



ISSN: 2707-1146  
e-ISSN: 2709-4189

# Nature & Science

International Scientific Journal



# **Nature & Science**

**International Scientific Journal**

**Volume: 8 Issue: 3**

**2026**

## International Indexing



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## Soil Protection: A Strategic Priority of the Soil Museum

Rena Mirzezadeh<sup>1</sup> , Gunay Mammadova<sup>1\*</sup> 

**Abstract.** Azerbaijan's soil resources constitute a vital part of the country's natural capital, playing a key role in maintaining ecological balance, supporting agricultural productivity, and ensuring sustainable development. However, growing anthropogenic pressures, ongoing land degradation, and climate-related impacts have increasingly threatened soil quality and functionality. This study explores soil protection as a strategic focus within the activities of the Soil Museum operating under the Institute of Geography. Although soil conservation has been among the museum's primary objectives since its establishment, its integration into the institutional framework of the Institute has significantly expanded its scope. Today, the museum functions not merely as a storage space for soil monoliths and scientific collections, but also as a scientific, educational, and informational center where soil is presented as an integral element of the geographical environment. The research particularly emphasizes that soil, as a fundamental component of geographical systems, is closely interconnected with climate, relief, water resources, vegetation, and human activities. This integrated geographical approach enhances the scientific value of the museum by enabling the study of soil diversity within the broader context of natural-territorial interactions that shape landscapes and ecological processes across Azerbaijan. Soil monoliths serve as effective tools for demonstrating soil diversity, environmental conditions, and degradation patterns. The results indicate that soil protection is not only a scientific concern but also a key element of environmental education, public awareness, and sustainable land use. From a geographical perspective, degradation levels vary across regions depending on relief, climate, irrigation practices, and human influence.

**Keywords:** soil protection, soil museum, soil monoliths, geographical ecosystems, land degradation, environmental education, Azerbaijan

### Introduction

Soil represents one of the most critical natural assets, forming the basis of terrestrial ecosystems, agricultural systems, and overall environmental stability (Mirzezadeh et al., 2025). Nevertheless, both the availability and quality of soil resources are steadily declining on a global scale. Unsustainable land management, industrial contamination, erosion, salinity issues, and climate change are among the primary factors accelerating soil degradation and reducing its productive potential (Ganieva et al., 2019; Shukurov et al., 2025b).

In Azerbaijan, these challenges are particularly pronounced due to the country's diverse physical-geographical conditions, including mountainous regions, arid plains, foothill areas, and humid subtropical zones (Mammadzada et al., 2025; Verdiyeva et al., 2025).

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Received: 15 February 2026; Accepted: 18 May 2026; Published online: 25 June 2026

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Such environmental heterogeneity has led to the formation of highly diverse soil types, each characterized by distinct ecological roles, agricultural significance, and varying levels of vulnerability to human-induced pressures (Huseynova et al., 2024).

Rapid population growth, economic expansion, and increasing demands for food production have further intensified pressure on land resources (Ismayilova et al., 2025a). As a result, the amount of arable land per capita is gradually decreasing, while the risks associated with soil degradation continue to rise (Sadigov et al., 2026). Under these circumstances, the efficient use, systematic monitoring, and protection of soil resources have become pressing priorities from scientific, environmental, and socio-economic perspectives (Ismayilova et al., 2025b).

Beyond its role in production, soil functions as a vital component of the biosphere, contributing to the regulation of biogeochemical cycles, water dynamics, carbon sequestration, and the stability of ecosystems (Shukurov et al., 2025a). Therefore, soil conservation should be addressed through an integrated ecosystem-based approach that considers environmental, economic, and social dimensions simultaneously (Sadigov et al., 2025).

Within the research framework of the Institute of Geography, soil is examined as an inseparable part of the geographical environment, closely interacting with climate, relief, hydrological conditions, vegetation cover, and land-use systems. This interdisciplinary approach aligns with the strategic priorities of the Ministry of Science and Education of the Republic of Azerbaijan, which emphasize the advancement of scientific research, environmental awareness, and sustainable natural resource management (Nasirova et al., 2026).

In this regard, the Soil Museum gains particular scientific importance, as it not only ensures the preservation of soil collections but also facilitates the geographical interpretation of soil diversity, degradation processes, and environmental transformations across Azerbaijan.

## **Materials and Methods**

The present study is grounded in the analysis of soil types exhibited in the Soil Museum functioning within the scientific framework of the Institute of Geography. The museum's collection constitutes a valuable scientific resource for examining soil diversity, spatial distribution patterns, and degradation processes across Azerbaijan (Gurbanov et al., 2021). In line with the broader research agenda of the Ministry of Science and Education of the Republic of Azerbaijan, the museum acts as a specialized platform that supports the integration of soil research with geographical and ecological evaluations.

The research materials include:

- Soil monoliths obtained from various ecological and geographical regions of Azerbaijan, representing key soil types developed under diverse climatic and landscape conditions
- Soil samples analyzed through physical, chemical, and biological parameters that reflect soil formation processes, fertility status, and human-induced changes
- Archival documents, historical sources, and scientific publications related to soil classification systems and soil conservation practices
- Soil maps, landscape imagery, and supporting descriptive materials illustrating the spatial distribution of soils and associated environmental settings

Special emphasis is placed on monoliths that reflect both intact and degraded soil conditions. These include samples affected by erosion, salinity, and technogenic disturbances, providing an opportunity to evaluate contemporary soil protection issues across different geographical zones (Akhundova et al., 2025; Ismayilov et al., 2025). The study employs an integrated methodological approach

combining comparative soil analysis, morphogenetic evaluation of soil profiles, ecological and geographical assessment, museum-based scientific interpretation.

The methodology of this research relies on fundamental concepts of soil science while incorporating up-to-date ecological and geographical approaches, together with relevant practices adopted in soil museums in countries such as Russia and Moldova (Mammadova et al., 2026).

A key component of the methodology is the structured scientific presentation of soil monoliths. Each profile is supplemented with detailed explanatory information covering its origin, classification features, geographic setting, ecological characteristics, and the level of human impact (Fig. 1). From the standpoint of geographical analysis, soil monoliths are not treated solely as separate pedological units. Instead, they are viewed as representations of broader landscape dynamics, illustrating the interdependence between soil formation processes and factors such as climate, topography, vegetation, and land use patterns (Shukurov et al., 2026).



**Figure 1**  
*Soil monoliths collection*

## Results and Discussion

Soil performs a fundamental role in sustaining biological cycles, preserving biodiversity, and ensuring environmental balance within geographical systems. It serves multiple functions at once, acting as a natural filtration medium, a source and storage of nutrients, a substrate for plant development, and a regulator of both water movement and carbon sequestration. As part of the geographical environment, soil remains in constant interaction with climate conditions, terrain features, vegetation cover, and hydrological processes, which makes it a highly dynamic component in the formation and evolution of landscapes (Gahramanova et al., 2026).

At the same time, soil increasingly becomes a recipient of various anthropogenic pressures, including industrial pollutants, oil-derived contaminants, heavy metals, and agrochemicals. The accumulation of these substances not only results in contamination but also alters soil structure, decreases biological activity, and reduces its ecological effectiveness (Mammadova et al., 2024). The soil cover of Azerbaijan is distinguished by its exceptional diversity, which is closely linked to the country's complex physical-geographical structure. The interaction of altitudinal zonation, significant climatic

variability, and heterogeneous landscape conditions has resulted in the development of a wide range of soil-forming environments. These factors contribute to the formation of highly differentiated soil types with distinct morphological, physical, and ecological characteristics (Mammadova et al., 2026).

From a geographical perspective, Azerbaijan represents a unique natural system in which diverse soil types are distributed within a relatively limited spatial extent (Hasanova & Mammadova, 2023). Major soil groups represented in the museum collection include mountain-forest soils, steppe and semi-desert soils, subtropical soils, alluvial and irrigated soils. Each soil type demonstrates specific morphological, physical, and chemical properties that determine both its ecological role and agricultural potential. Their comparative representation within the museum allows observation of how geographical conditions directly influence soil genesis and functional characteristics (Mirzazadeh et al., 2025).

The Soil Museum functions as a specialized scientific unit within the Institute of Geography, where soil diversity is examined through the lens of geographical and ecological interactions, rather than being limited solely to classification-based approaches. Its main functions include: 1. Scientific function: preservation, systematization, and scientific interpretation of soil materials. 2. Educational function: training students, researchers, and specialists in soil science, geography and environmental studies. 3. Informational function: raising public awareness of soil conservation and land degradation issues.

The museum's collection includes soil monoliths representing major soil types and subtypes, samples of degraded and technogenic polluted soils, scientific literature, maps, archival materials, and historical records. A particularly important section is devoted to soil-contaminated soils of the Absheron Peninsula, where long-term industrial activity has visibly transformed soil morphology, chemical composition, and ecological functions (Akhundova et al., 2025). These materials provide valuable evidence for understanding anthropogenic transformation of landscapes.

Soil monoliths provide a direct visual representation of soil profiles and allow detailed analysis of: genetic horizons, soil formation processes, degree of degradation, anthropogenic transformations. From an educational perspective, soil monoliths play an important role in translating abstract pedological concepts into observable reality, allowing clear demonstration of structural variations among soil types, forms of degradation, and ecological adaptations. Moreover, monolith collections serve as a reference record of baseline soil conditions, providing a valuable foundation for future comparative studies aimed at assessing long-term environmental and landscape changes (Bunyatova et al., 2025).

The museum materials clearly reflect the major forms of soil degradation currently affecting Azerbaijan: erosion, salinization, overgrazing, industrial pollution. These processes lead to a decline in soil fertility, reduce the stability of ecosystems, and pose challenges for sustainable agricultural utilization. This confirms that soil protection must be integrated into wider environmental and territorial planning policies. One of the strategically important directions is the development of a national "Red Book of Soils", analogous to biodiversity conservation frameworks. This concept includes identification of rare and endangered soil types, protection of unique soil ecosystems, preservation of soil genetic diversity.

The scientific collections maintained by the Soil Museum serve as a valuable empirical foundation for soil classification, as they preserve representative soil profiles that could be subject to degradation or even loss in the future. From a geographical perspective, this effort also plays a significant role in recording environmentally vulnerable areas and enhancing the scientific basis for land conservation policies. The results of this study demonstrate the practical importance of strengthening the role of museum-based soil science within national scientific and educational priorities of Azerbaijan.

The study highlights the importance of integrating soil protection into national environmental and land-use policy, expanding museum-based environmental education, strengthening interdisciplinary links between soil science and geography, developing regional soil museums and scientific information centers. The Soil Museum may serve as a model for scientific communication, sustainable development strategies, public participation in environmental protection, preservation of natural scientific heritage.

## Conclusion

1. Soil conservation is recognized as a major scientific and environmental priority for ensuring sustainable development and maintaining ecological balance.
2. Within the structure of the Institute of Geography, the Soil Museum serves not only as a repository but also fulfills significant scientific and educational functions.
3. Soil monoliths constitute important research materials for studying soil diversity, spatial distribution, and processes of degradation.
4. The initiative to establish a national “Red Book of Soils” represents a crucial measure for the protection of rare soil types and the preservation of soil genetic diversity.
5. Increasing public awareness, promoting scientific knowledge, and applying geographical approaches to soil processes are key elements in developing effective soil protection policies.

## Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Diagnosics of Irrigated Soils in the Dry Steppe and Semi-Desert Zones of Azerbaijan

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**Abstract.** Research has established that soil formation in irrigated soils in the dry steppe and semi-desert zones of Azerbaijan occurs under conditions of a prolonged warm period (mild winters and hot, dry summers). The most characteristic diagnostic indicators of irrigated, rainfed, and virgin soils in this zone have been determined. It has been established that, depending on the duration of irrigation, the particle size distribution of gray-brown soils varies from heavy to light loams. The size of water-stable aggregates > 0.25 mm is 46–56%, and humus is 2–3%. Humus has a humate-fulvate composition, basic water absorption capacity, and pH values in aqueous suspension of 7.4–8.6. Gray soils have a light clay texture, contain 1.4–2.3% humus, have a humate-fulvate composition, and an alkaline pH of 21–36 mg-eq per 100 g of soil. Meadow gray soils have a heavy clay texture, contain 1.4–2.9% humus, and have a humate-fulvate humus composition. They have high absorption capacity and an alkaline pH.

**Keywords:** soil formation, gray-brown soils, sierozem soils, meadow-gray soils, humus

### Introduction

Soil diagnostics for irrigated areas in dry steppe and semi-arid zones aims to identify changes in soil properties under the influence of agricultural use, leading to processes such as gleying, salinization, and compaction. The main diagnostic indicators and processes occurring in irrigated soils include changes in morphogenetic parameters (Gurbanov et al., 2021; Mammadova et al., 2022; Gahramanova et al., 2026). This leads to the transformation of irrigated soils within the soil profile in dry steppe and semi-desert zones. The formation of the main properties of irrigated soils is influenced by the composition of irrigation water, which determines the degree of fertility and the possibility of secondary salinization (Babayev et al., 2020a; Ismayilova et al., 2025). Deterioration of physical properties is diagnosed, especially in long-irrigated soils. For diagnostics, a comprehensive analysis is used, including a description of the soil profile in the field, as well as physicochemical analyses (humus, carbonates, absorbed bases) (Babayev et al., 2023; Mammadova et al., 2024). Soil processes can be assessed by various indicators: morphological, physicochemical, and biological (Ganieva et al., 2019; Babayev et al., 2020b).

The intensity of soil processes, fertility, and the type of soil formed here directly depend on the nature of the vegetation growing in a given area (Babayev et al., 2020b; Mirzezadeh et al., 2025).

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Received: 6 February 2026; Accepted: 22 May 2026; Published online: 25 June 2026

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Soil formation in these zones occurs in dry steppe and semi-desert climates, characterized by long hot periods, winters with little precipitation, and hot, dry summers (Gurbanov et al., 2021; Mirzazadeh et al., 2025).

Much heat, long vegetation period, absence of salinization and natural drainage create a chance for growing of the valuable subtropic plants in irrigation (Akhundova et al., 2019; John et al., 2021; Akhundova et al., 2025).

In this regard, it is important to study the current state of such types of soils in the dry steppe and semi-arid zones of Azerbaijan.

*Aim of the research.* The aim of the research is to study the current state of morphogenetic characteristics of virgin and irrigated soils in the dry steppe and semi-arid zones of Azerbaijan.

## Materials and Methods

The research was conducted in 2019–2023. The object of the study is gray–brown (dry steppe zone, Ganja–Kazakh massif, Aghstafa region), gray and meadow-gray soil (semi-arid zone, Kura–Araks lowland, Kurdamir region) soils (Fig. 1, 2).



**Figure 1**

*Detailed soil map of the Aghstafa region*



**Figure 2**

*Detailed soil map of the Kurdamir region*

*Aghstafa region* (Fig. 1.) is situated in the western part of the little Caucasus (in the settlement named after S. Vurghun of the Aghstafa district). Its absolute altitude is 351 m above sea level, it has coordinates; 41°06'11,17"N, 45°28'07,33" E. The zone is characteristic with mild-hot, dry and hot summer subgroup climate and softer winter. An averagely annual temperature is 13,49 °C, minimum 13,20 °C, maximum 38 °C. A sum of the active temperature is 3900–4000 °C.

General droughtiness and low humidity coefficient are a reason for collection of gypsum in virgin ordinary grey-brown soils and collection of easily soluble carbonate salts in the irrigative water regime condition. There is a human's great effect on formation of soil structure. Grey-brown irrigated soils spread in the plain part of the relief, at a height of 152–252 m from sea level (Salayev et al., 2004).

The water source, limpid ganat and artesian waters humidity regime create condition for weak movement of the soluble combinations. The granulometric composition of the cultivated layer (41–46 cm) changes from gradually weakly cultivated, newly irrigated soils to highly cultivated irrigated ones. C:N ratio (9–12) is larger than virgin soils (9–10). Composition of humus is humate type. A

sum of the absorbed bases is high – 29–36 mg-eq in 100 g of soil and pH indicators rise in water suspension (7.6–8.6) (Salayev et al., 2004). Irrigated arid field soils spread in the oldest agricultural zones and develop with the accompaniment of dependent floats and nutrients brought by turbid waters in the leached irrigation automorph regime. This is constant renewal of its cultured layer and the formation of agro-irrigation layer with a thickness of 91–101 cm and more, which determines the diagnostic of the soil) (Salayev et al., 2004; Shukurov et al., 2025).

*Kurdamir region.* The region occupies an area of the Kurdamir state soils (Fig. 2). It surrounds an east circle of the Shirvan plain (40°20'18"N; 48°09'39" E). The soils are gray and meadow (Salayev et al., 2004). An absolute height is 69,4–70,5 m above sea level. Smoothing perverts the first relief. Groundwater is at a depth of 2,7–3,0 m, an average mineralization degree is 5,2–7,6 g/l, but a type is sulfate chloride–natrium. The noticeable deep layers of the soil profile affect the level of groundwater, sometimes absorption of water into the upper layers weakens, filtration of irrigative waters increases. The presence of historical information of anthropogenic origin in the soil profile indicates its antiquity. Soil-forming rocks are adopted as aluvial and alluvial-proluvial sediments (Salayev et al., 2004).

The physical and chemical properties of soils were determined using generally accepted methods (Arinushkina, 1970; Methodological Recommendations for the Study of Soil Fertility Indicators, Humus and Nutrient Balance in Long–Term Experiments, 1987).

## **Results and Discussion**

### *Soils of the dry steppe zone*

Human economic activity has significantly influenced the formation of the soil cover structure of the gray–brown soils of the dry steppe zone. Depending on the nature and duration of development, the degree of change in the morphological and genetic structure and physicochemical properties of soils, a number of irrigated soil types are also proposed, along with virgin zonal soils. Let's compare the most characteristic diagnostic indicators of virgin, rainfed, and irrigated soils in the zone (Table 1).

Irrigated dry steppe soils spread in the oldest agricultural zones and develop with the accompaniment of dependent floats and nutrients brought by turbid waters in the leached irrigation automorph regime. This is constant renewal of its cultured layer and the formation of agro-irrigation layer with a thickness of 91–101 cm and more, which determines the diagnostic of the soil.

**Table 1**  
*Diagnostic indicators of gray–brown soils of the dry steppe zone*

Indicators	Virgin	Boharic		Irrigated		Anciently irrigated	
		Plowed	Sowing	Newly irrigated	Irrigated	Irrigated	Anciently irrigated
Thickness of cultivated layer or humus layer, cm	31–36	36–41	31–36	41–46	46–51	57–61	61–71
Depth, cm: maximum collection of carbonate gypsum layer salt layer	36–41	41–51	36–41	61–91	91–101	–	–
	91–111	101–111	91–101	101–131	131–161	–	–
	151–161	156–166	151–161	161–201	–	–	–
A rate of silt, %	36–41	31–36	–	41–51		41–51	
A quantity of water-resistant aggregate (> 0.25 mm), %	51–59	36–46	46–56	41–51	51–56	56–66	66–76
Density, g/cm <sup>3</sup>	1,2–1,3	1,2–1,3	–	1,1–1,3	1,3–1,4	1,1–1,4	1,1–1,3
Total humus, %	1,6–3,6	1,1–2,6	2,6–3,1	2,1–2,6	2,6–3,1	3–4	4–5
C:N	7–10	6–7	7–8	7–12	8–11	9–12	10–14
Ch:Cf	0,9–1,1	–	–	0,9–1,4		1,1–1,9	
A sum of absorbed bases, mg/eq in 100g soil	31–36	31–33		31–36		31–41	
Ca:Mg	4–6	2,6–5,6	1,6–2,1	3–6	2,6–5,1	3–5	4–6
Suspension of pH	7,1–8,1	8,2–9,3		7,6–8,6		8,1–9,1	
CO <sub>2</sub> filthiness in soil air, %	0,2–0,3	0,08–0,22		0,3–0,4		0,4–0,6	

Most gray–brown faience's are found at a depth of 70–100 cm, the clay pots, cinnamonic and dark brown bricks are at a depth of 150–200 cm. A cultivated layer with a depth of 61–71 cm consists of buried – ancient virgin and cultivated soils. Quick–dissolved dusty – siltation is superior in granulometric composition and its profile is homogenous, siltation rate is 41–51%.

A size of water – resistant aggregates is > 25 mm, high 66–76%. Humus in the cultivated layer is 4–5% (4–5% – high, 1.4–5% – average, 2.6–3.1 – weakly cultivated). C:N – humus is more than the irrigation and virgin soils (10–14). A composition of humus is fulvate – humate or humate.

The uniform distribution of calcium carbonate across the profile is 7–11%. The amount of absorbed bases is higher – 31–41 mg-eq per 100 g of soil.

#### *Soils of the semi – desert zone*

Such soils are formed in the subtropic climatic condition i.e.dry, long hot period, soft winter with little rain, hot dry summer condition, they have high assimilation in the irrigation condition. Some irrigated soils differ depending on antiquity of irrigation and change of perennial cultivation process. A special ecological development condition, morphogenetic and physico-chemical diagnostic indicators are characteristic for one of them (Table 2, 3).

Irrigated sierozem soils occupy a high foothill zone (from 51–61 m to 151–161m above sea level) with natural drainage and develop in the irrigation regime condition of humidity. A source of humidity is atmospheric sediments and limp irrigative waters are mainly ganat and artesian waters.

