effective and environmentally safe biopesticides, reducing dependence on imported products and contributing to sustainable pest management in Kazakhstan.

Keywords: Bacillus thuringiensis, biopesticide, lepidopteran pests, Hyponomeuta malinella, leafroller, biological control, Kazakhstan

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INTRODUCTION

The biological approach to agriculture has become a significant global trend. However, discussions about environmental sustainability must be supported by scientific advancements. Chemical treatments are commonly used without considering their inevitable consequences: soil erosion, decline in humus content, destruction of soil flora and fauna, emergence of pesticide-resistant pest strains, negative impacts on wildlife, and ultimately, adverse effects on human health. The range of harmful consequences linked to pesticide use is not diminishing - it is growing, and this trend is expected to continue. Ultimately, the only beneficiaries of widespread chemical pesticide use are the manufacturers themselves [1, 2].

In recent years, there has been a global intensification of research on the development of biological products based on spore-forming bacteria of the genus *Bacillus thuringiensis* (Bt). These Bt-based biopesticides are considered an ecologically safe alternative to synthetic pesticides for crop protection.

One of the key advantages of Bt compared to other microbial agents is its natural origin—commonly isolated from soil, insect cadavers, or other natural substrates—and its wide geographic distribution. Bacillus species possess characteristics that make them highly suitable for the development of biological plant protection agents. These include:

1. The ability to produce antibiotics and insecticidal toxins, which is the primary rationale for their use as bioagents.
2. Spore formation, which enhances their survivability and stability in natural environments.
3. Their natural occurrence in soils.
4. Their safety for humans and vertebrate animals [3, 4].

Unfortunately, Kazakhstan remains entirely dependent on imported biopesticides, leading to high costs and vulnerability to external economic and political factors. The absence of domestic production capabilities for biopesticides based on entomopathogenic microorganisms poses a serious risk to agriculture and the availability of safe pest management tools [5, 6].

The widespread adoption of biological control methods in Kazakhstan is hindered by the lack of local production and the limited efficacy of existing bioproducts, which is often affected by competition with native microflora and regional edaphoclimatic conditions. Taking these challenges into account, the biotechnology laboratory of the Kazakh Research Institute for Plant Protection and Quarantine is working on the development and implementation of bacterial and fungal biopesticides based on local microbial strains, tailored to the phytopathological conditions of agricultural crops [7, 8, 9].

Despite global advances, Kazakhstan significantly lags behind developed countries in the use of biological pesticides. The application of biopesticides to crop fields, plantations, and orchards remains minimal. Furthermore, there is currently no industrial production of domestic biopesticides. Meanwhile, an unfavorable phytosanitary situation is recorded on more than 70% of the country's cultivated lands, and pest resistance to chemical pesticides is widespread. This situation has resulted in decreased yields, substantial economic losses, and pushes agricultural systems closer to the point of no return in pest management [10, 11, 12].

Given this context, the development of local biopesticides based on indigenous microbial strains with high virulence against agricultural pests becomes imperative [13].

OBJECTIVE

The aim of this study is to develop a liquid culture-based biopesticide using the entomopathogenic bacterium *Bacillus thuringiensis* for the biological control of lepidopteran pests under the conditions of the Almaty region.

MATERIALS AND METHODS

Standard methods in microbiology and plant protection were employed throughout the study. Research objects included *Bacillus thuringiensis* isolates collected from insects showing signs of bacterial infection and from natural substrates (soil, fallen leaves, tree bark). Test insects included the leafroller (*Tortricidae*) and larvae of the apple ermine moth (*Hyponomeuta malinella* L.).

Research was conducted in the biotechnology laboratory of the Zh. Zhiembaev Kazakh Research Institute for Plant Protection and Quarantine and in forest zones of the Ile-Alatau State National Nature Park (SNNP).

Results

Insects with symptoms of bacterial infection and natural substrates were collected from ribbon pine forests of the state nature reserves "Ertis Ormany" in Pavlodar region and "Semey Ormany" in Abay region. Additional sampling was conducted in Borovoe forests (Akmola region) and Eastern Kazakhstan. In Almaty region, samples were collected from the Ile-Alatau SNNP and forest belts of Kegen, Enbekshikazakh, and Karasai districts.

These areas were selected due to their rich biodiversity and minimal anthropogenic impact, such as pesticide exposure, deforestation, pollution, and urbanization.