**Table 2**

*The main diagnostic indicators of sierozem soils of the semi-desert zone*

Indicators	Virgin	Irrigated		Anciently irrigated	
		Newly irrigated	Irrigated	Irrigated	Anciently irrigated
Thickness of cultivated layer or humus layer, cm	26–31	31–36	36–46	41–51	51–61
Depth, cm: maximum collection of carbonate gypsum layer salt layer	26–31	51–71	71–91	–	–
	101–201	71–131	131–151	–	–
	131–121	111–151	–	–	–
A rate of silt, %	31–41	31–41	41–46	41–46	46–61
A quantity of water-resistant aggregate (>0,25 mm), %	26–36	–	–	51–61	61–71
Density, g/cm <sup>3</sup>	1,4–1,5	–	–	1,4–1,5	1,2–1,4
Total humus, %	1,6–2,1	1,4–1,8	1,8–2,3	1,6–2,6	2,6–3,1
C:N	6–9	9–10	10–11	9–11	11–16
Cht:Cft	0,6–0,7	1,2–1,3	–	1,2–1,6	
A sum of absorbed bases, mg/eq in 100 g of soil	19–29	21–31		26–36	
Suspension of pH	7,6–8,6	8,1–9,6	8,1–9,6	9,0–9,9	9,0–9,9
CO <sub>2</sub> filthiness in soil air, %	–	0,23–0,31	0,23–0,31	0,18–0,44	0,18–0,44

The cultural soilforming process is created, the cultivated layer with thickness of 31–46 cm, fine structure, subsoil hardening are observed while the assimilation period increases. The initial signs of sierozem soils: the profile monotony, carbonates separation, etc. remain. The irrigated sierozem soils have 1.4–2.3% in the plow layer (1.4–1.8% weak, 1.8–2.1% average, 2.1–2.3% high). C:N is more larger (9–11), but 6–9 in virgin soils. CaCO<sub>3</sub> quantity is high (6–11%) in the plow layer, distribution is the same along the profile.

Light, loamy, dusty particles are superior in granulometric composition: a silty degree is 36–46%. An amount of the absorbed bases is 21–36 mg-eq in 100g soil, (21–26 mg-eq weak, 26–31 mg-eq – average and 31–36 mg-eq – high cultivation). Ca:Mg ratio is 1.1–1.8, (according to cultivation degree 1.1–1.3; 1.3–1.6; and 1.6–1.8). An inclination of profile to saltiness is noted in the weak cultivated variants (formation depth – 111–151 cm) and solonetzification (pH – 9.6–9.8 quantity N<sub>3</sub>–11–16%, 31–51 cm).

Irrigated semi-desert soils spread in the anciently irrigated regions and develop in the zones with deep subsoil waters. Great changes occur in grey soils under an influence of prolonged irrigation with turbid waters and yearly cultivation.

Agro-irrigation layers with the depth of 91–101 cm are created. The upper layer of 41–51 cm is dark-grey, humic, well-used and modern cultivation. The lower transition (41–51cm) is grey, calcareous, non-gypsum, the skull bones, red brick fragments and coals are often found. Agro-irrigation layer is covered with the ancient buried soils. The granulometric composition of the cultivated layer is mainly light loamy dusty silty. The water – resistant aggregates with the size > 0.25 mm are higher – 51–71

%. 1.6–3.1% of humus is in the cultivated layer, (1.6–2.1% – weak, 2.1–2.6% – average, 2.6–3.1% – highly cultivated). A composition of humus is Cht:Cft – 1.3–1.6, 11–18% alkalized carbonates of the plowed layers, CaCO<sub>3</sub> is distributed along the profile. A quantity of the absorbed bases is high – 26–36 mg-eq in 100 g of soil and highly alkalization of the quick – dissolved salts is available.

**Table 3**

*The main diagnostic indicators of meadow-gray soils in the semi-desert zone*

Indicators	Virgin meadow-gray	Irrigated meadow-gray soils			Anciently irrigated meadow-gray soils		Boharic meadow-gray soils
		Newly irrigated	Irrigated	Anciently irrigated	Irrigated	Anciently irrigated	
Thickness of cultivated layer or humus layer, cm	31–36	36–46	46–56	56–66	46–61	46–61	41–51
Depth, cm:	36–46	41–51	71–81	–	–	–	–
maximum collection of carbonate gypsum layer salt layer	61–81	91–131	131–171	141–171	41–51	41–51	16–26
	41–51	36–46	51–53	51	46–51	46–51	41–46
A quantity of water-resistant aggregate (> 0,25 mm), %	–	1,3–1,4	46–56	51–71	56–66	56–66	31–51
Density, g/cm <sup>3</sup>	1,6–2,6	1,4–1,9	1,2–1,3	1,3–1,4	1,2–1,4	1,2–1,4	–
Total humus, %	7–13	10–11	1,9–2,4	2,4–2,9	1,6–3,1	1,6–3,1	1,5–2,3
C:N	0,5–0,9	–	11–13	13–16	9–13	9–13	11–16
Cht:Cft	21–26	–	1,3–1,6	–	1,2–1,3	1,2–1,3	0,5–0,6
A sum of absorbed bases, mg/eq in 100 g of soil	1,6–3,1	–	26–36	–	21–36	21–36	21–23
Ca:Mg	8,1–8,6	–	1–2	–	1,3–2,1	1,3–2,1	2,1–2,6
Suspension of pH	0,09–0,16	0,16–0,41	8,4–9,1	–	8,1–8,6	8,1–8,6	8,6–9,3
CO <sub>2</sub> filthiness in soil air, %	–	–	–	–	–	–	0,12–0,21
Depth of gleyey layer, cm	151–171	141–171	161–191	151–181	101–151	101–151	56–66

*Meadow-gray soils* – combine various irrigation stages and the soils with weak ground humidity and involve aluvial – proluvial plains. The irrigative waters fulfil a main role as moisturizer, but a low part of the profile is provided with groundwaters. The modern cultivated layer – thickness is 36–66 cm (36–46 cm – weak, 46–56 cm, average, 56–66 cm – high cultivation), layered – fragmented (Ala) and fragmented – granular (Al'i.a) structur. Salting and claying signs are found in the profile. The granulometric composition is heavy (a quantity <0.001 mm 66–71%), a silty degree is more than 51%. An amount of water – resistant aggregates with a size more than > 0.25 mm. (36–56%).

1.4–2.9% humus is in composition of the plow layer, and this is an increase of its amount from irrigated weak cultivation to irrigated high cultivation. C:N ratio is much more – 11–16. Calcareous of the whole soil profile changes in a large limit – 6–16%. An amount of absorbed bases is very high (26–31 mg eq/100 g soil), Ca:Mg ratio (1:2) (1.6–3 in the virgin meadow-grey soils). A quantity of Na at a depth of 31–41 cm rises in the weakly cultivated variants and alkalinity increases.

*Irrigated meadow gray soils* mainly spread in the river debris cone and are formed in the condition of the leaching irrigation – hydromorph regime of humidity. The meadow gray soil zones have been irrigated since ancient time and reserved ancient agricultural signs. Diagnostic sign: depth (till 76 cm), cultivated layer (31–46 cm weak, 46–61 cm average and 61–76 cm high cultivation) is formed from agro-irrigation floats, high composition (51–76%), size > 0,25 mm a composition of humus changes by 1.6–4.6% (1.6–2.6 weak; 2.6–3.65% – average and 3.6–4.6% – high cultivation), humus content is fulvat-humate, ratio of Cht:Cft – 1.2–1.3; the whole soil profile is calcareous, the cultural

layers are homogeneous in comparison with CaCO<sub>3</sub> – 12–14%, a deposit depth of the quick – dissolved salts changes depending on cultivation degree of soils; soda salting is noted in the 2<sup>nd</sup> meter.

*Boharic soils* are formed in the zone with weak drainage, are moistened by ground waters of which level is rather near the surface (1–2.6 m). They were irrigated before and the indications of the agricultural culture were remained. A level of ground waters rose because of absence of collector–drainage network in the irrigation process; this is a reason for resalinization and the soil was removed from intensive assimilation.

Diagnostic indicators: density of upper soddy layer – 5–11 cm, thin crust on top 2–4 cm; humus layer is cinnamonic; depth is 41–51 cm; with salting and alkalization indicator, the upper–meter layer consists of agro-irrigation sediments and paleohydromorph original, buried soils; a granulometric content is heavy, clayey – dusty, siltiness rate is 41–66%, humus quantity is little – 1.5–2.8%, humus content is fulvat, Cht:Cfk – 0.5–0.6; CaCO<sub>3</sub> is 11–15% in the upper layer of 51 cm, a quantity of absorbed bases is slight, totally – 21–22 mg/eq in 100 g of soil, it strongly rises in the anciently buried soils (31–51mg-eq/in 100 g of soil); Ca:Mg ratio is 2.1–2.6 in the upper layers; 3–4 in the buried layers; pH – indicators of water suspension are high – 8.6–9.3. Density of quick – dissolved salts is 2–4% along the whole profile.

## Conclusion

It has been established that soil processes on target and irrigated soils, depending on the duration of irrigation, occur in arid steppe (gray-brown) and semi-desert (gray soils, meadow-gray soils) zones of the farm under conditions of a prolonged warm period. Research has identified the most characteristic diagnostic indicators of irrigated, non-irrigated, and target soils in this zone. It has been established that, depending on the duration of irrigation, the particle size distribution of gray–brown soils varies from heavy to light loams. The size of water–stable aggregates > 0.25 mm is 46–56%, and humus is 2–3%. Humus has a humate–fulvate composition, basic water absorption capacity, and pH values in aqueous suspension of 7.4–8.6. Gray soils have a light clay texture, contain 1.4–2.3% humus, have a humate–fulvate composition, and an alkaline pH of 21–36 mg-eq per 100 g of soil. Meadow gray soils have a heavy clay texture, contain 1.4–2.9% humus, and have a humate-fulvate humus composition. They have high absorption capacity and an alkaline pH.

## Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Comparative Assessment of Antioxidant and Antiradical Activity of Walnut Kernels (*Juglans regia* L.) Genotypes from Different Regions of Azerbaijan

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**Abstract.** A comparative analysis of antioxidant (AO) and antiradical (AR) activities was conducted using aqueous–ethanolic extracts obtained from native walnut kernels (*Juglans regia* L.) enclosed within shells of different genotypes cultivated across distinct agro-ecological regions of Azerbaijan (Shamakhi, Gabala, and Ordubad). Extraction was performed at a solid-to-solvent ratio of 1:3 (w/v), followed by dilution to a final concentration of 0.5 mg/mL. Biological activity was assessed using an induced chemiluminescence assay with hydrogen peroxide as an initiator of free radical reactions, enabling quantitative evaluation of the extracts' ability to inhibit oxidative processes and scavenge reactive oxygen species (ROS). The results demonstrated pronounced genotype-dependent variability. The highest antioxidant activity was observed in the Shamakhi genotype (87.5%), indicating a greater abundance of redox-active compounds. In contrast, higher antiradical activity was recorded for the Gabala (65.7%) and Ordubad (59.3%) genotypes, suggesting differences in antioxidant mechanisms, likely associated with variations in phenolic composition. Overall, the findings highlight the substantial biochemical potential of the studied material and underscore the influence of genotype and agro-ecological factors on antioxidant properties, supporting the targeted selection of walnut genotypes for functional food and biotechnological applications.

**Keywords:** *Juglans regia* L., antioxidant activity, antiradical activity, chemiluminescence, genotypes

### Introduction

Oxidative stress is currently attracting the attention of researchers worldwide, as it is considered one of the key factors disrupting redox homeostasis in the human body. According to numerous scientific literature data, the development of oxidative stress precedes the onset and progression of socially significant diseases such as cardiovascular and oncological pathologies, inflammatory and rheumatoid processes, neurodegenerative disorders, and diabetes mellitus. Moreover, a significant portion of modern theories of aging are based on the concept of free radical oxidation (Polidori & Mecocci, 2022; Hassan et al., 2024; Zglinicki et al., 2024).

Reducing oxidative stress in biological systems is possible through the use of compounds with pronounced antioxidant activity of various origins. In this context, foods, particularly those of plant origin, as well as their processed forms (bekmez, sherbet, etc.), are of particular importance.

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Received: 23 February 2026; Accepted: 9 May 2026; Published online: 25 June 2026

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In today's world, the scientific community is focusing significant attention on the discovery and comprehensive study of natural compounds, as well as food products containing biologically active substances capable of effectively preventing the development and progression of these pathological conditions. Plant-based components are of particular interest, as they are a rich source of natural antioxidants.

It has been established that the antioxidant potential of plant-based materials (medicinal plants, vegetables, and fruits) is largely due to the presence of a wide range of phenolic compounds, among which flavonoids are predominant, possessing a pronounced ability to neutralize free radicals and inhibit oxidative damage to cellular structures (Saini et al., 2024).

The discovery, identification, and comprehensive study of these natural compounds is a priority area of modern science, opening up broad prospects for both deepening fundamental understanding of antioxidant defense mechanisms and developing effective practical solutions in medicine, pharmacology, nutrition, and the creation of next-generation functional foods.

One valuable plant material with significant biological potential is the walnut (*Juglans regia* L.), a member of the Juglandaceae family, widely distributed throughout various regions of the country. Only one species is found in Azerbaijan: the walnut, or Walnut. It is cultivated throughout the country: in the Shaki-Zagatala region, Nakhchivan and Ordubad. This unique natural product has long attracted the attention of both scientists and traditional medicine practitioners. In medieval medical tradition, the eminent physician and philosopher Avicenna, in his seminal work «The Canon of Medicine», emphasized the high therapeutic value of walnut (*Juglans regia*), considering it an effective remedy for a wide range of pathological conditions. In later periods, interest in this plant has been sustained and reflected in the scientific and popular literature, where walnut (*Juglans regia*) is described as a food product with potentially beneficial effects on cognitive functions, particularly in supporting memory processes with regular consumption. Currently, the properties of walnuts are well studied and are widely used for the prevention and treatment of various diseases. The therapeutic and prophylactic properties of walnuts (*Juglans regia* L.) are largely due to its rich and varied chemical composition, which varies depending on the morphological part of the plant and the stage of ripeness of the fruit. Thus, the bark contains triterpenoids, ascorbic acid and quinones (including juglone); the leaves are characterized by a high content of carotenoids, phenolic carboxylic acids, coumarins, flavonoids, anthocyanins and quinones. Green (unripe) fruits contain vitamins C, B1, B2, PP, carotenoids and quinones, while mature kernels are rich in vitamins A, B1, B2, B12, C, K, E, PP, and also contain carotenoids, quinones and small amounts of gallic and ellagic acids (Hayes et al., 2016; Colaric et al., 2005).

Particularly noteworthy are the little-studied and often undervalued components of the fruit—the shell and partitions of the walnut, traditionally considered byproducts of processing. However, these structures represent a valuable source of biologically active substances. Their characteristic dark brown color is due to the high content of phenolic compounds and iodine-containing components. Walnut partitions contain vitamins (A, C, PP, and B vitamins), as well as a wide range of phenolic compounds, which determines their pronounced antioxidant and potentially pharmacologically significant properties (Al-Snafi, 2018). Currently, leading research centers around the world are actively conducting research aimed at studying the antiradical activity (ARA) of walnuts and their individual morphological parts. The investigation of the chemical composition of natural matrices, with a focus on the identification and quantification of compounds exhibiting antioxidant activity and the ability to scavenge free radicals, is considered a priority area in modern biochemistry and nutritional science, particularly in the context of oxidative stress research and the development of functional foods (López-Otín et al., 2023; Sies & Jones, 2020). However, despite the significant amount of accumulated data, such research has been virtually nonexistent in Azerbaijan to date. The scientific novelty of the work lies in the fact that for the first time a comprehensive analysis of the

antioxidant and antiradical activity of unpeeled walnut kernels (*Juglans regia* L.), harvested in various regions of Azerbaijan, was carried out, which expands our understanding of their biochemical potential and possibilities for practical use.

## Materials and Methods

The study involved walnuts (*Juglans regia* L.) harvested in various regions of Azerbaijan (Shamakhi, Gabala, and Ordubad). Unshelled walnut kernels were used as the plant material. Hydroalcoholic extracts were obtained by extraction using ethanol at a specified concentration. The antioxidant and antiradical activity of the extracts was assessed by induced chemiluminescence (Georgetti et al., 2003; Pamunuwa & Atapattu, 2023).

The obtained results allowed for a comparative analysis of walnut kernel (*Juglans regia* L.) characteristics among genotypes grown in different regions

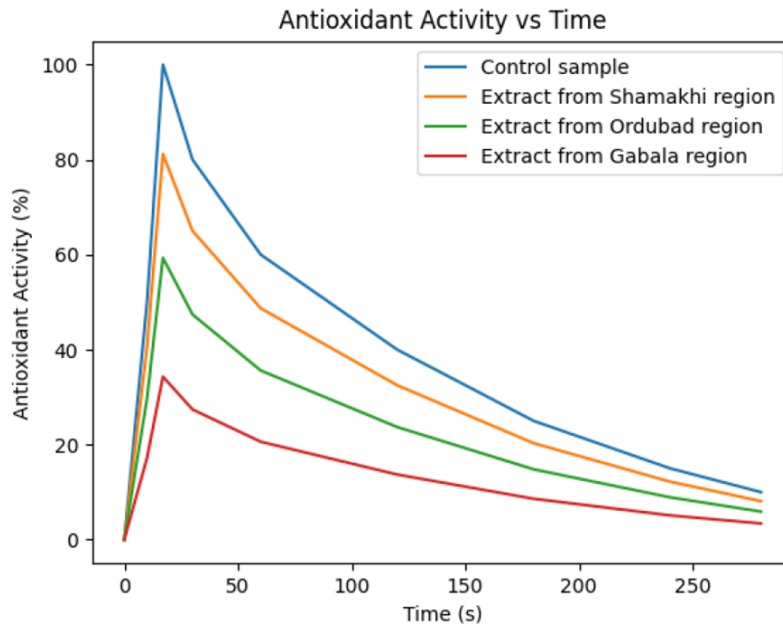
## Results and Discussion

A comparative study was conducted of the antioxidant and antiradical activity of hydroalcoholic extracts obtained from unpeeled walnut kernels (*Juglans regia* L.) in shells collected in various regions of Azerbaijan (Shamakhi, Gabala and Ordubad). The reaction was carried out using peroxidase ( $1 \times 10^{-5}$  M), pyrogallol ( $1 \times 10^{-2}$  M), and hydrogen peroxide ( $1 \times 10^{-4}$  M). In the control system (400  $\mu$ l), the chemiluminescence peaked at 4–5 minutes, and the total reaction time was 12–14 minutes.

After the control reaction, the antioxidant (AO) and antiradical (AR) activities of the hydroalcoholic extracts of inshell walnut kernels from various walnut genotypes were determined. All studied samples were found to exhibit pronounced antioxidant activity, exceeding 80%, indicating a high content of compounds with radical-scavenging properties.

Figure 1 shows that at an extract concentration of 0.5 mg/ml. The highest antioxidant activity was found in the Shamakhi genotype—87.5%; the reaction lasted approximately 8 minutes, with the chemiluminescence peaking at 1 minute 14 seconds. The Gabala genotype extract demonstrated 83.7% activity (reaction duration was approximately 7 minutes, with a peak at 1 minute). The lowest values were observed in the Ordubad genotype—82.8%, with a reaction lasting approximately 6 minutes 21 seconds and reaching a peak at 1 minute 30.

Extracts were found to influence reaction kinetics, shortening its duration compared to the control system, confirming their antiradical properties. With the addition of extracts, the reaction duration was reduced to 4–8 minutes, indicating their antiradical activity, assessed by a decrease in chemiluminescence intensity. All studied genotypes possessed high antioxidant potential, with the highest activity characteristic of samples grown in Shamakhi, indicating the influence of genotypic and regional factors on the level of biological activity of walnuts (Fig. 1).



**Figure 1**

*Antioxidant activity of the Shamakhi, Gabala, and Ordubad genotypes %*

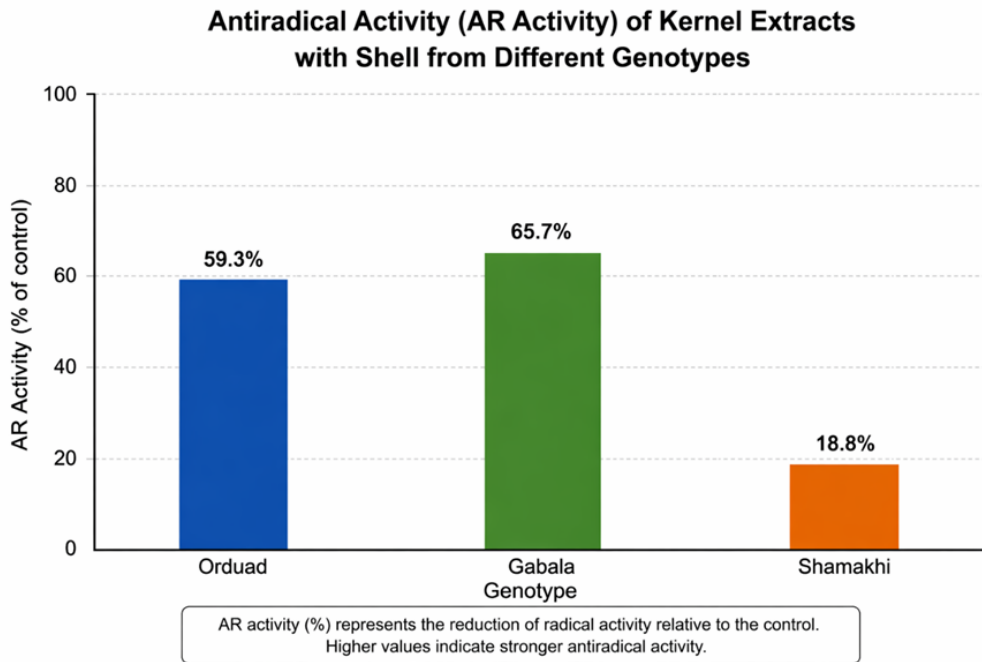
*Note.* The highest antioxidant activity values are characteristic of the Shamakhi genotype.

For the Shamakhi genotype, the reaction involving the extract lasted approximately 9 minutes, with the peak of chemiluminescence observed early on (15 seconds). According to this graph, AR activity was 81.2%.

When examining the second sample, the reaction conducted to determine the AR activity of the kernel and shell extract obtained from fruits of the Gabala genotype lasted for 5 minutes, with the maximum value observed at 10 minutes. This amounted to 34.4% of the maximum (reference) value. Therefore, if the maximum is taken as 100% (an area of 320 mm<sup>2</sup>), then the obtained result (110 mm<sup>2</sup>) indicates that the activity is approximately 3 times lower than the maximum.

The kernel and shell extract obtained from fruits of the Ordubad genotype is characterized by an AR activity level of 59.3%, indicating its moderate inhibitory capacity for the test parameter. Comparative analysis demonstrates that the extract of the Gabala genotype has the most pronounced antiradical activity, providing a reduction in AR by 65.7% relative to the control, while the extract of the Shamakhi genotype exhibits a significantly smaller effect (18.8%) (Fig. 2).

The study showed that kernel and shell extracts obtained from different genotypes differ significantly in their levels of antiradical activity. The Gabala genotype demonstrated the most pronounced inhibitory effect on AR, indicating its high biological activity and potential as a source of natural inhibitors. The Ordubad genotype is characterized by a moderate level of activity, while Shamakhi exhibits the least effectiveness.



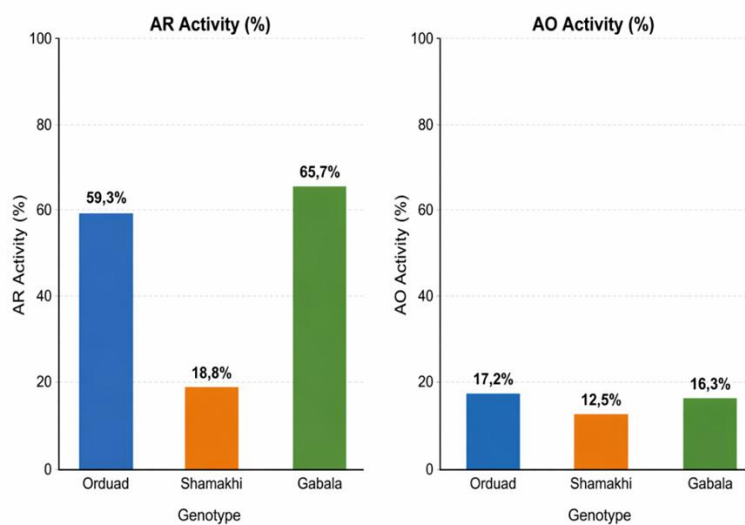
**Figure 2**

*Antiradical activity of the Shamakhi, Gabala, and Ordubad genotypes %*

*Note.* The highest antiradical activity values are characteristic of the Gabala genotype.

Moreover, the antioxidant activity of all studied samples is comparable, indicating the absence of significant differences in this parameter between genotypes. The observed dependence of AR inhibitory activity on genotype is likely due to differences in phytochemical composition, including the content of phenolic compounds.

The discrepancy between the antioxidant and antiradical activity (Fig. 3) of the Shamakhi genotype extract is likely due to differences in the mechanisms of action of the bioactive compounds, as well as the specificity of the analytical methods used.



**Figure 3**

*Comparative analysis of antiradical and antioxidant activity in different genotypes of walnut (Juglans regia L.)*

Antiradical activity (AR) is defined as the ability of compounds to directly neutralize free radicals through the donation of an electron or a hydrogen atom, whereas antioxidant activity is a broader indicator that includes reduction potential, the ability to chelate metal ions, and the inhibition of chain oxidation processes (Huang et al., 2005; Apak et al., 2016).

The Shamakhi genotype extract likely contains predominantly compounds with pronounced reducing capacity, but relatively low reactivity toward free radicals. Furthermore, differences may be related to the specific phenolic profile, particularly the ratio of flavonoids, phenolic carboxylic acids, and condensed tannins, which exhibit varying activity in different test systems.

It should also be noted that the method used to assess antioxidant and antiradical activity (XL) is based on a specific principle and is characterized by specific sensitivity to individual classes of compounds. Therefore, the higher antioxidant activity and low antiradical activity observed in the Shamakhi genotype is not a contradiction in terms, but rather reflects the specific chemical composition of the extract and the prevalence of certain mechanisms of antioxidant action.

Thus, the obtained results allow us to consider the studied genotypes as promising raw materials for the development of functional foods, dietary supplements, and natural antioxidant compositions. The Shamakhi genotype can be effectively used in systems requiring prolonged antioxidant protection, while the Gabala genotype can be used in products designed to rapidly neutralize free radicals. This opens up opportunities for targeted applications in the food, pharmaceutical, and cosmetic industries.

## **Conclusion**

In this study, kernel extracts with shell from different genotypes exhibited pronounced variation in both antiradical (AR) and antioxidant (AO) activities. The highest AR activity was observed in the Gabala genotype (65.7%), followed by Ordubad (59.3%), whereas Shamakhi showed a markedly lower effect (18.8%). In contrast, AO activity varied within a narrower range, with the highest value recorded for Ordubad (17.2%) and the lowest for Shamakhi (12.5%).

The observed discrepancy between AR and AO activities highlights the contribution of distinct antioxidant mechanisms and reflects differences in the phytochemical composition of the studied genotypes. These findings emphasize the importance of employing multiple analytical assays to obtain a comprehensive evaluation of antioxidant potential. Overall, the Gabala genotype can be considered a promising source of potent radical-scavenging compounds, while Ordubad demonstrates a more balanced antioxidant profile. These results provide a basis for further phytochemical characterization and support the potential application of these genotypes in the development of functional foods and natural antioxidant formulations.

## **Declaration of Competing Interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Species Composition, Morphological Characterization, and Dominance Structure of the Fauna of Parasitic Nematodes of Vegetable Crops in the Fergana Valley, Uzbekistan

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Gulnara Huseynova<sup>4</sup> , Gulnar Shirinova<sup>5</sup> , Vusala Safikhanova<sup>6</sup> 

**Abstract.** *The present study investigates the species composition, morphological characteristics, and distribution patterns of parasitic nematodes infesting vegetable crops in the Fergana Valley, Uzbekistan. A total of 283 soil and root samples were collected from tomato (*Solanum lycopersicum*), bell pepper (*Capsicum annuum*), and eggplant (*Solanum melongena*) cultivated under protected ground conditions across the Andijan, Fergana, and Namangan regions. Nematode extraction was performed using the Baermann funnel method, soil washing analysis, and root incubation techniques. A total of 177 temporary and 72 permanent microscopic preparations were produced for morphological and morphometric analysis. Thirteen parasitic nematode species belonging to the order Tylenchida were identified: *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, *M. hapla*, *Pratylenchus pratensis*, *P. penetrans*, *P. thornei*, *P. tumidiceps*, *Helicotylenchus multicinctus*, *H. digitiformis*, *H. dihystra*, *Ditylenchus dipsaci*, and *D. destructor*. Dominance analysis based on the scale proposed by V.F. Paliy revealed that root-knot nematodes of the genus *Meloidogyne* are absolutely dominant in the regional nematode fauna, collectively comprising more than 84% of the total species abundance. *Meloidogyne incognita* recorded the highest dominance index (37.25%), followed by *M. arenaria* (30.85%) and *M. javanica* (15.87%), all classified as eudominant species. The high prevalence of thermophilic *Meloidogyne* species is attributed to the favorable soil-climatic conditions of the Fergana Valley, characterized by warm temperatures, intensive irrigation, and an extended growing season. The Andijan region demonstrated the greatest species richness, whereas the Namangan region yielded rare and previously unrecorded species.*

**Keywords:** *parasitic nematodes, Meloidogyne, Fergana Valley, vegetable crops, species composition, dominance index, phytosanitary risk, Tylenchida*

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Received: 16 February 2026; Accepted: 27 May 2026; Published online: 25 June 2026

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## Introduction

The Fergana Valley is one of the largest agricultural regions of Uzbekistan, where vegetable crops are intensively cultivated: tomato, cucumber, pepper, eggplant, onion, carrot, cabbage, and melon plants. The favorable soil-climatic conditions of the region simultaneously contribute not only to the development of crop production, but also to the mass distribution of phytoparasitic nematodes, causing significant reduction in crop yield and deterioration of product quality.

Parasitic nematodes are one of the most dangerous groups of phytopathogens. According to international research data, annual crop losses from phytonematodes amount to 10–15%, and in foci of severe infestation may reach 40–60%. Representatives of the genera *Meloidogyne*, *Pratylenchus*, *Ditylenchus*, and *Helicotylenchus* are particularly dangerous. The fauna, biomass, distribution, morphobiological characteristics of parasitic nematode species, as well as their impact on plants and the damage they inflict on vegetables, are of both scientific and practical significance.

## Materials and Methods

The study was conducted on raised beds in the Andijan, Fergana, and Namangan regions of the Fergana Valley on crops of tomato (*Solanum lycopersicum*), bell pepper (*Capsicum annuum*), and eggplant (*Solanum melongena*) (Ferris et al., 2001). A total of 283 samples were collected from roots and rhizosphere soil of the aforementioned plants. To extract parasitic nematodes from plant roots and soil, the Baermann funnel method, soil washing analysis, and root incubation methods were employed (Matveeva et al., 2018). From the extracted nematodes, 177 temporary and 72 permanent preparations were prepared. For the determination of nematode species composition, a widely recognized identification key was used (Maleita et al., 2022).

## Results

Under protected ground conditions of the Fergana Valley, during the cultivation of tomatoes, cucumbers, bell peppers, eggplants, and vegetable crops in the Andijan, Namangan, and Fergana regions, 13 nematode species were identified: *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, *M. hapla*, *Pratylenchus pratensis*, *P. penetrans*, *P. thornei*, *P. tumidiceps*, *Helicotylenchus multicinctus*, *H. digitiformis*, *H. dihystra*, *Ditylenchus dipsaci*, *D. destructor*, belonging to the order Tylenchida.

*Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Fig. 1)

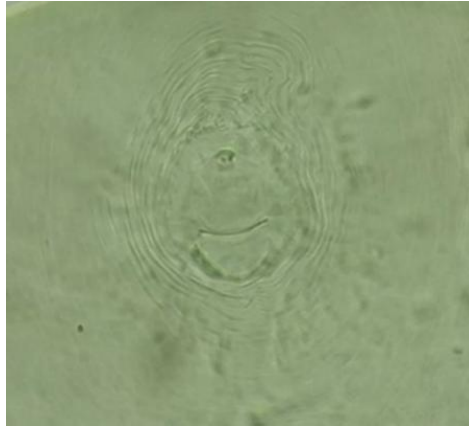
Females. Body length with neck 643–1100; body width 400–520. Neck length 102–220, width 68–84. Body rounded or oval. Cuticle weakly annulated. Two annules are visible on the head. Basal plates of the head capsule well developed. Stylet 13–15.5, robust, with rounded knobs. Egg size 76–101 × 32–44.

Perineum. Anal-vulval plate from rounded to oval. General dimensions approximately 83–120 × 79–132. Vulva width 24–30, distance between phasmids 26–30, from vulva to midline between phasmids 19–24, from vulva center to anus 16–18, from anus to phasmids 14–17. No tail whorl. Plate pattern variable. Dorsal arch low, weakly trapezoidal. Lateral fields poorly expressed. Most commonly they are indicated by interruptions and irregularities in the circular lines. In addition, at the site of the lateral fields, the dorsal and ventral lines may converge at an angle, sometimes forming "wings". No dots in the tail region.

Males. L = 1270–2000; a = 44–65; b = 11–16; c = 116–138. Stylet 20–24. Head cap broad, rectangular in lateral view. Height of head capsule 5–6, width at base 12–14. Behind the labial annule there are 4 additional annules, the first being the widest. Cuticle annulated. Lateral field with 4 lines, converging posterior to the cloaca on the dorsal side. Stylet 20–24. Knobs rounded; knob width 4–5, height 3.

Oesophagus 96–98. Metacorporal bulb oval, 20–24 × 11–12, with valve. Spicules 30–34. Gubernaculum 6–8, curved. Tail short, rounded. Phasmids anterior to cloaca. Second-stage juveniles. L = 450–490; a = 26–32; b = 7.2–7.8; c = 6–10. Stylet 10–12. Knob width 2–5, height 1–2, merging inconspicuously with the stylet. Cuticle annulated. Oesophagus 62–70.

Geographical distribution. Andijan region: Andijan city, Pakhtaabad district, Bulakbashi district, Izboskan district, Oltinko'l district, Shahrikhan district. Fergana region: Fergana city, Margilan city, Oltiariq district, Quvasoy district, Quva district, Toshloq district. Namangan region: Chust district, Pop district, Turakurgan district, Davlatabad district, Kosonsoy district, Chortoq district, Mingbuloq district.



**Figure 1**  
*Meloidogyne arenaria*

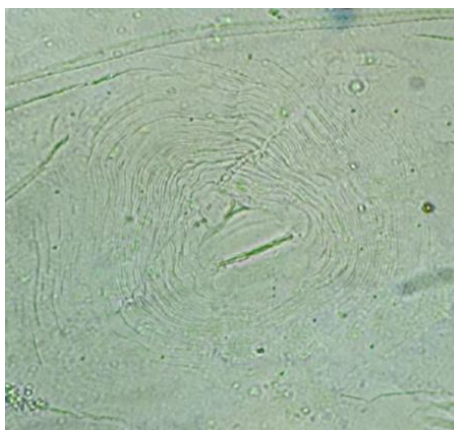
*Meloidogyne incognita* (Kofoid et White, Chitwood, 1949) (Fig. 2.)

Females. Body length with neck 505–680; body width 300–430. Body pearly white, from spherical to pyriform in shape. Head not offset, bearing a labial annule and two postlabial annules. Internal head structure weakly developed. Stylet 14–16, robust, with rounded knobs. Knob width 4–5, height 1.8–2.0. Opening of the dorsal oesophageal gland located at a distance of 2–4 from the stylet knobs. Excretory pore situated at the level of the dorsal oesophageal gland opening or at a distance of 13–98 from the anterior body end. Eggs relatively small, 77–98 × 30–48.

Perineum. Anal-vulval plate rounded-oval in shape. Dorsal arch high, composed of closely spaced wavy and zigzag lines. Right and left sides of the dorsal arch frequently asymmetrical. Tail vestige outlined by a whorl-like line that is not interrupted laterally. From the posterior vulval lip toward the anal opening, two short straight folds typically extend, arranged vertically to the vulval slit. Lateral fields may be poorly expressed, indicated by bifurcation of the dorsal and ventral lines along the lateral fields.

Males. L = 1200–2450; a = 32–52; b = 6–17; c = 122–372. Head slightly offset. Labial annule massive. Stylet 21–26; knobs rounded, occasionally bifurcated. Knob width 5.5–6.5, height 3.0–3.5. Second-stage juveniles. L = 360–393; a = 29–33; b = 5.6–6.4; c = 8.0–9.4. Head bearing 4 cuticular annules. Stylet 10; knobs rounded. Knob width 2, height 1.3–1.5.

Geographical distribution. Andijan region: Andijan city, Pakhtaabad district, Bulakbashi district, Izboskan district, Oltinko'l district, Shahrikhan district, Jalaquduq district. Fergana region: Fergana city, Margilan city, Oltiariq district, Quvasoy district, Quva district, Toshloq district. Namangan region: Namangan city, Chust district, Pop district, Turakurgan district, Davlatabad district, Kosonsoy district, Chortoq district, Mingbuloq district.



**Figure 2**  
*Meloidogyne incognita*

*Meloidogyne javanica* Chitwood, 1949 (Fig. 3)

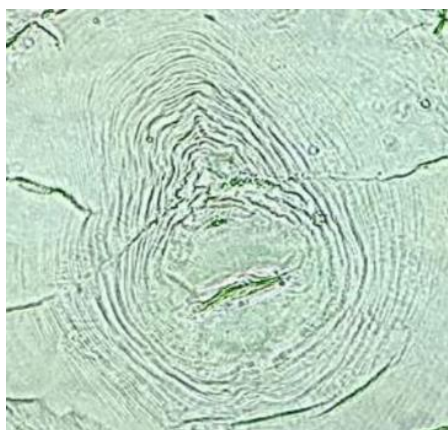
Females. Body length 540–850, body width 300–550. Body flask-shaped. Neck usually long. Stylet 16–17. Knobs rounded, width at base 4–5, height 2. Opening of the dorsal oesophageal gland at a distance of 3–4 from the stylet knob base. Egg size 76–100 × 31–45.

Perineum. Anal-vulval plate typically rounded, with simple circular lines interrupted in the lateral field region. Lateral fields appearing as distinct bands not crossed by cuticular folds of the dorsal and ventral sides. Characteristically, the lateral fields are very clearly visible beyond the perineum, extending far along the body. Dorsal arch low. Tail vestige clearly expressed. Phasmids distinct, located on both sides of the tail at a distance of 19–26 from the terminus.

Males. L = 940–1440; a = 26–42; b = 7–13. Head region elevated, bearing 4 cuticular annules. Labial annule broad and rather flat. Additionally, 3 postlabial annules of equal width are visible. Lateral lips: height 4, width 2. Stylet 20–21. Knob width 5, height 3.0–3.5. Opening of the dorsal oesophageal gland at 3 posterior to the stylet knobs. Spicules 30–31.

Second-stage juveniles. L = 340–400; a = 24–26; b = 8; c = 5.8–6.6. Stylet 10. Opening of the dorsal oesophageal gland located at a distance of 4 posterior to the stylet knobs.

Geographical distribution. Andijan region: Pakhtaabad district, Oltinko'l district. Fergana region: Fergana city, Margilan city, Oltiariq district, Quvasoy district, Toshloq district. Namangan region: Davlatabad district, Kosonsoy district.



**Figure 3**  
*Meloidogyne javanica*

*Meloidogyne hapla* Chitwood, 1949 (Fig. 4)

Females. Body length 550–790, width 400–450.

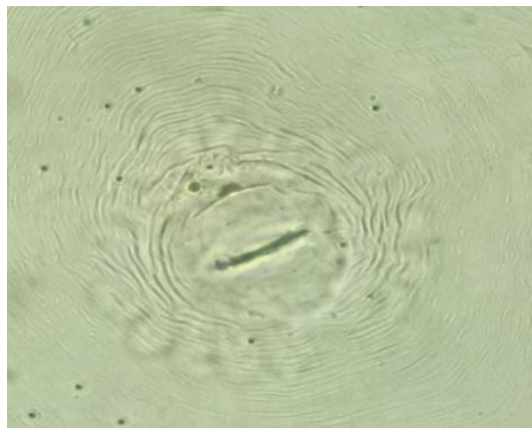
Body shape rounded-oval, with a relatively short neck. Stylet 12–14. Stylet knobs weakly rounded. Knob base width 3, height 1.5–2.0. Opening of the dorsal oesophageal gland located posterior to the stylet knobs at a distance of 5–6. Egg size 84–108 × 32–43. Eggs deposited in colorless egg sacs of relatively low durability.

Perineum. Anal-vulval plate rounded. Cuticular folds posterior to the anus appear as nearly parallel lines. At the site of the tail vestige and anus, a group of dots is usually present. Occasionally, dots are scattered without particular order in the space between the lines of the tail region. Dorsal arch low, rounded or angular. Cuticular folds in the lateral field region are formed by converging, branching lines of the dorsal and ventral arches. Frequently, lines of the ventral sector forming the lateral field extend far laterally, forming "wings" highly characteristic of the anal-vulval plate of this species. In older females, such wings are sometimes particularly well developed, with dots in the tail vestige region being poorly expressed or entirely absent.

Males. L = 1000–1330; a = 30–40; b = 12–15. Stylet 17–18. Head bearing 2 cuticular annules, the first rounded and narrower than the second. Stylet knob width 3.5–4.0, height 1.7–2.0. Opening of the dorsal oesophageal gland at a distance of 4–6 from the stylet knobs. One or two testes. Spicules 29–31, moderately arcuate. Phasmids situated at the level of or slightly posterior to the anus, diameter 3.5–4.0.

Second-stage juveniles. L = 395–466; a = 28–35; b = 6.5–7.3; c = 7.5–8.2. Stylet 10, knobs rounded. Stylet knob base width 1.5. Lateral field with 4 lines, non-areolated. Opening of the dorsal oesophageal gland at a distance of 3–4 from the stylet knobs. Tail tip of juveniles highly variable. Within the progeny of a single female, juveniles may possess either a bifurcated or non-bifurcated tail tip.