A total of 203 natural substrate samples and 334 insect cadavers from various orders were collected. Among these, 241 insect cadavers were analyzed (Figure 1).

Figure 1 – Insect cadavers with signs of bacterial infection

From these samples, 30 bacterial isolates were obtained and identified at the species level. Their morphological, cultural, and select biochemical characteristics were studied. Their biological activity against larvae of leafrollers, apple ermine moth, greater wax moth, cabbage butterfly, and geometrid moths was tested under laboratory conditions.

From the laboratory trials, five newly isolated strains and six collection strains were selected for small-plot experiments against apple ermine moth and hawthorn leafroller larvae. Experimental results are shown in Figures 2 and 3.

Experiments on apple trees were conducted in mesh cages using spore concentrations of 1×10^7 and 1×10^8 CFU/mL. Figure 2 presents data on the biological activity of these strains against H. malinella.

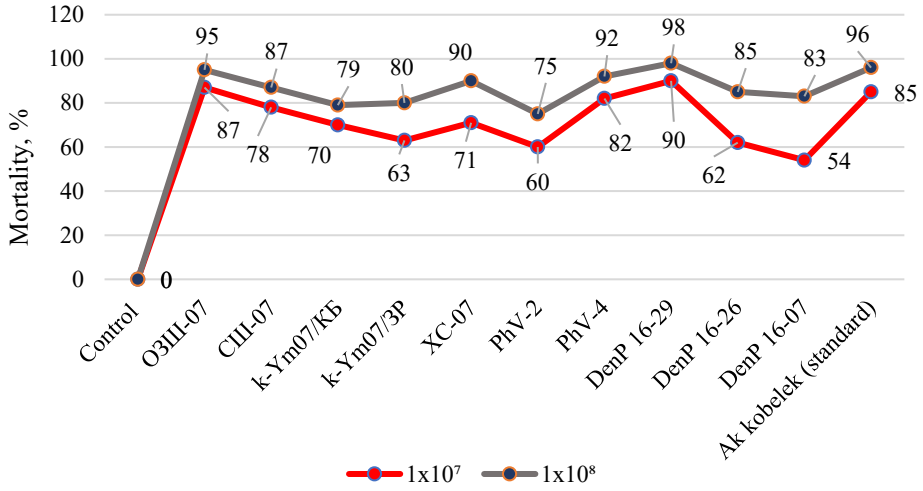

Figure 2 – Biological activity of *Bacillus thuringiensis* strains against H. malinella larvae (2023–2024 small-plot trials)

As shown in Figure 2, the 1×10^7 CFU/mL concentration yielded lower mortality compared to 1×10^8. Mortality at the lower concentration ranged from 54% to 87%. At 1×10^8, mortality increased to 79–98%. The highest mortality rates were recorded for isolates OZSh-07 (95%), Hs-07 (90%), PhV-2 (92%), Den 16-29 (98%), and the reference strain "Ak Kobelek" (96%). For field trials, isolates OZSh-07 and Den 16-29 were selected. Similar results were obtained for hawthorn leafroller larvae (Figure 3).

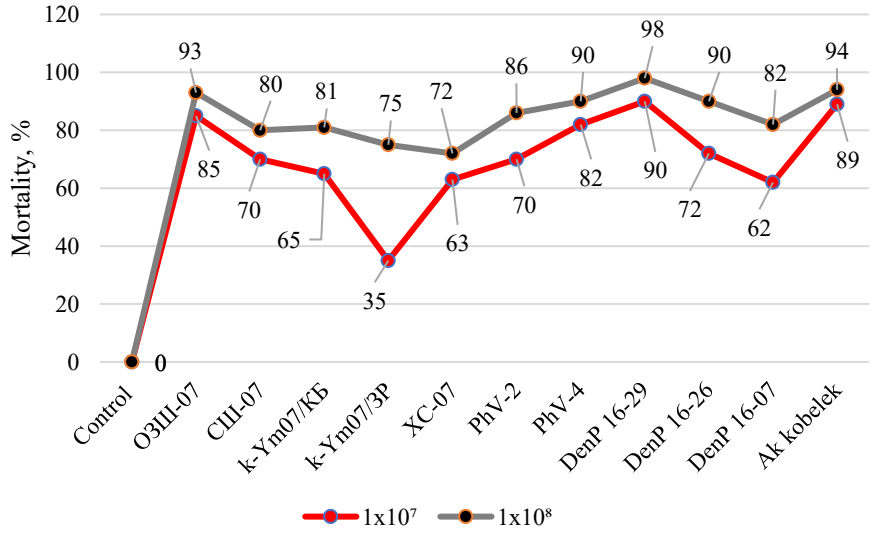

Figure 3 – Biological activity of *Bacillus thuringiensis* strains against hawthorn leafroller larvae (2023–2024 small-plot trials)