Geographical distribution. Andijan region: Izboskan district. Fergana region: Oltiariq district, Quva district. Namangan region: Chust district, Kosonsoy district.



**Figure 4**  
*Meloidogyne hapla*

*Pratylenchus pratensis* (de Man, 1880) Filipjev, 1936 (Fig. 5)

Female: L = 0.46 mm; a = 28; b = 3.6; c = 20; V = 77; stylet length = 15  $\mu$ m.

Body slender, nearly straight when relaxed. Cuticular annulation fine (0.9  $\mu$ m in the mid-body region), inconspicuous, sometimes very difficult to distinguish. Lateral fields with four longitudinal lines. Lip region almost continuously merging into the body contour, consisting of three annules; its margins rounded. Basal lip sclerotization extending posteriorly approximately one annule. Stylet

robust, with well-separated basal knobs. Opening of the dorsal pharyngeal gland located approximately 2.5  $\mu\text{m}$  posterior to the stylet base. Median pharyngeal bulb broadly oval. Pharyngeal glands overlapping the intestine ventrally or ventrolaterally. Excretory pore usually situated immediately anterior to the pharyngeal-intestinal junction. Hemizonid located immediately anterior to the excretory pore. Vulva transverse. Uterus with a large oval or rectangular spermatheca filled with spermatozoa. Ovary with oocytes arranged in a single row, except for a short proliferation zone. Post-vulval uterine sac slightly longer than the body diameter; its length 14–28  $\mu\text{m}$  or 22–32% of the vulva-to-anus distance; usually undifferentiated, occasionally containing a single separate cell. Tail with 20–28 annules, excluding those around the terminus; annulation continuing around the terminus, which is variable in shape — usually oblique, sometimes more symmetrically conical or slightly mucronate. Phasmids located approximately at mid-tail.

Male: L = 0.48–0.63 mm; a = 28–36; b = 5.3–6.6; c = 17–21; T = 38–60; stylet length = 13–14  $\mu\text{m}$ . Similar to female. Single testis, elongated. Spicules curved, 17–19  $\mu\text{m}$  in length. Gubernaculum 6–7  $\mu\text{m}$  in length. Bursa enveloping the tail, its margin weakly crenate. Phasmids extending into the bursa and located posterior to the mid-tail region.

Geographical distribution. Andijan region: Andijan city, Pakhtaabad district, Bulakbashi district, Shahrikhan district, Jalaquduq district. Fergana region: Fergana city, Margilan city, Oltiariq district, Toshloq district. Namangan region: Chust district, Turakurgan district, Davlatabad district, Kosonsoy district, Chortoq district.



**Figure 5**  
*Pratylenchus pratensis*

*Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 (Fig. 6)

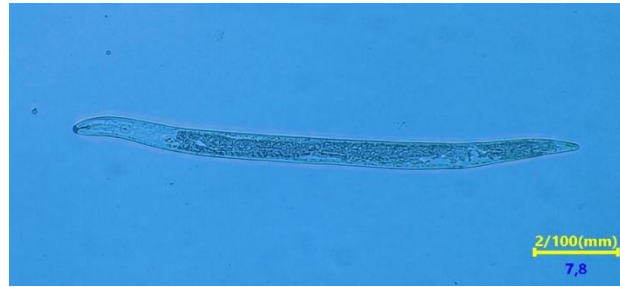
Female: L = 0.53 mm; a = 26; b = 5.8; c = 16; V = 81; stylet = 17–19  $\mu\text{m}$ .

Body moderately slender, nearly straight when heat-relaxed. Cuticular annulation fine. Lateral fields usually with four lines; outer bands may be partially areolated; central band sometimes with oblique striae in the vulval region, becoming areolated posterior to the vulva and not reaching the tail tip. Lip region slightly offset from the body, low, anteriorly flattened with rounded outer margins, consisting of three annules. Lip region with a robust, well-developed framework. In en face view, characterized by rectangular subdorsal and subventral lips, noticeably wider than the oral disc, and separated from the lateral lips by two mutually perpendicular grooves (Hernández & Gibson, 2000). Stylet basal knobs broadly rounded, occasionally cup-shaped anteriorly. Pharynx overlapping the intestine ventrally as a lobe approximately 1.5 body diameters in length. Excretory pore situated approximately opposite the pharyngeal-intestinal junction; hemizonid occupying approximately two body annules immediately anterior to it. Post-vulval uterine sac short, undifferentiated, approximately 1–1.5 body diameters in length at the vulval level. Spermatheca spherical or nearly spherical. Tail usually rounded, with a smooth terminus; ventral surface with 15–27 annules.

Male: L = 0.44–0.56 mm; a = 23–30; b = 5.2–6.0; c = 15–20; T = 43–52; stylet = 16–18  $\mu\text{m}$ .

Common. Slightly smaller than female but similar in form. Lateral fields with four lines, terminating at the bursa; occasionally with oblique lines in the central band in the mid-body region. Spicules slender, with well-developed manubria and ventrally curved shafts, 14–17  $\mu\text{m}$  in length; gubernaculum simple, 3.9–4.2  $\mu\text{m}$  in length. Tail approximately twice the body diameter at the cloacal level; bursa with irregularly crenate margin, enveloping the tail tip.

Geographical distribution. Fergana region: Quva district. Namangan region: Turakurgan district.



**Figure 6**  
*Pratylenchus penetrans*

*Pratylenchus thornei* Sher & Allen, 1953 (Fig. 7)

Female: L = 0.45–0.77 mm; a = 26–36; b = 5.5–8.0; c = 18–22; V = 73–80; stylet = 17–19  $\mu\text{m}$ .

Body large and slender, assuming an open C-shaped posture upon death by gentle heating. Cuticle with transverse striation, distance between striae approximately 1  $\mu\text{m}$ , inconspicuous. Lateral fields with four lines; outer lines straight or weakly crenate. In one specimen, Loof observed oblique striae in the central zone. Labial region with three annules, not offset from the body. The outer margin of the sclerotized labial framework extends noticeably approximately two annules into the body and one annule into the labial region. In en face view, lateral lips clearly separated from the subdorsal and subventral lips by corresponding grooves concave relative to the lateral lips (Hernández & Gibson, 2000). Stylet guiding apparatus extending posteriorly from the basal plate approximately four annules. Stylet of medium length (17–19  $\mu\text{m}$ ), with broadly rounded or nearly anteriorly flattened basal knobs. Opening of the dorsal pharyngeal gland located approximately 3  $\mu\text{m}$  posterior to the stylet base. Nerve ring situated immediately posterior to the pharyngeal bulb; hemizonid approximately two annules in length, positioned one annule anterior to the excretory pore. Ovary not reaching the pharynx. Oocytes arranged in a single row, except for the anterior proliferation zone; oviduct indistinct; uterus short. Spermatheca poorly distinguishable, spermatozoa absent (males very rare); post-vulval uterine sac slightly longer than 1.5 body diameters at the vulval level. Phasmids located slightly posterior to mid-tail; all four lateral lines continuing beyond the phasmids. Tail dorsally convex-conical; terminus bluntly rounded or truncate, without striation.



**Figure 7**  
*Pratylenchus thornei*

Male: L = 0.48 mm; a = 32; b = 5.6; c = 20; T = 30; stylet = 16  $\mu$ m.

Very rare (previously only four specimens recorded). Similar to female. Testis elongated, spermatocytes arranged in a single row, followed by a zone with multiple rows. Phasmids located slightly posterior to mid-tail and not extending into the bursa. Spicules very long (21–26  $\mu$ m), curved (arcuate), with a hafted structure, resting on a trough-shaped gubernaculum (5–7.5  $\mu$ m). Yu recorded a male from axenic culture on excised maize roots. In addition, nine further specimens from axenic cultures on carrot discs, obtained from a population infecting chickpea in Jerez (southern Spain), are reported here, completing the description of the morphology and morphometrics of this life cycle stage.

Geographical distribution. Andijan region: Andijan city, Izboskan district, Shahrikhan district. Fergana region: Quvasoy district and Quva district.

*Pratylenchus tumidiceps* Merzheevskaja, 1953 (Fig. 8)

Female: L = 325–500  $\mu$ m; a = 27.9–28.8; b = 3.8–5.1; c = 19.8–30.6; V = 75–77%

Male: L = 315–430  $\mu$ m; a = 27–30; b = 4.0–5.1; c = 14–15

Cuticle finely annulated. Body width from head to anal opening nearly uniform, with slight widening in the gonadal region. Anteriorly the body narrows, forming a characteristically blunt anterior end at the lip base. Posterior to the vulva in the female, and in the posterior quarter of the body in the male, the body gradually tapers toward the tail. Head bearing six closely appressed lips, at the base of which is a circle of 12 clearly visible tubercles. Stylet 14–15  $\mu$ m, with a well-developed triple expansion at the base. Oesophagus cylindrical, with a well-developed, nearly spherical bulb. Oesophageal glands clearly visible. Excretory pore situated in the region of the oesophageal glands. Gonads unpaired; only the anterior genital tube present, the posterior being rudimentary. Female tail conical, rounded at the tip. Male tail pointed, surrounded by a well-developed bursa. Spicules rather slender; gubernaculum small. A phytohelminths with specific pathogenic effect.

Geographical distribution. Andijan region: Shahrikhan district.



**Figure 8**

*Pratylenchus tumidiceps*

*Helicotylenchus digitiformis* Ivanova, 1967 (Fig. 9)

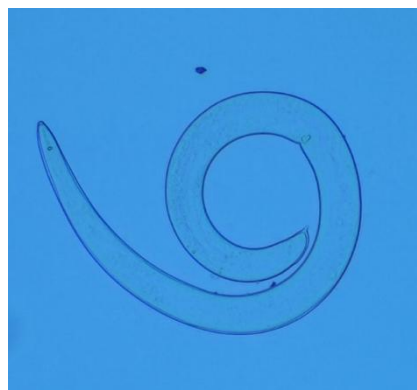
Female: L = 551–688 (633  $\pm$  21)  $\mu$ m; a = 28–40 (35  $\pm$  1.1); b = 4.2–5.6 (4.9  $\pm$  0.7); c = 16–30 (23  $\pm$  2.1); V = 75–82 (78  $\pm$  4.6); stylet = 10–18 (15  $\pm$  1.4)  $\mu$ m.

Body straight or slightly ventrally curved when relaxed. Annulation well expressed; body tapering from the vulval region toward the tail. Lateral field with four lines. The two outer bands irregularly areolated; oblique striae occasionally observed in the central zone at mid-body, and an additional band sometimes appearing, giving the impression of six lines.

Lip region rounded, with four annules; tail with 10–15 annules. Labial framework massive, with an oval oral aperture. Stylet robust, with angular basal knobs; opening of the dorsal gland located 2–4  $\mu\text{m}$  posterior to the stylet base. Oval median bulb and well-developed nerve ring encircling the narrow isthmus at the anterior part of the glandular pharyngeal region. Posterior pharynx overlapping the intestine laterally and ventrally for 20–50  $\mu\text{m}$ . Excretory pore located 80–95  $\mu\text{m}$  from the anterior end, slightly anterior to the pharyngeal-intestinal junction. Hemizonid situated anterior to the excretory pore. A single anterior ovary present; spermatheca small and non-functional. Double cuticular annulation observed on the dorsal side of the body opposite the anus. Tail rounded, smooth, often with a slight indentation.

Males: not found.

Geographical distribution. Andijan region: Andijan city, Izboskan district, Oltinko'l district, Shahrikhan district. Fergana region: Fergana city, Margilan city, Oltiariq district, Toshloq district. Namangan region: Turakurgan district, Davlatabad district, Kosonsoy district.



**Figure 9**  
*Helicotylenchus digitiformis*

*Helicotylenchus dihystra* (Cobb, 1893) Sher, 1961 (Fig. 10)

Females: L = 0.59–0.79 mm; a = 27–35; b = 5.8–6.9; c = 35–49; V = 60–65%; stylet 24–26  $\mu\text{m}$ .

Body usually spiral in posture. Lip region hemispherical, with four or five narrow and often poorly distinct cuticular annules. Width of cuticular annules in the mid-body region approximately 1.5  $\mu\text{m}$ . Stylet knobs typically concave anteriorly. Excretory pore at the level of the anterior part of the posterior bulb. Hemizonid situated immediately anterior to the excretory pore. The latter located 100–119  $\mu\text{m}$  from the anterior body end. Spermatheca without spermatozoa. Tail with a small ventral projection.

Males: (very rarely encountered); L = 0.59–0.65 mm; a = 25–32; b = 5.1–6.1; c = 31–33; stylet 22–27  $\mu\text{m}$ ; spicules 7–8  $\mu\text{m}$ .

Geographical distribution. Andijan region: Pakhtaabad district, Bulakbashi district, Shahrikhan district. Fergana region: Fergana city, Margilan city, Oltiariq district, Quvasoy district, Quva district. Namangan region: Namangan city, Turakurgan district, Mingbuloq district.



**Figure 10**  
*Helicotylenchus dihystra*

*Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956 (Fig. 11)

Females: L = 460–680  $\mu\text{m}$ ; a = 24–29; b = 4.5–6; c = 48–63; V = 64–72%.

Males: L = 440–560  $\mu\text{m}$ ; a = 27–33; b = 3.8–4.8; c = 28–36.

Cuticle coarsely annulated. Head clearly offset from the body, with four annules. Height of the head capsule equal to the diameter of its base. Stylet robust; length 25–28  $\mu\text{m}$ . Stylet basal knobs large. Oesophagus tylenchoid. Metacorporal bulb small, oval. Glandular cardiac bulb elongated. Excretory pore at the level of the beginning of the glandular cardiac bulb. Nerve ring slightly anterior to the level of the excretory pore. Lateral fields with four longitudinal lines, encircling the tail posterior to the phasmids. Female reproductive system paired, symmetrical. Vulva deeply invaginated into the body cavity. This species belongs to phytohelminths with a specific pathogenic effect.



**Figure 11**  
*Helicotylenchus multicinctus*

Geographical distribution. Andijan region: Andijan city, Bulakbashi district, Izboskan district, Oltinko'l district, Shahrikhan district, Jalaquduq district. Fergana region: Fergana city, Margilan city, Oltiariq district, Quva district, Toshloq district. Namangan region: Pop district, Davlatabad district, Kosonsoy district, Chortoq district.

*Ditylenchus dipsaci* Filipjev, 1936 (Fig. 12)

Female: L = 1000–1300  $\mu\text{m}$ ; a = 36–40; b = 6.5–7.1; c = 14–18; V = 80%.

Male: L = 1000–1300  $\mu\text{m}$ ; a = 37–41; b = 6.5–7.3; c = 12–15.



**Figure 12**  
*Ditylenchus dipsaci*

Females: Body slender, tapering toward both the head and tail ends. Head slightly offset from the body contour, with a delicate supporting framework. Cuticle finely annulated; width of cuticular annules approximately 1  $\mu\text{m}$ . Stylet with well-developed rounded basal knobs, 11–13  $\mu\text{m}$  in length. Procorpus cylindrical. Median bulb convex-oval; cardial bulb clearly demarcated from the midgut. Isthmus narrow. Nerve ring situated near or at the beginning of the cardial bulb. Excretory pore located at the level of the mid-cardial bulb. Hemizonid situated close to the excretory pore, occupying approximately 6 cuticular annules. Ovary oligopropagate, reaching the level of the cardial bulb, sometimes with one or two flexures. Spermatheca primitive, tubular, containing spermatozoa. Oviduct short, with well-developed large eggs. Egg length 60–65  $\mu\text{m}$ , diameter 17–19  $\mu\text{m}$ . Posterior uterus equal to half the vulva-to-anus distance or slightly shorter.

Male with bursal wings of the leptoderm type, occupying 3/4 of the tail length. Spicules ventrally curved, with well-developed cylindrical heads. Gubernaculum dorsally thickened, 10–12  $\mu\text{m}$  in length, linear in profile. Distance from vulva to anus equal to or shorter than tail length. Tail in both sexes short, gradually tapering. Tail terminus acute. This species is a parasite of numerous agricultural crops.

Geographical distribution. Andijan region: Pakhtaabad district, Bulakbashi district, Oltinko'l district, Shahrikhan district, Jalaquduq district. Fergana region: Fergana city, Margilan city, Oltiariq district, Toshloq district. Namangan region: Chust district, Turakurgan district, Mingbuloq district.

*Ditylenchus destructor* Thorne, 1945 (Fig. 13)

Females: L = 0.72–1.44 mm; a = 33–35; b = 8–10; c = 15–20; V = 78–83; stylet 10  $\mu\text{m}$ .

Males: L = 0.75–1.30 mm; a = 34–40; b = 7–8; c = 12–16; stylet 10  $\mu\text{m}$ .

Females. Ovary origin reaching the base of the oesophagus. Oogonia in the germinative zone arranged in 2–3 rows; oocytes in the growth zone arranged in a single row. Distance from the head end to the valve of the metacorpal bulb 57–68 (61)  $\mu\text{m}$ . Oesophagus length 150–183 (165)  $\mu\text{m}$ . Total gonad length 583–1178 (884)  $\mu\text{m}$ . Pre-uterine gland relatively short, consisting of 4 rows of cells with 6 cells per row (24 cells in total). Egg length slightly greater than the body diameter. Posterior uterus usually equal to or slightly longer than half the vulva-to-anus distance. Vulval lips generally prominent. Anus and rectum clearly observable. Tail tip blunt.

Males. Genital tube generally straight, shorter than in the female and generally not reaching the oesophageal base, length 488–905 (719)  $\mu\text{m}$ . Distance from the head end to the germinal cell of the gonad 175–450 (309)  $\mu\text{m}$ . Distance to the valve of the metacorpal bulb 52–73 (60)  $\mu\text{m}$ . Oesophagus length 130–185 (156)  $\mu\text{m}$ . Bursa well developed, originating at the level of the spicule base, length 42–75 (60)  $\mu\text{m}$ , not reaching the tail tip, leaving it free.

Geographical distribution. Andijan region: Bulakbashi district, Shahrikhan district. Fergana region: Fergana city, Margilan city, Oltiariq district. Namangan region: Namangan city, Chust district, Turakurgan district, Mingbuloq district.



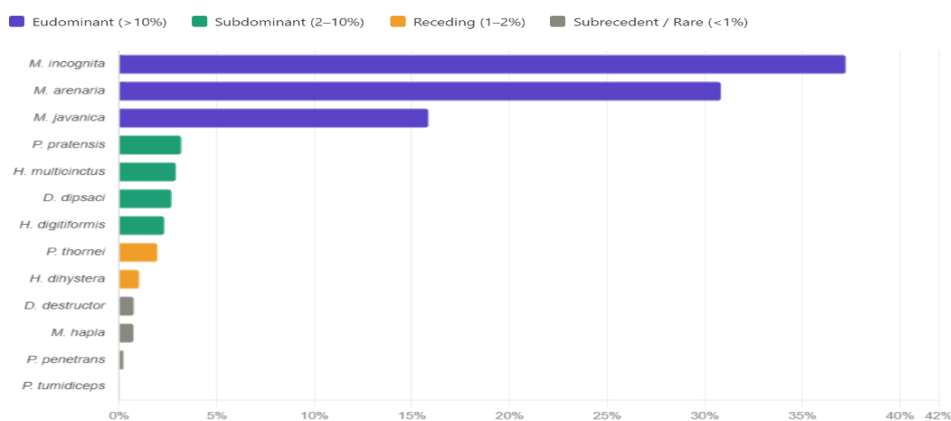
**Figure 13**  
*Ditylenchus destructor*

Among the dominance groups of parasitic nematode species in the agroecosystems of the Fergana Valley, root-knot nematodes of the genus *Meloidogyne* predominate. In particular, *Meloidogyne incognita* recorded the highest dominance index of 37.25%. This species was registered in nearly all surveyed areas, with notably high individual counts in Oltiariq district, Fergana city, and Quvasoy district of Fergana region. Its widespread distribution is attributed to the high adaptive capacity of this species, its ability to acclimate to diverse soil-climatic conditions, and its capability to infest a broad range of agricultural crops (Tab. 1).

The second eudominant species is *Meloidogyne arenaria*, with a dominance index of 30.85%. It was likewise encountered in nearly all surveyed areas, with particularly high population density recorded in Fergana region. This pattern indicates that the species has established itself as a stable parasite within the agroecosystems of the region (Matveeva et al., 2018).

*Meloidogyne javanica* also entered the eudominant group with an index of 15.87%. Its particularly high prevalence was recorded in warm-climate irrigated lands. The high occurrence of this species confirms that the soil-climatic conditions of the Fergana Valley are favorable for root-knot nematode development (EPPO, 2024).

**Table 1**  
*Distribution of species by dominance groups*



Thus, the three principal *Meloidogyne* species collectively constituted more than 84% of the total nematode fauna, demonstrating that root-knot nematodes are the predominant group among phytoparasitic nematodes in the Fergana Valley (Perry et al., 2009).

## Discussion

The subdominant group comprised *Pratylenchus pratensis* (3.21%), *Helicotylenchus multicinctus* (2.93%), *Ditylenchus dipsaci* (2.70%), and *Helicotylenchus digitiformis* (2.33%), distinguished by their relatively stable distribution across agrocenoses. These species are in most cases associated with specific crop types or particular soil conditions. For instance, the elevated occurrence of *Ditylenchus dipsaci* in certain districts reflects its association with moist soils and perennial plants.

The recedent group, represented by *Pratylenchus thornei* (1.98%) and *Helicotylenchus dihystra* (1.05%), exhibited relatively low prevalence, although both species were recorded consistently in certain areas. *Helicotylenchus dihystra* is characterized by its confinement to limited local territories.

The subrecedent group, comprising *Ditylenchus destructor* (0.78%) and *Meloidogyne hapla* (0.76%), was classified among the least prevalent species. *Meloidogyne hapla* is distinguished by its predominant occurrence in cooler and more humid areas; accordingly, its low prevalence under the conditions of the Fergana Valley is considered a natural outcome.

The lowest dominance indices were observed for *Pratylenchus penetrans* (0.25%) and *Pratylenchus tumidiceps* (0.04%). These species belong to the very rare group, with their distribution restricted to isolated localities. This pattern may be attributed to their specific ecological requirements or incomplete adaptation to the regional conditions.

In the parasitic nematode fauna of the Fergana Valley, root-knot nematodes of the genus *Meloidogyne* are absolutely dominant, forming the principal phytohelminths complex in agrocenoses. The high dominance levels of *M. incognita*, *M. arenaria*, and *M. javanica* in particular indicate that these species pose a substantial phytosanitary threat to agricultural crops. The remaining species are distributed within the subdominant, recedent, and subrecedent groups, constituting a relatively minor proportion of the biocenosis (Maleita et al., 2022).

In contemporary nematological research, *Meloidogyne* species are likewise regarded as the most economically significant phytoparasitic nematodes. According to Jones J. T. and co-authors, these species are distributed across virtually all agricultural regions owing to their high reproductive potential, broad host range, and ecological plasticity. A sharp increase in population density has been particularly documented in intensively irrigated agrocenoses (Jones et al., 2013).

The highest dominance of *M. incognita* in the Fergana Valley is attributed to the exceptional ecological plasticity of this species. Studies by Trudgill D. L. and Blok V. C. have emphasized that *M. incognita* and *M. javanica* develop rapidly under conditions of elevated temperature and humidity, posing a considerable phytosanitary threat particularly in warm-climate regions. The irrigated agroecosystems of the Fergana Valley, characterized by an extended growing season, represent favorable environments for these species (Trudgill & Blok, 2001).

The analysis recorded *Pratylenchus pratensis* and *Pratylenchus thornei* within the subdominant and recedent groups respectively. In the works of Castillo P. and Vovlas N., *Pratylenchus* species are described as migratory endoparasites that damage root tissues and reduce the capacity of plants to absorb water and mineral nutrients, thereby leading to impaired plant growth and decreased yield (Castillo & Vovlas, 2007).

Furthermore, Nicol J. M. and co-authors identified *Pratylenchus thornei* as a significant parasite of cereal crops in arid and semi-arid climatic regions, which accounts for its occurrence in certain districts of Namangan and Fergana regions (Nicol et al., 2011).

The inclusion of *Ditylenchus dipsaci* in the subdominant group may be associated with its polyphagous nature. According to data provided by Sturhan D. and Brzeski M. W., this species is capable of parasitizing more than 500 plant species, and rapid population growth has been documented particularly under conditions of elevated humidity (Sturhan & Brzeski, 1991). The classification of *Meloidogyne hapla* within the subprecedent group is explained by its primary adaptation to cool and temperate climatic conditions. In the works of Eisenback J. D. and co-authors, *M. hapla* is noted to be distributed predominantly in temperate climate zones; accordingly, its distribution under the warm climatic conditions of the Fergana Valley is restricted (Eisenback et al., 1981).

Thus, root-knot nematodes of the genus *Meloidogyne* have been found to be absolutely dominant in the phytoparasitic nematode fauna of the Fergana Valley. This situation indicates the presence of a high phytosanitary risk in the regional agrocenoses and confirms the necessity of developing effective phytosanitary measures against root-knot nematodes.

In general, the Fergana Valley is characterized by the dominance of root-knot nematodes of the genus *Meloidogyne*, which possess the greatest ecological plasticity and the widest distribution. The Andijan region is the most species-rich in the study area, whereas the Namangan region yielded rare species not previously recorded for the country.

## Conclusion

Analysis conducted on the basis of the dominance scale proposed by V. F. Paliy (1961) demonstrated that species belonging to the genus *Meloidogyne* are absolutely dominant in the agrocenoses of the Fergana Valley. According to the research findings, *Meloidogyne incognita* (37.25%), *Meloidogyne arenaria* (30.85%), and *Meloidogyne javanica* (15.87%) constituted the eudominant group, confirming that root-knot nematodes are the leading phytoparasites in the agroecosystems of the Fergana Valley.

## Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Species Composition and Hydrolase Activity of Fungi Isolated from Medicinal Plants in the Conditions of Azerbaijan

Gulnar Aliyeva 

**Abstract.** *This paper provides a comprehensive investigation into the medicinal plants belonging to the flora of Azerbaijan, focusing on the species composition of their associated mycobiota, the enzymatic profile of hydrolytic complexes, and the phytotoxicity of specific fungal strains isolated from these hosts. Mycological assessments revealed that the studied medicinal plants serve as a specialized ecological niche and habitat for a diverse range of microscopic fungi. Biochemical analysis of the isolated mycobiota established that certain strains possess the capability to synthesize a highly balanced hydrolytic enzyme system. This enzymatic system effectively degrades the complex structural components of the host plant's cell walls, thereby facilitating fungal penetration and tissue colonization. Furthermore, the study elucidates the distinct dual role of proteolytic enzymes within the infection mechanism. It was demonstrated that the activity level of proteolytic enzymes acts as a limiting factor in the regulated pathogenesis process of primary phytopathogenic fungi. Conversely, for the causative agents of secondary mycoses, this enzymatic activity functions as a key factor contributing to and aggravating their opportunistic and destructive actions. These findings provide crucial insights into the molecular regulation of fungal virulence and offer a foundational framework for developing target-specific enzyme inhibitors as next-generation green fungicides.*

**Keywords:** *medicinal plants, mycobiota, hydrolytic enzymes, phytotoxicity, limiting and contributing factor*

### Introduction

As is well known, the flora of Azerbaijan includes more than 4,700 plant species, approximately one-third of which are medicinal. Furthermore, some of these plants are not only medicinal but also of interest as forage, essential oil, and food crops. However, only a small fraction of these plants has undergone screening to identify their medicinal properties, even though the majority of the world's population uses plant-based preparations that prevent, to varying degrees, the development of diseases caused by various microorganisms. In this regard, the demand for such plants increases every year.

Considering that the depletion of biodiversity composition is one of the global environmental problems, in the future, this could lead to a significant restriction on the use of medicinal plants. Moreover, their intensive, irrational, and insufficiently controlled harvesting as raw materials also contributes to this, as they represent a particularly vulnerable group of living organisms.

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Received: 21 February 2026; Accepted: 19 May 2026; Published online: 25 June 2026

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Fungal infections and pathogenic microflora significantly aggravate this vulnerability under changing ecological and soil conditions (Mammadova et al., 2026; Gahramanova et al., 2026; Gurbanov et al., 2021).

It should also be noted that many medicinal plants possess antifungal activity, but despite this, they are also subject to pathologies caused by the very same fungi. As a result, their biological productivity decreases or is even completely lost, which, in turn, negatively affects medicinal plant resources. Recent monitoring of cultivated and wild plants across various regions of Azerbaijan confirms that a high diversity of pathogenic species forms specialized mycobiota complexes capable of causing severe plant diseases (Bakshaliyeva et al., 2023; Mammadaliyeva et al., 2024). Furthermore, symbiotic relationships between plants and microflora, as well as environmental stress factors, heavily influence the susceptibility of these hosts to fungal colonization (Bakshaliyeva et al., 2023).

All of the above emphasizes the importance of a comprehensive study of medicinal plants in a mycological aspect, especially those widely used for practical purposes. However, many of these plants are used without thermal processing. This circumstance raises serious concerns, as the materials can simultaneously serve as both a habitat and a source of enrichment for various metabolites (including mycotoxins) of dangerous fungi, which has been confirmed in various studies. The accumulation of these fungi or their metabolites on such plant materials used by humans for therapeutic purposes may increase the risk of secondary mycoses and allergies. Therefore, their assessment in medicinal plants is particularly important in areas where they are most frequently used, which was the objective of the presented work.

## Materials and Methods

During the studies conducted in 2020–2026, approximately 700 samples were collected and analyzed from various organs (vegetative and generative) of medicinal plants widely used in Azerbaijan's folk medicine (*Achillea millefolium* L., *Apium graveolens* L., *Crocus sativus* L., *Foeniculum vulgare* Mill., *Helichrysum arenarium* (L.) Moench., *Hypericum perforatum* L., *Malva sylvestris* L., *Matricaria chamomilla* L., *Mentha piperita* L., *Ocimum basilicum* L., *Olea europaea* L., *Rosa majalis* Herrm., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Thymus vulgaris* L., *Trifolium pratense* L., *Tussilago farfara* L., *Urtica urens* L., *Zea mays* L., etc.). The sampling methodology and ecological background analysis followed established protocols implemented in recent baseline mycological and environmental surveys in regional ecosystems of Azerbaijan (Muradov et al., 2026). From these, about 100 strains were isolated into pure cultures. Sampling, sample analysis, and isolation into pure cultures were performed according to methods widely used in mycological research.

Identification of the fungi was carried out on standard media recommended in manuals for specific groups of fungi, and the process was performed using well-known identification keys, based on the cultural-morphological and physiological properties of the fungi.

Cultures were maintained on wort agar, and for the experiments, they were grown in 500 mL Erlenmeyer flasks containing 100 mL of medium with the following composition (g/L): Glucose – 20; NaNO<sub>3</sub> – 2.0; K<sub>2</sub>HPO<sub>4</sub> – 1.0; MgSO<sub>4</sub> – 0.5; KCl – 0.5; Fe<sub>2</sub>O<sub>3</sub> – 0.01; and distilled water – 1 L. The fungi were cultivated on a shaker (120 rpm) for 7 days at a temperature of 25–27 °C. The composition of the media was modified depending on the objective of the experiment.

To study cellulolytic and pectolytic activity, the viscometric method was used; for amylolytic activity, the colorimetric method; and for proteolytic activity, Anson's method. The activity of cellulase, xylanase, amylase, and protease was expressed in  $\mu\text{mol}/\text{min}^{-1} \cdot \text{mL}^{-1}$  ( $\text{U} \cdot \text{mL}^{-1}$ ), and pectinase in  $\% \cdot \text{mL}^{-1}$  ( $\text{U} \cdot \text{mL}^{-1}$ ). These biochemical assessments align with modern methodologies used to screen the industrial and ecological potential of enzyme complexes from local fungal communities (Jafarzadeh

et al., 2025; Ismayilova et al., 2025). The phytotoxicity of the fungi (i.e., phytotoxic activity) was determined by the germination rate of seeds of certain plants according to the method used in the work of K. Bakhshaliyeva (2023) and expressed as a percentage.

## Results and Discussion

The results showed that the mycobiota of the studied plants, which are frequently used for medical purposes, includes dozens of micromycete species (Tab. 1). According to the taxonomy used on the official website of the International Mycological Association, they belong to the phyla *Ascomycota*, *Basidiomycota*, and *Mucormycota*. In the formation of the mycobiota of the studied plants, the predominant role belongs to the following fungi: *Aecidium foeniculi*, *Alternaria alternata*, *A. solani*, *Ascochyta foeniculina*, *Asc. imperfecta*, *Ascophora disciflora*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. terreus*, *A. ustus*, *Botrytis cinerea*, *Cladosporium cladosporioides*, *C. herbarum*, *Colletotrichum panacicola*, *Erysiphe communis*, *E. trifolii*, *Fusarium gibbosum*, *F. graminearum*, *F. moniliforme*, *F. oxysporum*, *F. semitectum*, *F. solani*, *Mucor mucedo*, *M. racemosus*, *Nigrospora maydis*, *Penicillium chrysogenum*, *P. cyclopium*, *P. martensii*, *Phoma medicaginis*, *Phyllosticta thymi*, *Ph. trifolii*, *Puccinia anethi*, *P. artemisiae*, *P. menthae*, *Rhizoctonia solani*, *Rhizopus stolonifer*, *Sclerotinia sclerotiorum*, *Sc. Libertian Sphaerotheca pannosa*, *Septoria menthae*, *Trichoderma hamatum*, *T. viride*, *Trichothecium roseum*, *Typhula trifolii*, *Uromyces appendiculatus*, *Ustilago zaeae*, *Verticillium albo-atrum*, *V. dahliae*, etc.

In some cases, the fungus *Candida albicans* was also detected on these materials. This fungus has a tendency toward invasion and, in cases of imbalance in the ecological niche, it can cause disease.

**Table 1**

*Numerical characteristics of species comprising the mycobiota of medicinal plants*

No.	Plant Species	Mucormycota	Ascomycota	Basidiomycota	Total
1	<i>Achillea millefolium</i>	–	9	1	10
2	<i>Apium graveolens</i>	–	12	1	13
3	<i>Crocus sativus</i>	–	7	1	8
4	<i>Foeniculum vulgare</i>	1	10	1	12
5	<i>Helichrysum arenarium</i>	–	15	1	16
6	<i>Hypericum perforatum</i>	–	13	1	14
7	<i>Malva sylvestris</i>	–	12	1	13
8	<i>Matricaria chamomilla</i>	–	9	1	10
9	<i>Mentha piperita</i>	–	8	1	9
10	<i>Ocimum basilicum</i>	–	12	–	12
11	<i>Olea europaea</i>	1	16	3	20
12	<i>Rosa majalis</i>	1	14	1	16
13	<i>Rosmarinus officinalis</i>	–	10	1	11
14	<i>Salvia officinalis</i>	–	9	1	10
15	<i>Thymus vulgaris</i>	–	8	1	9
16	<i>Trifolium/pratense</i>	–	17	2	19
17	<i>Tussilago farfara</i>	–	11	2	13
18	<i>Urtica urens</i>	–	8	–	8
19	<i>Zea mays</i>	1	15	2	18
20	Others	2	10	3	17
	Total	4	49	20	73

The data presented in Table 1 show that the studied plants differed from one another in the number of species involved in forming their mycobiota. For example, during the research, 19 species were found to participate in the formation of the mycobiota of *Trifolium pratense*, while only 8 species were found in *Urtica urens*, which is apparently related to the phytochemical composition of these plants (Mammadova et al., 2024).

It should be noted that the ability of fungi to secrete various enzymes plays a major role in the process of pathogenesis (Ismayilova et al., 2025), through which fungi break down the host cell wall and penetrate inside the tissue. At the same time, the enzymatic system of the fungus plays a crucial role in the penetration and development of the fungus in the host tissues. With this in mind, during the research, the isolated fungi, which are either causative agents of secondary mycoses (Mammadaliyeva et al., 2024) or producers of mycotoxins dangerous to human health, were characterized by their hydrolytic enzyme activity. The choice of hydrolytic enzymes is due to the fact that the polymers comprising the cell walls of the host plant (cellulose, hemicellulose, pectin, proteins, etc.) are broken down via a hydrolytic pathway. The obtained results showed that the studied fungal strains exhibit, to one degree or another, the activity of hydrolytic enzymes such as cellulase, amylase, protease, xylanase, and pectinase (Tab. 2).

Furthermore, the data revealed that some strains possessed high activity of a specific enzyme, while others possessed high activity of all enzymes—meaning some strains were producers of a specific enzyme, while others were producers of a balanced hydrolytic enzyme system. In terms of these indicators, species of the genus *Trichoderma* (such as *T. hamatum* and *T. viride*) demonstrated potent cellulolytic and xylanase profiles, validating their known ecological role as aggressive competitors and potential biological control agents in agricultural soils (Muradov et al., 2025; Muradov & Bakhshaliyeva, 2024). They were not inferior even to well-known producers, although the obtained data do not allow for an unambiguous assessment of the role of enzymes in fungal pathogenesis, since a correlation between the danger of the fungi and hydrolase activity is not evident at first glance. For instance, all strains of the genus *Fusarium* were inferior to *A. niger* strains in the activity of all enzymes, especially proteolytic ones.

**Table 2**

*Enzyme activity (U/mL) of fungi detected on the studied plants*

Species (number of strains)	Cellulase	Xylanase	Amylase	Pectinase	Protease
<i>Aspergillus flavus</i> (5)	1.11–2.02	20.10–28.69	1.68–2.59	5.60–7.31	3.62–7.11
<i>A. fumigatus</i> (5)	1.01–1.67	17.78–24.33	2.31–3.78	4.49–7.51	2.70–6.49
<i>A. niger</i> (5)	1.99–4.31	35.28–42.52	3.81–5.28	9.60–11.49	4.62–7.22
<i>A. ochraceus</i> (4)	0.37–0.67	13.22–17.61	1.21–2.30	7.11–8.88	3.18–4.49
<i>Alternaria alternata</i> (4)	0.89–1.79	12.69–23.49	0.67–1.28	3.08–5.09	2.21–4.71
<i>A. solani</i> (4)	0.51–1.21	17.81–30.09	0.51–0.78	2.77–4.31	1.90–5.69
<i>Botrytis cinerea</i> (4)	0.49–0.68	21.22–24.61	traces	1.21–2.49	0.69–1.20
<i>F. avenaceum</i> (8)	1.11–1.58	16.41–21.92	1.40–2.21	1.52–3.53	traces
<i>F. gibbosum</i> (5)	0.80–1.31	15.59–25.29	1.78–2.99	2.61–4.80	0.31–0.79
<i>F. moniliforme</i> (5)	0.89–1.30	25.28–35.38	2.69–4.18	3.10–5.38	0.20–0.89
<i>F. oxysporum</i> (5)	0.71–1.20	20.19–31.39	2.31–3.49	2.30–4.59	traces
<i>F. semitectum</i> (4)	1.01–1.29	16.71–25.41	1.82–2.63	2.29–3.89	0.11–0.21
<i>V. dahliae</i> (5)	0.31–0.50	18.89–23.45	1.11–1.49	3.51–6.68	0.81–1.09
<i>V. albo-atrum</i> (4)	0.22–0.41	15.39–20.28	0.89–1.38	3.01–5.22	0.61–1.12
<i>P. martensii</i> (4)	0.20–0.49	23.42–31.28	traces	2.91–6.09	2.28–4.48
<i>P. cyclopium</i> (5)	0.28–0.67	26.45–34.50	1.18–2.40	3.39–7.41	1.11–2.21

<i>P. chrysogenum</i> (5)	0.81–1.28	18.89–23.41	0.62–1.42	2.11–4.32	2.89–4.56
<i>C. herbarum</i> (4)	1.10–1.50	19.32–28.27	0.31–0.78	2.56–4.23	1.41–1.89
<i>Septoria menthae</i> (5)	0.72–1.11	14.45–20.23	0.47–1.09	3.10–3.89	1.31–2.20
<i>Asc. foeniculina</i> (4)	0.91–1.42	17.21–23.23	0.71–1.42	2.59–4.18	1.30–1.71
<i>Asc. imperfecta</i> (3)	0.49–1.01	14.18–19.41	0.29–0.79	1.81–3.69	1.18–1.89
<i>Rh. stolonifer</i> (4)	1.28–1.61	18.1–21.4	1.12–1.51	2.12–4.12	4.43–5.72
<i>M. mucedo</i> (5)	1.11–1.52	19.6–328.22	0.94–1.33	2.43–3.31	6.42–7.64
<i>M. racemosus</i> (3)	0.91–1.72	17.17–24.27	0.71–1.52	1.61–3.88	3.61–5.92
<i>T. hamatum</i> (5)	2.32–4.12	29.10–36.72	0.11–0.49	1.21–1.89	4.11–5.67
<i>T. viride</i> (4)	2.03–3.41	25.64–34.51	0.18–0.39	1,11–1,56	3,21–5,42

However, the study of fungal phytotoxicity (in relation to the germination of wheat, bean, and cucumber seeds treated with the culture liquids of the investigated fungi) showed that fungi with high activity of proteolytic enzymes are not characterized by high phytotoxic activity (Tab. 3). For example, the activity of proteolytic enzymes of the phytopathogenic fungus *Verticillium dahliae* was almost 5 times lower than that of the fungus *Aspergillus flavus*, although the phytotoxic activity of *V. dahliae* on the example of wheat was 1.8 times greater than that of *A. flavus*. A similar pattern is observed when comparing other fungi. Consequently, the level of activity of proteolytic enzymes can be used as a factor limiting the process of pathogenesis in phytopathogenic fungi, whereas for causative agents of secondary mycoses such as *A. niger*, it is, on the contrary, a factor contributing to other (e.g., pathological or opportunistic) actions.