On day 5 after treatment with a 1×10^7 spore concentration, mortality ranged from 35% to 90%. The lowest efficacy was observed with strain k-Ym07/ZR (35%). Other strains—SSh-07, k-Ym07/KB, Hs-07, PhV-2, DenP 16-26, and DenP 16-07—demonstrated mortalities of 70%, 65%, 63%, 70%, 72%, and 62%, respectively.

At 1×10^8 CFU/mL, all tested strains showed high activity. Strains OZSh-07, PhV-4, DenP 16-29, and DenP 16-26 resulted in over 90% mortality. The reference strain "Ak Kobelek" yielded 94%.

Based on these results, isolates OZSh-07 and DenP 16-29 were selected for preparation of experimental culture liquids.

Field treatments were conducted on wild Malus sieversii apple trees in the Ile-Alatau SNNP at an elevation of 1800 meters in Talgar district, Almaty region. Tree infestation rates reached up to 100%. Application volume was 5 L per tree, with a spore titer of 1×10^8 CFU/mL. Infestations were patchy and localized. Treatments were applied using Solo 450 backpack sprayers. Results are presented in Figure 4.

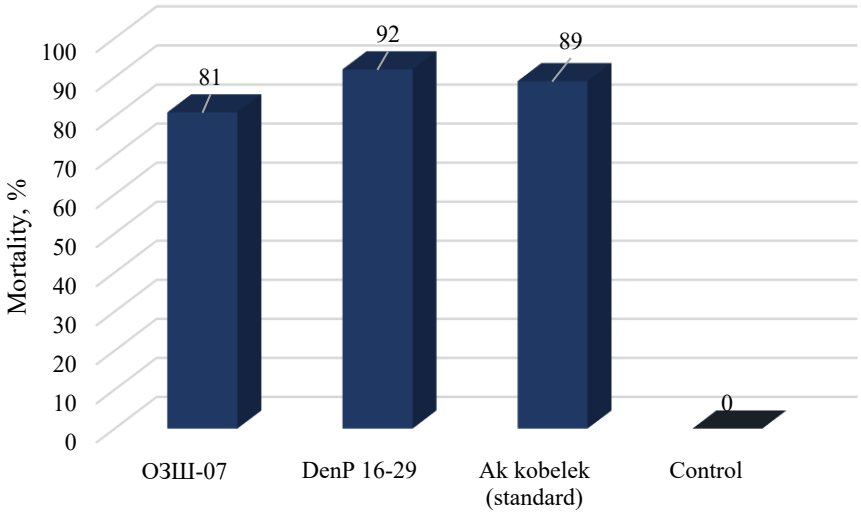

Figure 4 – Biological efficacy of culture-liquid formulations based on B. thuringiensis strains, 2024

According to Figure 5, B. thuringiensis strain DenP16-29 caused 92% mortality, OZSh-07 caused 81%, and the reference strain “Ak Kobelek” 89%. No mortality was observed in the control group.

These findings confirm the high virulence of DenP16-29 against various lepidopteran larvae on fruit and vegetable crops. In particular, the culture-liquid formulation demonstrated significant efficacy against apple ermine moth larvae in field conditions on wild M. sieversii. Further studies are planned to continue this line of research.

CONCLUSION AND DISCUSSION

One of the key aspects of effective pest management is early detection. This allows for timely intervention and minimizes damage. The use of pheromone traps enables early pest detection. Regular monitoring can identify early signs such as leaf damage or the presence of larvae and adults. These and other proactive measures help control phytophagous pests at the initial stages, improving control effectiveness and reducing the need for chemical pesticides.

Thus, early detection is not merely a strategy - it is an essential condition for successful and environmentally responsible pest management.

At the same time, the development of biopesticides based on entomopathogenic microorganisms is a cornerstone in modern pest control. These products provide not only effective pest suppression but also serve as ecologically sound alternatives to chemical pesticides. The use of bacteria such as *Bacillus thuringiensis* helps reduce environmental impact and preserves biodiversity.

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