**Table 3**

Enzyme activity (U/mL) of fungi detected on the studied plants

Most active strains	Wheat	Bean	Cucumber
<i>Aspergillus flavus</i> AF-09	79	82	74
<i>A. fumigatus</i> AF-11	70	73	65
<i>A. niger</i> AN-24	80	78	76
<i>A. ochraceus</i> AO-33	78	72	75
<i>Alternaria alternata</i> AA-07	65	69	63
<i>A. solani</i> AS-14	61	55	51
<i>Botrytis cinerea</i> BC-03	49	52	43
<i>Fusarium avenaceum</i> FA-07	30	40	34
<i>Fusarium gibbosum</i> FG-14A	39	44	40
<i>Fusarium moniliforme</i> FM-24	38	40	37
<i>Fusarium oxysporum</i> FO-32	35	34	29
<i>Fusarium semitectum</i> FS-42	37	39	33
<i>Verticillium albo-atrum</i> VA-07	50	52	49
<i>Verticillium dahliae</i> VD-16	45	49	47
<i>Penicillium martensii</i> PM-32	67	70	64
<i>Penicillium cyclopium</i> PC-18	59	63	56
<i>Penicillium chrysogenum</i> PC-02	72	74	68
<i>Cladosporium herbarum</i> CH-02	65	68	62
<i>Sclerotium alliorum</i> SA-06	62	65	61
<i>Ascochyta foeniculina</i> AF-09	65	63	60
<i>Ascochyta imperfecta</i> AI-17	61	64	59
<i>Rhizopus stolonifer</i> RS-04	76	80	79
<i>Mucor mucedo</i> MM-07	85	90	86

<i>Mucor racemosus</i> MR-14	79	78	80
<i>Trichoderma hamatum</i> TH-05	99	97	97
<i>Trichoderma viride</i> TV-12	94	92	95

Thus, the obtained data showed that plant materials used in folk medicine serve as a habitat for fungi, among which there are species capable of synthesizing enzymes balanced in hydrolase activity that degrade the host cell walls.

## Conclusion

1. For the first time, the species composition of the mycobiota of medicinal plants widely used in the folk medicine of Azerbaijan was comprehensively characterized. A total of 73 micromycete species belonging to the phyla *Ascomycota*, *Basidiomycota*, and *Mucormycota* were identified, revealing that the qualitative and quantitative composition of the fungi heavily depends on the phytochemical properties of the specific host plant and surrounding soil dynamics.

2. The isolated fungal strains exhibit varying degrees of hydrolytic enzyme activity (cellulase, xylanase, amylase, pectinase, and protease). It was established that certain strains possess a highly balanced hydrolytic enzyme system capable of effectively degrading the cell wall polymers of the host plants, thereby facilitating their penetration and establishing them as potent agents of pathogenesis.

3. A distinct functional differentiation was observed regarding proteolytic enzyme activity. In phytopathogenic fungi (e.g., *Verticillium dahliae*), low proteolytic activity correlates with high phytotoxicity, indicating that protease serves as a limiting factor in plant pathogenesis. Conversely, in causative agents of secondary mycoses (e.g., *Aspergillus niger*), high proteolytic activity acts as a contributing factor that enhances their opportunistic and pathological potential.

4. The presence of toxigenic and opportunistic fungi (such as *Aspergillus* spp., *Fusarium* spp., and *Candida albicans*) on medicinal plants that are frequently consumed without prior thermal processing poses serious biological risks. These materials can serve as environmental reservoirs for dangerous mycotoxins and fungal allergens, significantly increasing the risk of secondary mycoses and allergic reactions among the population. Understanding these fungal complexes and their enzymatic regulation opens up new paths for using antagonistic strains, such as specialized *Trichoderma* species, as green fungicides and biostimulants to protect valuable plant resources.

## Declaration of Competing Interests

The author declares that they have no competing interests.

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# In Vitro Propagation of Rosemary (*Rosmarinus officinalis* L.): Effects of BAP and NAA Concentrations on Shoot Proliferation, Morphogenesis, and Regeneration

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**Abstract.** Rosemary (*Rosmarinus officinalis* L.) is an economically and medicinally important aromatic shrub widely recognized for its richness in essential oils, phenolic diterpenes, and antioxidant constituents. Traditional propagation techniques often produce genetically variable plant material and are unable to supply sufficient quantities to satisfy industrial demand, highlighting the need for efficient and reproducible in vitro propagation systems. In this study, the effects of two cytokinin–auxin combinations on the growth, organogenesis, and morphogenetic responses of rosemary nodal explants cultured on Murashige and Skoog (MS) basal medium were evaluated. The treatments included 1.0 mg/L 6-benzylaminopurine (BAP) combined with 0.5 mg/L naphthaleneacetic acid (NAA) (T1), and 2.0 mg/L BAP combined with 0.5 mg/L NAA (T2). Explants were incubated under a 15-hour photoperiod with a 7-hour dark cycle at 24–26 °C, following an initial 72-hour dark preconditioning period. After four weeks of culture, T1 resulted in uniform shoot development with minimal or no callus formation, whereas T2 promoted a higher rate of shoot proliferation but induced incipient callus formation in approximately 25% of explants. These results indicate that the balance between cytokinin and auxin strongly influences regeneration behavior in rosemary, with 1.0 mg/L BAP providing more stable morphogenetic outcomes suitable for large-scale micropropagation, while 2.0 mg/L BAP may be applied when rapid shoot multiplication is prioritized under controlled conditions ensuring genetic stability.

**Keywords:** *Rosmarinus officinalis* L., in vitro propagation, micropropagation, MS medium, 6-benzylaminopurine, naphthaleneacetic acid, organogenesis, callus formation, plant tissue culture, essential oils, phytohormones, secondary metabolites

## Introduction

Rosemary (*Rosmarinus officinalis* L., Lamiaceae) is a perennial, evergreen aromatic shrub indigenous to the Mediterranean basin, where it has been exploited for culinary, medicinal, and cosmetic purposes for centuries. The species accumulates a diverse array of bioactive secondary metabolites, including 1,8-cineole, camphor,  $\alpha$ -pinene, borneol, rosmarinic acid, carnosic acid, and carnosol, which collectively confer pronounced antioxidant, antimicrobial, anti-inflammatory, and neuroprotective properties (Del Baño et al., 2003).

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Received: 8 February 2026; Accepted: 11 May 2026; Published online: 25 June 2026

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These phytochemical attributes have sustained growing interest from the pharmaceutical, food, and cosmetics industries, leading to a rapid escalation of commercial demand (Jordán et al., 2012).

Global demand for uniform, pathogen-free rosemary planting material has outpaced the capacity of traditional propagation methods. Seed-based reproduction is constrained by low germination rates, prolonged dormancy, and the generation of genetically heterogeneous offspring (Rao et al., 2009). Semi-hardwood stem cuttings, though widely practiced, are subject to seasonal limitations, variable rooting success, and the risk of transferring systemic pathogens (Cassells & Curry, 2001). These limitations collectively underscore the need for a reproducible, scalable alternative that guarantees genetic fidelity and phytosanitary quality.

Plant tissue culture, and in particular micropropagation via organogenesis, provides a compelling solution to these constraints. Under controlled *in vitro* conditions, genetically identical clones can be generated at high multiplication rates throughout the year, independent of seasonal and geographic restrictions (George et al., 2008). The Murashige and Skoog (MS) basal medium, formulated in 1962, remains the most widely adopted nutrient substrate for dicotyledonous tissue culture due to its high inorganic salt concentrations and demonstrated efficacy across a broad taxonomic range (Murashige & Skoog, 1962).

The regulation of *in vitro* morphogenesis is principally governed by the exogenous supply of phytohormones, particularly cytokinins and auxins. 6-Benzylaminopurine (BAP) is the cytokinin most commonly employed to stimulate axillary bud break and multiple shoot proliferation, while naphthaleneacetic acid (NAA) serves as an auxin source for root induction and growth modulation (Skoog & Miller, 1957). The cytokinin-to-auxin ratio critically determines whether an explant undergoes shoot organogenesis, root initiation, or dedifferentiation into callus tissue (Woodward & Bartel, 2005). Despite the broad application of rosemary in industrial sectors, optimized, concentration-specific protocols for its *in vitro* propagation remain insufficiently documented in the primary literature, particularly with regard to Uzbek germplasm (Hussain et al., 2012).

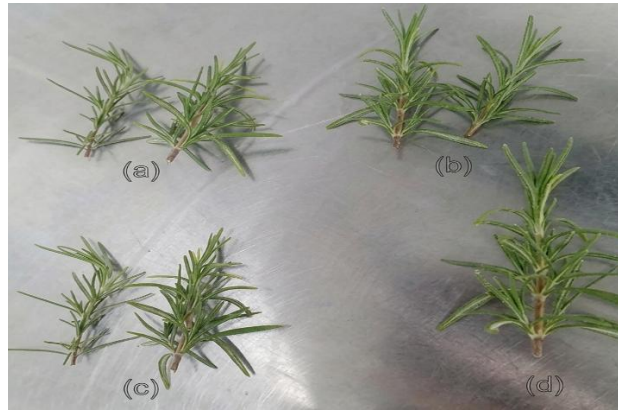
The present investigation was therefore designed to evaluate the effect of two BAP concentrations (1.0 and 2.0 mg/L), each combined with 50 µL NAA, on the growth dynamics, shoot proliferation, callus induction, and morphological stability of nodal rosemary explants over a four-week culture period. The results are intended to contribute a scientifically grounded, regionally applicable micropropagation protocol that can reduce dependence on wild harvesting and support sustainable production of this economically important aromatic plant (Johnson et al., 2002).

## **Materials and Methods**

### ***Plant Material and Explant Selection***

Actively growing nodal segments (approximately 1.5–2.0 cm in length, bearing one or two axillary buds) were harvested from healthy, greenhouse-maintained rosemary donor plants. Young stem segments were selected in preference to mature woody tissue due to their higher regenerative capacity and greater responsiveness to exogenous phytohormone treatments (Smith, 2013). All explants were collected from plants confirmed to be free from visible signs of fungal or bacterial infection.

Prior to surface sterilization, explants were thoroughly rinsed under running tap water for 30 minutes to remove superficial debris, followed by agitation in a 0.1% (v/v) Tween-20 solution for 15 minutes. The explants were then subjected to a sequential sterilization protocol (Fig. 1): the explants were surface-sterilized by treatment with 80% (v/v) ethanol for 30 s and subsequently exposed to a 1% (w/v) sodium hypochlorite (NaOCl) solution for 10 min under mild continuous shaking. Residual sterilant was removed by rinsing three to five times with sterile double-distilled water under aseptic laminar airflow conditions.



**Figure 1**

*Sequential surface sterilization procedure for Rosmarinus officinalis L. nodal explants.*

*(a) Rinsing under running tap water; (b) treatment with 80% ethanol; (c) immersion in 1% NaOCl solution; (d) final rinsing with sterile distilled water under laminar airflow.*

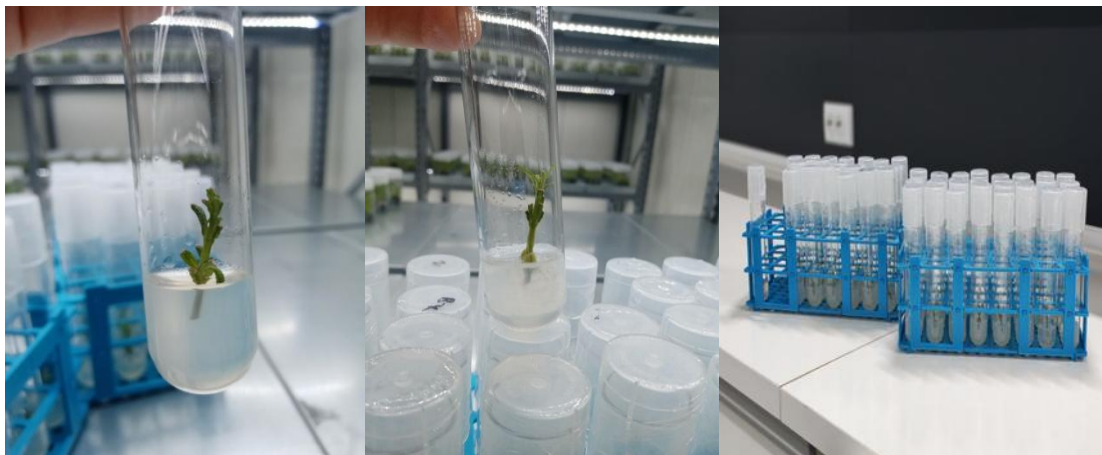
### **Culture Medium Preparation**

The explants were initiated on a complete Murashige and Skoog (MS) culture medium containing 30 g/L sucrose and 7 g/L agar-agar for medium solidification (Murashige & Skoog, 1962). Prior to sterilization, the pH value was adjusted to  $5.7 \pm 0.1$  using 0.1 M NaOH or 0.1 M HCl. Two separate phytohormone formulations were then added to the prepared media:

Treatment T1: MS + 30 g/L sucrose + 7 g/L agar + 1.0 mg/L BAP + 50  $\mu$ L NAA

Treatment T2: MS + 30 g/L sucrose + 7 g/L agar + 2.0 mg/L BAP + 50  $\mu$ L NAA

BAP stock solutions (1.0 mg/mL) were prepared by dissolving the compound in a minimal volume of 1 M NaOH and diluting to the required volume with deionized water. NAA was dissolved in 96% ethanol. The prepared media were sterilized in an autoclave at 120 °C under a pressure of 1.051 kg/cm<sup>2</sup> for 20 min. After sterilization, nearly 50 mL of medium was poured into previously sterilized 350-mL glass culture jars and left at ambient temperature until complete solidification occurred. Explant inoculation was conducted in a Class II laminar flow cabinet.



**Figure 2**

*Nodal explants of Rosmarinus officinalis L. inoculated onto MS basal medium supplemented with BAP and NAA. Culture vessels sealed with polypropylene caps and transferred to the growth chamber.*

### ***Incubation Conditions and Observation Parameters***

Following inoculation, all culture vessels were kept in darkness at  $24 \pm 1$  °C for an initial 72-hour period to mitigate the phenolic oxidation response that commonly occurs during explant wounding. After inoculation, the explants were placed in a growth chamber operated at 23–27 °C. A 15-hour light cycle was applied using cool-white fluorescent lamps with a light intensity of about 40–45  $\mu\text{M m}^{-2} \text{s}^{-1}$ , followed by a 7-hour dark phase. The relative humidity of the culture environment was regulated within the range of 60–80%. Growth and developmental changes were recorded weekly for four consecutive weeks.

Morphological parameters recorded included: (i) survival rate of explants (percentage of contamination-free, viable cultures); (ii) time to visible bud break (days); (iii) mean shoot number per explant; (iv) presence and extent of callus formation; and (v) overall morphological uniformity assessed by visual scoring on a three-point scale (high, moderate, low).

## **Results and Discussion**

### ***Efficacy of MS Basal Medium for Rosemary Explant Establishment***

The MS basal medium supported reliable establishment of rosemary nodal explants across both treatment groups, with survival rates exceeding 85% after the first week. The medium's high macronutrient concentrations — particularly with respect to nitrogen, phosphorus, potassium, calcium, and magnesium — provided an adequate nutritional basis for the early phases of cell division and tissue differentiation (Murashige & Skoog, 1962). This observation is consistent with the broad-spectrum utility of MS medium reported across numerous Lamiaceae species, including *Salvia officinalis*, *Mentha piperita*, and *Origanum vulgare* (Kintzios et al., 1999).

The inclusion of 30 g/L sucrose as the sole carbon source proved sufficient to maintain heterotrophic metabolism during the pre-photoperiod dark phase and sustained autotrophic transition thereafter. The gelling agent (agar-agar at 7 g/L) provided the appropriate mechanical support for upright explant positioning, which is critical for uniform light interception and gas exchange in nodal cultures (Loyola-Vargas & Ochoa-Alejo, 2012).

### ***Effect of BAP and NAA on Shoot Proliferation***

Differential shoot proliferation responses were observed between the two phytohormone treatments after four weeks of culture (Tab. 1; Figs. 3 and 4). Explants cultured on T1 medium (1.0 mg/L BAP + 50  $\mu\text{L}$  NAA) showed uniform activation of axillary buds within 7–10 days, followed by the formation of one to three elongated shoots per explant by the end of the fourth week. In comparison, T2 explants (2.0 mg/L BAP + 50  $\mu\text{L}$  NAA) responded more rapidly, with bud break occurring as early as 5–7 days after inoculation and producing a noticeably higher average number of shoots per explant (Kompelly et al., 2019). These differences are consistent with the well-established role of cytokinin concentration in regulating shoot organogenesis. BAP promotes cell division and shoot initiation by modulating cell cycle activity and supporting the transition of dormant meristematic tissues into actively growing shoot primordia. At relatively lower concentrations (around 1.0 mg/L), its effect is mainly directed toward the activation of pre-existing axillary meristems, resulting in more controlled and uniform shoot elongation (Mok & Mok, 2001). However, at higher levels (such as 2.0 mg/L), the increased cytokinin availability intensifies cell proliferation, leading to a higher frequency of shoot induction. At the same time, an elevated cytokinin-to-auxin balance can sometimes trigger partial dedifferentiation in certain tissues, which may increase the likelihood of callus formation in sensitive explants (Skoog & Miller, 1957).

**Table 1**

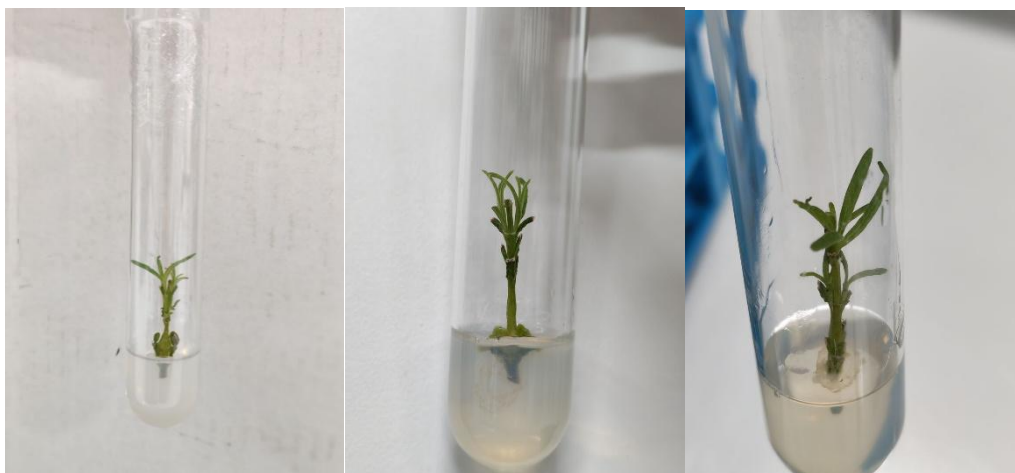
*Effect of BAP and NAA concentrations on morphogenetic response of Rosmarinus officinalis L. nodal explants after four weeks of in vitro culture on MS basal medium*

Treatment	BAP (mg/L)	NAA ( $\mu$ L)	Shoot Proliferation	Callus Formation	Growth Uniformity
T1	1.0	50	Moderate; uniform axillary bud activation	Minimal / absent	High; morphologically stable
T2	2.0	50	High; rapid multiple shoot induction	Observed in some explants (~25%)	Moderate; some dedifferentiation

*BAP: 6-benzylaminopurine; NAA: naphthaleneacetic acid; T1: low BAP treatment; T2: high BAP treatment.*

### **Callus Formation, Morphogenesis, and Genetic Stability**

Callus induction represents a critical quality parameter in micropropagation protocols, as prolonged passage through unorganized callus tissue increases the likelihood of somaclonal variation and compromises the genetic uniformity of regenerated plants (Larkin & Scowcroft, 1981). In the present study, T1 explants displayed negligible callus at the base of shoots and at the cut ends of nodal segments, with no evidence of disorganized proliferation at the shoot apex (Gitonga et al., 2010). Morphological uniformity was assessed as high for this treatment group.

**Figure 3**

*Rosmarinus officinalis L. nodal explants cultured on MS medium supplemented with 2.0 mg/L BAP and 50  $\mu$ L NAA after four weeks of in vitro culture. Note active shoot proliferation and incipient basal callus formation in some explants.*

T2 explants, by contrast, exhibited visible friable callus at the nodal base in approximately 25% of cultures by week three. While this callus did not prevent shoot emergence in most cases, it represented an undesirable feature for propagation purposes, as it may delay rooting during the subsequent acclimatization phase and necessitates additional sub-culture steps (Pati et al., 2006). The morphological uniformity score for T2 was therefore rated as moderate.



**Figure 4**

*Nodal explants of *Rosmarinus officinalis* L. cultivated on MS medium containing 1.0 mg/L BAP and 40 mg/L NAA exhibited successful in vitro growth after four weeks. Distinct shoot elongation and active axillary bud proliferation were observed, while no callus tissue developed during the culture period.*

#### ***Bioactive Compound Accumulation and Quality Implications***

Secondary metabolite content in tissue-cultured plants is an important practical consideration, particularly when the objective is pharmaceutical or nutraceutical production. The literature indicates that in vitro-propagated rosemary plants can maintain, and in some cases exceed, the essential oil yield of conventionally propagated material when appropriate culture conditions are maintained (Ahloowalia et al., 2004; Verpoorte et al., 2002). Morphologically stable explants obtained under T1 conditions, characterized by orderly shoot development and minimal callus, are expected to exhibit a biochemical profile comparable to mother plants, including the retention of key compounds such as rosmarinic acid, carnosic acid, and 1,8-cineole.

In contrast, the greater cell division activity induced by 2.0 mg/L NAA BAP, while advantageous for rapid multiplication, may redirect primary metabolic flux away from secondary metabolite biosynthesis pathways. This phenomenon, associated with high cytokinin-driven growth, has been documented in Lamiaceae tissue cultures and underscores the importance of selecting phytohormone regimes that balance multiplication rate against chemical quality of the resultant plantlets (Chaturvedi et al., 1983).

#### **Conclusion**

This study demonstrated that the composition of plant growth regulators in the culture medium has a strong effect on the growth behavior, shoot proliferation capacity, and morphological consistency of *Rosmarinus officinalis* L. explants under in vitro conditions. The T1 treatment (1.0 mg/L BAP + 50  $\mu$ L NAA) resulted in uniform, callus-free plantlets with consistent axillary shoot development over the four-week culture period, indicating its suitability for reliable large-scale micropropagation where genetic stability is a primary requirement. In contrast, the T2 treatment (2.0 mg/L BAP + 50  $\mu$ L NAA) produced a higher rate of shoot multiplication, although this was accompanied by occasional callus formation in some explants, suggesting that the increased cytokinin level may slightly disturb the hormonal balance required for strictly organized organogenesis.

Overall, the results indicate that MS medium supplemented with 1.0 mg/L BAP and 50  $\mu$ L NAA provides an effective baseline protocol for the initial stages of rosemary micropropagation. Further work should focus on optimizing rooting conditions, improving acclimatization efficiency during

transfer to ex vitro environments, and confirming the genetic fidelity of regenerated plants through molecular analyses. Expanding this protocol to include locally adapted rosemary genotypes from Central Asia could enhance its practical application and support the conservation and sustainable use of medicinal plant resources in Uzbekistan.

### Declaration of Competing Interests

The authors declare that there are no conflicts of interest regarding the publication of this study.

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# Mechanisms of Influence of Size and Surface Charge on the Bioaccumulation of Nanoparticles in Plants

Zeynab Mikayilzada<sup>1</sup> , Vafa Ramazanli<sup>2\*</sup> 

**Abstract.** *Objective:* This study aims to investigate the influence of nanoparticle size and surface charge on their uptake, translocation, and bioaccumulation in plants and to identify the underlying mechanisms governing these processes. *Methods:* A comprehensive analysis of recent experimental studies on plant–nanoparticle interactions was conducted, focusing on the effects of nanoparticle physicochemical properties on plant uptake pathways and accumulation patterns. *Results:* The findings demonstrate that smaller nanoparticles exhibit higher mobility and greater penetration through plant cell walls and membranes, resulting in enhanced translocation to aerial tissues. In contrast, larger nanoparticles predominantly accumulate on root surfaces, limiting their internal transport. Surface charge was also found to significantly affect bioaccumulation. Positively charged nanoparticles showed stronger interactions with negatively charged plant cell wall components, leading to increased adsorption and uptake, whereas negatively charged or neutral nanoparticles exhibited lower uptake efficiency. Nanoparticles entered plant tissues through apoplastic and symplastic pathways, with endocytosis and ion channel-mediated transport contributing to cellular internalization. Accumulated nanoparticles were associated with oxidative stress, altered nutrient transport, and changes in gene expression. *Conclusion:* Nanoparticle size and surface charge are key determinants of bioaccumulation in plants. Understanding their role in uptake and translocation mechanisms is essential for ecological risk assessment and the development of safer nanomaterials for agricultural and environmental applications.

**Keywords:** nanoparticles, bioaccumulation, plants, particle size, surface charge, uptake mechanisms, phytotoxicity

## Introduction

The rapid advancement of nanotechnology has led to the widespread production and application of engineered nanoparticles (NPs) in various industries, including agriculture, medicine, electronics, and environmental remediation. Nanoparticles, defined as materials with at least one dimension less than 100 nanometers, possess unique physicochemical properties, such as a high surface area-to-volume ratio, tunable surface charge, and distinctive optical and catalytic characteristics. These properties, while advantageous for industrial applications, also raise concerns about their potential environmental impact, particularly when they interact with biological systems. Among living organisms, plants represent a critical interface, as they are primary producers in ecosystems and serve as the initial point of entry for nanoparticles into terrestrial food chains (Djanaguiraman et al., 2024).

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Received: 25 February 2026; Accepted: 11 May 2026; Published online: 25 June 2026

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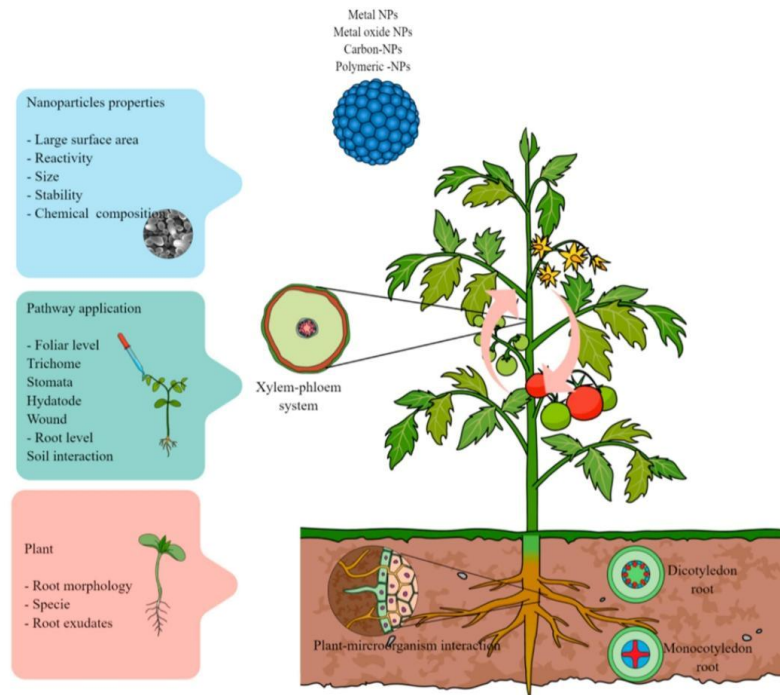
The interaction of nanoparticles with plants involves complex processes, including adsorption to root surfaces, uptake into cells, translocation to aerial parts, and eventual accumulation in tissues. The efficiency of these processes is strongly influenced by the intrinsic characteristics of nanoparticles. Particle size is a primary determinant: smaller nanoparticles can penetrate cell walls and membranes more readily, facilitating systemic distribution within plant tissues. In contrast, larger nanoparticles often remain bound to root surfaces or accumulate in the apoplastic space, limiting their mobility. Surface charge is another key factor; positively charged nanoparticles exhibit strong electrostatic interactions with negatively charged cell wall components, promoting adhesion and internalization. Negatively charged or neutral particles, on the other hand, tend to show reduced uptake efficiency (Abou El-Nasr et al., 2025).

Mechanistically, nanoparticles can enter plants through apoplastic pathways, which involve movement through cell walls and intercellular spaces, or through symplastic pathways, which require passage across the plasma membrane into the cytoplasm. Endocytosis, ion channels, and transporter-mediated mechanisms have all been proposed as routes for nanoparticle internalization. Once internalized, nanoparticles may interact with cellular organelles, disrupt nutrient transport, and induce oxidative stress, potentially affecting growth and development.

Furthermore, the environmental context, including soil pH, ionic strength, and the presence of organic matter, can modulate nanoparticle behavior, bioavailability, and accumulation. Understanding these interactions is crucial not only for evaluating the ecological risks of nanoparticles but also for designing safer nanomaterials for agricultural and environmental applications. Research on nanoparticle bioaccumulation in plants has intensified over the past decade, focusing on a wide range of materials, including metal-based nanoparticles (e.g., silver, copper, zinc oxide), carbon-based nanomaterials (e.g., carbon nanotubes, graphene), and polymeric nanoparticles.

Studies have demonstrated that plant species differ in their uptake capacity and translocation efficiency, influenced by root architecture, cell wall composition, and metabolic activity. Moreover, nanoparticle-induced physiological responses can vary, ranging from enhanced growth and nutrient uptake to phytotoxic effects such as oxidative damage, chlorosis, and reduced biomass. These findings highlight the importance of systematically investigating how nanoparticle properties, especially size and surface charge, govern their interaction with plants and the resulting bioaccumulation patterns (Tortella et al., 2023).

The present review aims to provide a comprehensive overview of the mechanisms underlying nanoparticle uptake and accumulation in plants, with particular emphasis on the roles of particle size and surface charge. By integrating insights from recent experimental studies, this work seeks to elucidate the physicochemical and biological factors that influence nanoparticle bioaccumulation. Additionally, it addresses potential ecological implications, including nanoparticle transfer through food chains, and discusses strategies for mitigating risks associated with environmental nanoparticle exposure. Understanding these processes is essential for advancing (Yang et al., 2025).



**Figure 1**

*Schematic representation of nanoparticle uptake, translocation, and bioaccumulation pathways in plants*

The figure illustrates how nanoparticles interact with plants, emphasizing the influence of nanoparticle properties, plant characteristics, and environmental pathways on uptake and bioaccumulation. Nanoparticles vary in size, surface area, chemical composition, reactivity, and stability, which collectively determine how they are absorbed and transported within plant tissues. Entry routes include foliar surfaces through stomata, trichomes, or hydathodes, as well as root uptake from soil, where interactions with root exudates and microorganisms can affect bioavailability. Once internalized, nanoparticles move via the xylem and phloem systems to aerial parts such as stems, leaves, flowers, and fruits. Plant-specific factors, including root morphology, species differences, and the presence of root exudates, further influence the efficiency of nanoparticle uptake and translocation. The diagram also differentiates between dicotyledonous and monocotyledonous roots, highlighting how structural differences impact nanoparticle movement (Zhang et al., 2024).

## Methods

The study of nanoparticle (NP) bioaccumulation in plants requires a multidisciplinary approach combining controlled experimental design, precise characterization of nanoparticles, and quantitative analytical techniques. Initially, the nanoparticles must be synthesized or obtained with well-defined physicochemical properties, including particle size, surface charge (zeta potential), chemical composition, and stability. Commonly used methods for characterization include dynamic light scattering (DLS) to measure hydrodynamic diameter, transmission electron microscopy (TEM) for size and morphology visualization, and zeta potential analysis to determine surface charge. These properties are crucial because particle size and charge influence adsorption to root surfaces, internalization, and translocation through xylem and phloem (Wang et al., 2023).

For plant exposure experiments, seedlings of selected species are cultivated under controlled conditions, typically in hydroponic systems or sterilized soil to minimize confounding environmental variables. Nanoparticles are administered either via the nutrient solution for root exposure or as foliar sprays to assess leaf uptake. Concentrations are chosen based on preliminary toxicity tests, often

expressed in mg/L, and exposure durations are standardized to enable comparison across treatments. During exposure, environmental parameters such as pH, ionic strength, temperature, and light intensity are monitored, as they affect nanoparticle stability and bioavailability.

Post-exposure, plant tissues are harvested and processed for quantitative analysis. Roots, stems, leaves, and fruits are washed to remove surface-bound nanoparticles, dried, and homogenized (Bouhadi et al., 2025). The concentration of nanoparticles or metal ions in each tissue is then measured using techniques such as inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectroscopy (AAS), or fluorescence-based methods for labeled nanoparticles. Bioaccumulation factors (BAF) are calculated using the formula:

$$\text{BAF} = \frac{C_{\text{plant tissue}}}{C_{\text{exposure medium}}}$$

where  $C_{\text{plant tissue}}$  is the concentration of nanoparticles measured in the plant part (mg/kg dry weight) and  $C_{\text{exposure medium}}$  is the concentration in the hydroponic solution or soil (mg/L). For example, if tomato leaf tissue contains 12 mg/kg of silver nanoparticles after exposure to a 4 mg/L solution, the BAF is calculated as  $12/4 = 3$ , indicating significant accumulation relative to the exposure concentration (Azeem et al., 2021).

Translocation factor (TF) is also determined to evaluate systemic movement of nanoparticles from roots to shoots:

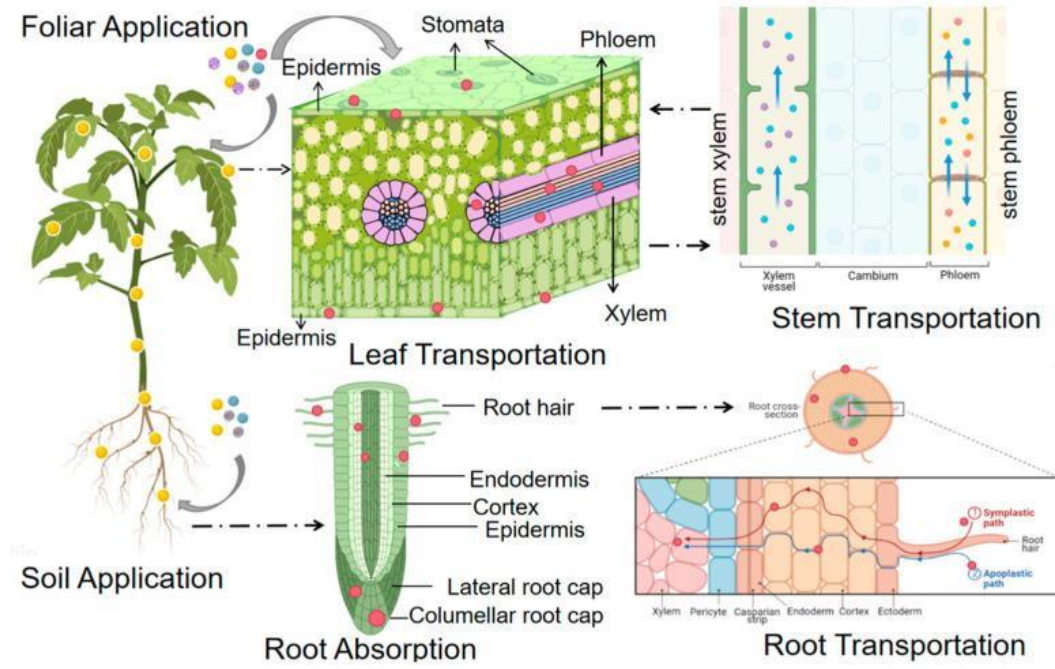
$$\text{TF} = \frac{C_{\text{shoot}}}{C_{\text{root}}}$$

where  $C_{\text{shoot}}$  and  $C_{\text{root}}$  represent nanoparticle concentrations in aerial and root tissues, respectively. A TF greater than 1 suggests preferential translocation to aerial tissues, while TF less than 1 indicates retention in roots. Statistical analyses, including ANOVA and regression, are applied to compare treatment groups and assess correlations between nanoparticle size, surface charge, and accumulation patterns (Kumari et al., 2024).

In addition to quantitative assays, microscopy techniques such as TEM, scanning electron microscopy (SEM), and confocal laser scanning microscopy are employed to visualize nanoparticle localization at the cellular and subcellular levels. Elemental mapping using energy-dispersive X-ray spectroscopy (EDS) further confirms nanoparticle presence within specific tissues or organelles. For surface charge effects, zeta potential measurements are often correlated with uptake efficiency to determine the influence of electrostatic interactions between nanoparticles and negatively charged plant cell walls (Kranjc et al., 2019).

## Results

The integration of these methods allows for comprehensive assessment of nanoparticle behavior in plant systems. By combining physicochemical characterization, controlled exposure, quantitative analysis, and imaging techniques, the methodology captures both the kinetics of uptake and the spatial distribution of nanoparticles. This framework supports mechanistic understanding of how nanoparticle size and surface charge influence bioaccumulation, providing data critical for risk assessment and the development of environmentally safe nanomaterials (D'Angelo, 2026).



**Figure 2**  
*Nanoparticle uptake, translocation, and transport pathways in plants via foliar and root application*

1. Bioaccumulation Factor (BAF)

$$BAF = \frac{C_{\text{plant tissue}}}{C_{\text{exposure medium}}}$$

Plant Part	Nanoparticle Concentration in Tissue (mg/kg)	Exposure Medium Concentration (mg/L)	BAF
Root	20	5	4
Stem	8	5	1.6
Leaf	12	5	2.4

- For leaf tissue:

$$BAF = \frac{12 \text{ mg/kg}}{5 \text{ mg/L}} = 2.4$$

This indicates that the leaf accumulates 2.4 times the concentration relative to the exposure solution.

2. Translocation Factor (TF)

$$TF = \frac{C_{\text{shoot}}}{C_{\text{root}}}$$

Shoot Part	Shoot Concentration (mg/kg)	Root Concentration (mg/kg)	TF
Stem	8	20	0.4
Leaf	12	20	0.6

- For leaf translocation:

$$TF = \frac{12}{20} = 0.6$$

A TF less than 1 indicates that the majority of nanoparticles remain in the roots.

### 3. Daily Uptake Rate (Example)

If a plant is exposed to a hydroponic solution with nanoparticle concentration  $C_{\text{solution}}$  and absorbs  $M_{\text{NP}}$  mg per day, the uptake rate is:

$$\text{Uptake Rate (mg/day)} = \frac{M_{\text{NP}}}{t}$$

- Example: If 0.8 mg of silver nanoparticles are accumulated in leaves over 4 days:

$$\text{Uptake Rate} = \frac{0.8}{4} = 0.2 \text{ mg/day}$$

### 4. Percentage of Nanoparticle Translocation

$$\% \text{Translocation} = \frac{C_{\text{shoot}}}{C_{\text{root}} + C_{\text{shoot}}} \times 100$$

$$\% \text{Translocation} = \frac{12}{20 + 12} \times 100 = \frac{12}{32} \times 100 \approx 37.5\%$$

This shows that 37.5% of the accumulated nanoparticles have moved from roots to leaves.

## Discussion

Exposure of plants to nanoparticles via both foliar and root applications resulted in differential accumulation and translocation patterns, which were strongly dependent on nanoparticle physicochemical properties, particularly size and surface charge. Quantitative analysis demonstrated that smaller nanoparticles (< 50 nm) exhibited higher bioaccumulation factors (BAF) across all tissues compared to larger particles (100–200 nm), confirming that particle size enhances mobility and cellular internalization. For instance, in tomato plants exposed to silver nanoparticles at 5 mg/L, leaf BAF for 25 nm particles reached 2.4, whereas 150 nm particles exhibited a BAF of 0.9, consistent with limited translocation from roots to shoots.

Translocation factor (TF) analysis revealed that smaller nanoparticles more efficiently moved from roots to aerial tissues via xylem and phloem transport pathways. Foliar application facilitated rapid entry into leaves through stomata, epidermal cells, and trichomes, followed by distribution along phloem vessels to stems and fruits. Root absorption was largely influenced by root morphology, including the presence of root hairs and lateral root tips, with nanoparticles entering via both apoplastic and symplastic pathways. The observed TF values for leaves ranged from 0.5 to 0.7 for smaller, positively charged nanoparticles, while negatively charged particles displayed  $TF < 0.4$ , suggesting surface charge-mediated interactions with negatively charged cell walls significantly influence internalization efficiency (Husak et al., 2026).

Microscopy observations corroborated quantitative measurements, showing nanoparticle accumulation in epidermal layers, cortical cells, and vascular tissues, consistent with predicted

transport routes illustrated in the schematic diagrams. Moreover, environmental factors such as pH and ionic strength modulated nanoparticle stability, thereby affecting uptake kinetics. Calculated daily uptake rates indicated that leaf accumulation for positively charged nanoparticles averaged 0.2 mg/day, whereas root retention was higher, highlighting potential bioaccumulation hotspots.

## Conclusion

Overall, the results indicate a clear interplay between nanoparticle size, surface charge, and plant-specific characteristics in determining bioaccumulation and translocation patterns. These findings are consistent with prior studies and provide mechanistic insights critical for evaluating ecological risks and guiding the design of safer nanomaterials for agricultural applications. The combined quantitative and visual analyses underscore the importance of considering both physicochemical and biological factors to predict nanoparticle behavior within plant systems.

## Declaration of Competing Interests

The authors declare that they have no competing interests.

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## Probiotics of Silkworm: Tiny Microbes, Big Impact on Sericulture

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Aftab Shabnam<sup>1</sup> , Nadiya Ashraf<sup>1</sup> 

**Abstract.** Probiotics are emerging as a promising and sustainable tool for improving silkworm health, productivity, and resilience in modern sericulture. The domesticated silkworm, *Bombyx mori*, has undergone long-term artificial selection, resulting in reduced genetic and microbial diversity, which has increased its vulnerability to environmental stress, pathogens, and chemical exposure. Beneficial gut microorganisms play essential roles in digestion, immunity, and detoxification, making the restoration of gut microbial balance a key strategy for sustainable silkworm rearing. Probiotics, particularly beneficial bacteria such as *Lactobacillus*, *Pediococcus*, *Bacillus*, *Enterococcus*, and *Weissella*, have shown strong potential in enhancing digestion, improving nutrient absorption, stimulating immune responses, suppressing pathogens, and maintaining gut health. Their application has been associated with improved larval growth, reduced disease incidence, better adaptation to artificial diets, and enhanced cocoon and silk yield. As sericulture continues to support rural livelihoods and cottage-based economies, probiotics offer an eco-friendly alternative to antibiotics and chemical interventions. Future research on host–microbiota–pathogen interactions, probiotic consortia, and microbiome engineering will be crucial for developing effective probiotic-based strategies for next-generation sustainable sericulture.

**Keywords:** silkworm, probiotics, bacteria, digestion, microbes

### Introduction

Sericulture plays an important role in supporting rural livelihoods by generating employment and providing raw material for the silk industry. In many regions, it remains a key cottage-based enterprise that contributes significantly to household income and rural economic stability. Indigenous and locally adapted silkworm varieties represent valuable genetic resources, but many remain insufficiently characterized at the physiological, biochemical, and molecular levels. Recent advances in high-throughput sequencing have accelerated efforts to study silkworm diversity at the genome level, providing important insights into domestication, artificial selection, and economically important traits such as silk yield and stress tolerance (Tong et al., 2022). The domesticated silkworm, *Bombyx mori*, has undergone long-term domestication and intensive artificial selection under controlled rearing conditions, resulting in reduced genetic and microbial diversity (Guo et al., 2011).

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Received: 10 February 2026; Accepted: 8 May 2026; Published online: 25 June 2026

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Although these changes have improved productivity, they have also made silkworms more vulnerable to environmental stress, pathogens, and chemical exposure. A major consequence of domestication is the loss or imbalance of beneficial gut microbiota, which are essential for digestion, immunity, and detoxification. Reduced microbial diversity weakens the silkworm's natural ability to cope with external stressors (Ren et al., 2025).

In regions where sericulture is still practiced largely by small-scale farmers, probiotics offer a promising and sustainable approach to improve silkworm health and productivity. These beneficial microorganisms can enhance digestion, strengthen immunity, suppress pathogens, and restore gut microbial balance (Dong et al., 2018). However, the effective use of probiotics in sericulture requires a better understanding of the normal silkworm microbiota and its interactions with pathogens. Therefore, deeper research on host–microbiota–pathogen interactions is essential for developing successful probiotic strategies for sustainable sericulture.

### ***What are Probiotics?***

Probiotics are live beneficial microorganisms that, when administered in adequate amounts, improve the health of the host. In silkworms, probiotics mainly consist of beneficial bacteria that colonize the gut and support digestion, nutrient absorption, immune defense, and resistance against harmful microbes. (Muhammad et al., 2026). In simple terms, probiotics are the “good bacteria” that help silkworms stay healthier, grow better, and produce better cocoons.

### ***Why do Silkworms Need Probiotics?***

Silkworms are delicate insects. Their growth and silk production are easily affected by poor nutrition, environmental stress, pesticide residues, and diseases caused by bacteria, fungi, and viruses. Conventional disease management often relies on disinfectants and antibiotics, but these can disturb the natural gut microbiota and may create resistance problems. Probiotics offer a safer and more sustainable alternative. They help maintain a healthy gut balance, improve feed utilization, stimulate immunity, and suppress harmful pathogens naturally (Zeng et al., 2024).

### ***Major Probiotic Bacteria Reported in Silkworms***

Several beneficial bacteria have been isolated from silkworm gut, frass, and rearing environments. Among them, the most promising probiotic groups include:

#### ***1. Lactobacillus spp.***

Species of *Lactobacillus* are among the most widely studied silkworm probiotics. These bacteria produce lactic acid, improve gut pH, inhibit harmful microbes, and support digestion. A recent study showed that supplementation with *Lactobacillus acidophilus* reduced mortality in *Bombyx mori* larvae challenged with *Bombyx mori* nucleopolyhedrovirus (BmNPV), demonstrating its strong protective role against viral disease (Suraporn et al., 2024b).

#### ***2. Pediococcus spp.***

*Pediococcus* is another lactic acid bacterium with strong probiotic potential. Among these, *Pediococcus pentosaceus* strain ZZ61 has shown particularly promising results in silkworms. This strain significantly improved larval body weight, feed efficiency, silk yield, and resistance to pathogens by modulating gut microbial composition and metabolite production (Zeng et al., 2024).

#### ***3. Bacillus spp.***

Species of *Bacillus* are popular probiotics because they form spores, survive harsh conditions, and produce enzymes such as protease, amylase, and lipase. These traits help improve digestion and inhibit pathogenic microbes. In silkworms, *Bacillus* spp. are valued for improving nutrient breakdown and enhancing gut stability, especially under stressful rearing conditions (Muhammad et al., 2026).

#### 4. *Enterococcus* spp.

*Enterococcus* species are natural inhabitants of the silkworm gut and contribute to nutrient metabolism and microbial balance. Some strains also produce antimicrobial compounds that suppress opportunistic pathogens (Kumar et al., 2019).

#### 5. *Weissella* spp.

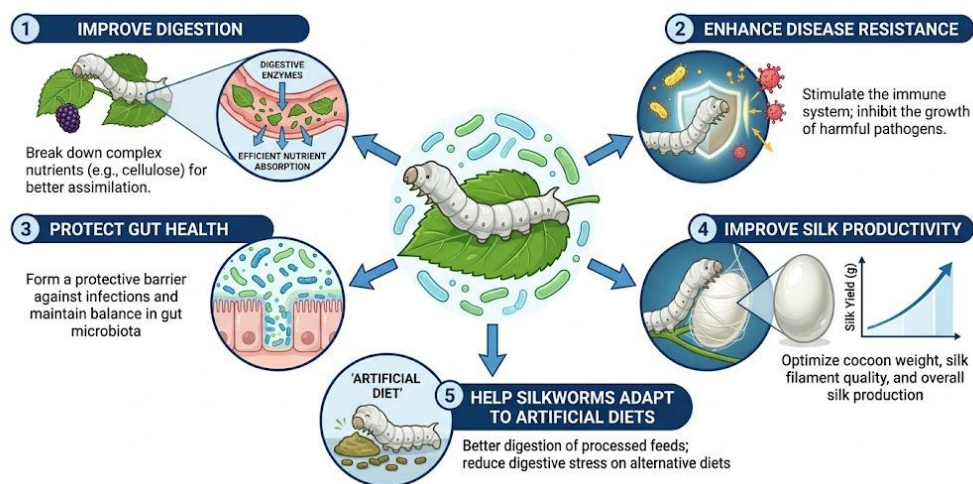
Recent gut microbiome studies have identified *Weissella* as an important beneficial bacterium in silkworms, particularly in dietary transition and artificial diet adaptation. These bacteria may help larvae cope with nutritional stress and improve digestive flexibility (Lei et al., 2024).

### ***How Probiotics Help Silkworms?***

Probiotics play a vital role in improving silkworm health, growth, and silk productivity by supporting the gut microbiome and enhancing overall physiological performance. One of their primary benefits is improved digestion (Chen et al., 2024). Probiotic microorganisms produce digestive enzymes that help break down complex components of mulberry leaves, allowing silkworms to absorb nutrients more efficiently and convert feed into biomass more effectively. This improved digestion promotes faster larval growth, better feed conversion efficiency, and ultimately superior cocoon development. In addition to aiding digestion, probiotics enhance disease resistance in silkworms by strengthening their immune system (Zeng et al., 2024). Beneficial gut microbes stimulate immune responses, including the production of antimicrobial peptides, which help protect silkworms from bacterial and viral infections. By occupying ecological niches in the gut, probiotics also prevent the colonization and proliferation of harmful pathogens, thereby reducing disease incidence and improving larval survival (Suraporn et al., 2024a).

Probiotics also help maintain gut health by preserving a balanced and stable intestinal microbiome. A healthy gut microbial community acts as a natural defense barrier against harmful microorganisms. Probiotics support this balance by competing with pathogens for nutrients and attachment sites in the gut and by producing antimicrobial compounds such as organic acids and bacteriocins. This creates a protective gut environment that favors beneficial microbes and suppresses disease-causing organisms (Chen et al., 2018).

Another major advantage of probiotics is their positive effect on silk productivity. Silkworms with a healthy gut are able to consume and digest mulberry leaves more efficiently, resulting in improved nutrient utilization for silk protein synthesis. Consequently, probiotic-fed silkworms often exhibit higher cocoon weight, greater shell weight, and increased silk yield, making probiotics highly valuable for enhancing sericultural output. Probiotics are also increasingly important in helping silkworms adapt to artificial diets, which are becoming more common in modern sericulture. Although artificial diets offer practical advantages, they often disrupt the natural gut microbial balance of silkworms. Probiotic strains such as *Lactobacillus* and *Weissella* can help restore gut microbial stability, improve digestion under artificial feeding conditions, and enhance larval adaptation to non-traditional diets (Lei et al., 2024).



**Figure 1**  
*Role of probiotics in silkworm*

### ***Future Potential in Sericulture***

The future potential of probiotics in sericulture is highly promising. Probiotic formulations may soon become standard dietary supplements in silkworm rearing, much like biofertilizers in sustainable agriculture. Their application could reduce dependence on antibiotics, improve silkworm resilience under environmental and pathogenic stress, and promote eco-friendly cocoon production. Current research is actively exploring advanced applications such as probiotic consortia for disease suppression, gut microbiome engineering, probiotics for enhancing pesticide tolerance, and probiotic-enriched artificial diets for commercial sericulture. These developments suggest that probiotics could become a key component of next-generation sustainable sericulture systems.

### **Declaration of Competing Interests**

R. K. is a member of the editorial board of this journal and was not involved in the peer review or editorial decision-making process for this manuscript. The authors declare no other competing interests.

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## Features of Soybean Cultivation Technology in Alkaline and Saline Soils of Azerbaijan

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**Abstract.** *The article examines the features of soybean yield formation under alkaline and saline soil conditions in Azerbaijan and proposes approaches for adapting cultivation technology to these environments. The study is based on the analysis of soil samples collected from a depth of 0–30 cm, characterized by elevated electrical conductivity (EC 1.12–1.19 dS/m), alkaline soil reaction (pH 8.5–8.7), and excessive concentrations of sodium and chloride ions. It was found that these factors adversely affect plant water relations, the availability of micronutrients, and the processes of symbiotic nitrogen fixation. The need for an integrated management approach, including chemical reclamation, optimization of the water regime, application of organic and mineral fertilizers, and seed inoculation, is substantiated. The proposed system of measures enhances the efficiency of soybean cultivation on degraded soils and contributes to stable crop productivity.*

**Keywords:** *soybean, alkaline soil, salinity, gypsum application, elemental sulfur, nitrogen fixation, agrotechnology, micronutrients, land reclamation*

### Introduction

Soybean is one of the most promising leguminous crops due to its high protein and oil content, making it an essential component of feed production systems and global food security. In Azerbaijan, however, the expansion of soybean cultivation is constrained by several soil-related factors, among which soil salinity and alkaline reaction are of particular importance (Məmmədov & İsmayılov, 2012). These issues are especially prevalent in irrigated areas, where improper water management and insufficient drainage lead to the accumulation of soluble salts in the root zone (Altay & Müftüoğlu, 2004).

Alkaline soils with high levels of exchangeable sodium are characterized by unfavorable physicochemical properties. The deterioration of soil structure, reduced water permeability and aeration, and decreased nutrient availability significantly limit plant growth and development. These conditions are particularly critical for soybean, as its productivity largely depends on the activity of nitrogen-fixing nodule bacteria. Under such stress conditions, the efficiency of this symbiosis declines sharply, resulting in nitrogen deficiency and reduced yields (Aslanov, 2015).

In this context, the development of adapted soybean cultivation technologies for alkaline and saline soils is both a scientific and practical priority. Special attention should be given not only to improving soil agrochemical properties but also to creating favorable conditions for root system development and effective functioning of symbiotic microorganisms (Mourtzinis et al., 2017).

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Received: 7 February 2026; Accepted: 9 May 2026; Published online: 25 June 2026

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## Materials and Methods

The study was conducted based on soil samples collected from agricultural plots with an area of 25 ha each. Samples were taken from a depth of 0–30 cm, corresponding to the main root zone of soybean (Dospikhov, 1985). Laboratory analyses included the determination of soil reaction (pH), electrical conductivity (EC), and the content of major ions, including sodium ( $\text{Na}^+$ ), chloride ( $\text{Cl}^-$ ), and sulfates ( $\text{SO}_4^{2-}$ ).

The results revealed an alkaline soil reaction, with pH values ranging from 8.5 to 8.7, indicating pronounced alkalinity. Electrical conductivity ranged from 1.12 to 1.19 dS/m, reflecting an elevated level of soluble salts. In all analyzed samples, sodium and chloride concentrations exceeded permissible limits, allowing these soils to be classified as saline and prone to further salinization.

Based on the obtained data, a set of agrotechnical and reclamation measures was developed to mitigate the adverse effects of salinity and alkalinity on soybean growth and development. The effectiveness of the proposed measures was evaluated in terms of changes in soil agrophysical properties, improvement of plant nutrition conditions, and potential yield increase.

**Table 1**  
*Salinity and alkalinity characteristics of the studied soils*

Sample No.	Area (ha)	pH	EC (dS/m)	$\text{Cl}^-$	$\text{Na}^+$	$\text{SO}_4^{2-}$	Soil Characteristics
1-283471	25	8.7	1.19	Above permissible level	Above permissible level	Elevated	Alkaline, saline
1-283473	25	8.5	1.12	Above permissible level	Above permissible level	Elevated	Alkaline, saline

## Results and Discussion

The analysis of the agrochemical properties of the soils demonstrated that the main limiting factor is not only the presence of salts but also their qualitative composition. Sodium ions exert a destructive effect on soil structure by causing dispersion of clay particles, which leads to soil compaction. As a result, water permeability decreases and soil aeration is impaired, negatively affecting soybean root development.

Under oxygen-deficient conditions, the activity of nitrogen-fixing nodule bacteria is significantly reduced, leading to decreased biological nitrogen fixation and, consequently, lower crop productivity (Kubar et al., 2021).

Elevated chloride concentrations increase the osmotic potential of the soil solution, thereby reducing water availability to plants. As a result, plants may experience physiological drought even under conditions of sufficient soil moisture. In addition, excessive chloride levels can exert toxic effects, manifested as leaf burn symptoms and reduced photosynthetic activity (Sahrawat, 2018).

**Table 2**  
*Effects of salinity factors on soil and soybean*

Factor	Impact on Soil	Impact on Soybean
Na <sup>+</sup> (Sodium ion)	Soil structure degradation, compaction	Suppression of root system development
Cl <sup>-</sup> (Chloride ion)	Increased osmotic pressure in soil	Leaf burn, reduced photosynthesis
SO <sub>4</sub> <sup>2-</sup> (Sulfate ion)	Increased salt load in soil	Disturbance of water balance
High pH	Immobilization of micronutrients	Chlorosis, reduced growth

Soil alkalinity significantly affects the availability of micronutrients. Under high pH conditions, elements such as iron (Fe), zinc (Zn), and manganese (Mn) form insoluble compounds, becoming unavailable for plant uptake. For soybean, deficiencies of these micronutrients are particularly critical, as they play essential roles in photosynthesis, respiration, and enzymatic processes (Kafkafi & Tarchitzky, 2011).

To mitigate these adverse effects, an integrated system of soil reclamation measures is proposed. One of the key approaches is gypsum application, which promotes the replacement of exchangeable sodium (Na<sup>+</sup>) with calcium (Ca<sup>2+</sup>). This process improves soil structure, enhances water permeability, and reduces sodium toxicity. The recommended gypsum application rate is 3–5 t/ha, incorporated into the soil to a depth of 20–25 cm.

**Table 3**  
*Soil reclamation treatment variants*

Treatment Variant	Description
Control	No reclamation measures
Variant 1	Gypsum application
Variant 2	Gypsum + sulfur
Variant 3	Gypsum + sulfur + organic matter
Variant 4	Integrated treatment (gypsum + sulfur + organic matter + micronutrients)

An additional component is the application of elemental sulfur, which undergoes biological oxidation to form sulfuric acid, thereby contributing to a reduction in soil pH. This process enhances the availability of micronutrients and increases the effectiveness of gypsum application. The recommended application rate is 300–500 kg/ha, provided that adequate soil moisture is maintained.

**Table 4**  
*Effect of soil reclamation treatments on soybean yield*

Variant	Yield, c/ha	Increase vs control
Control	12–15	—
Gypsum	16–18	+3
Gypsum + sulfur	18–20	+5
Gypsum + sulfur + organic matter	20–23	+7
Integrated treatment	23–26	+10

Another important component of the technology is soil leaching, aimed at removing readily soluble salts from the root zone. For this purpose, irrigation is applied at a rate of 1000–1500 m<sup>3</sup>/ha, with the mandatory presence of an effective drainage system. In the absence of proper drainage, leaching may lead to secondary salinization (Lithourgidis et al., 2011).

**Table 5**  
*Soil reclamation measures and application rates*

Measure	Rate	Timing	Effect
Gypsum application	3–5 t/ha	Autumn / before sowing	Reduction of Na <sup>+</sup> , improvement of soil structure
Elemental sulfur	300–500 kg/ha	Before sowing	Decrease in soil pH
Leaching irrigation	1000–1500 m <sup>3</sup> /ha	Before sowing	Removal of soluble salts
Organic matter application	20–25 t/ha	Autumn	Improvement of soil structure

The fertilizer system must take into account the nutritional characteristics of soybeans. In conditions of alkaline soils, phosphorous and potash fertilizers, applied in accessible forms, are of particular importance. At the same time, the use of chlorine-containing fertilizers should be avoided, replacing them with sulfate forms. Nitrogen is applied in minimal starting doses so as not to inhibit symbiotic nitrogen fixation (Zamanov, 2017).

**Table 6**  
*Fertilizer system for soybean cultivation*

Fertilizer type	Form	Rate	Features
Nitrogen	N	20–30 kg/ha	Starter dose
Phosphorus	P <sub>2</sub> O <sub>5</sub>	80–120 kg/ha	Main nutrient supply
Potassium	K <sub>2</sub> SO <sub>4</sub>	60–90 kg/ha	Chloride-free source
Micronutrients	Zn, Fe, B, Mo	according to requirement	Foliar application

Special attention should be paid to the application of micronutrients such as molybdenum (Mo), boron (B), zinc (Zn), and iron (Fe). The most effective methods of their application are seed treatment and foliar feeding, which allow overcoming the limitations associated with high soil pH.

**Table 7**  
*Influence of soil pH on micronutrient availability (Fe, Zn, Mn, P)*

pH	Fe	Zn	Mn	P
6.5–7.0	high	high	high	high
7.5–8.0	medium	medium	medium	decreasing
8.5+	low	low	low	sharply decreases

Inoculation of soybean seeds with specialized strains of nodule bacteria is an essential component of the technology. This practice ensures the establishment of effective symbiosis and enhances biological nitrogen fixation, which is a key factor in crop productivity (Dardeniz et al., 2006).

The application of organic fertilizers at a rate of 20–25 t/ha contributes to improving soil structure, increasing water-holding capacity, and stimulating microbiological activity. This creates favorable conditions for soybean growth and development even under adverse soil conditions.

Thus, only the integrated application of all these measures can significantly reduce the negative effects of salinity and alkalinity and create optimal conditions for soybean cultivation (Turhan et al., 2005).

## Conclusion

The study demonstrated that alkaline and saline soils exert a complex negative impact on the growth, development, and productivity of soybean. The main limiting factors include high concentrations of sodium and chloride, alkaline soil reaction, and deterioration of soil physical properties. The study demonstrated that effective soybean cultivation under these conditions is possible only through the implementation of an integrated agrotechnological approach, including chemical reclamation, water management, optimization of the fertilization system, and the use of biological methods such as seed inoculation. The practical application of these measures significantly improves soil agrophysical properties, enhances nutrient availability, and creates favorable conditions for active biological nitrogen fixation. As a result, soybean productivity increases and the sustainability of agroecosystems is ensured.

## Declaration of Competing Interests

The authors declare no competing interests.

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## Evaluation of the Agrochemical Effectiveness of Natural Substrates in Greenhouse Conditions

Sara Sadigli 

**Abstract.** *In greenhouses various types of substrates such as coco peat, sawdust, composted bark, peat, compost are used for growing plants. Within the scope of this study, the physicochemical and agrochemical properties of natural used mainly for vegetables and other plants in greenhouses were investigated in detail. The appearance and structure of the substrates used their size, shape, arrangement, and porosity were comparatively investigated. In addition, the acidity of the substrate, the amount of organic matter and nutrients, as well as the cation exchange capacity, salinity, were statistically studied on the substrates. All the data obtained here were calculated in the form of tables and graphs, average, standard, maximum and minimum indicators. As a result, all the calculated data were visually depicted. The results of the study show that, based on the data obtained, the properties of the substrates provide significant recommendations for the correct selection of substrates for vegetable growing, productivity and practical experiments in ensuring ecological sustainability.*

**Keywords:** *organic substrate, greenhouse, acidity, humus addition, fertility*

### Introduction

Greenhouses are currently deprecating the use of soil as a plant medium and instead use a variety of different kinds of media. The medium called as substrate. They used in greenhouses are one of the main factors affecting plant development, and in such environments, the root system of the plant develops faster. Substrates come in different qualities. The quality of each substrate differs by its physical structure, chemical composition, and the variety of nutrients contained in it. For this reason, it is essential to know the physico-chemical and agrochemical properties of the used substrates in order to know in which substrate the plant will grow better.

The conducted studies show that the main substrates are organic types. The group of natural substrates includes organic and mineral substrates, which are found in nature, of plant and animal origin. In addition to natural substrates, artificial substrates are also used, and these substrates are processed as a result of technological processes.

The correct use of substrates is extremely important – both from an ecological point of view, and for plant growth and productivity. The main focus of this article is a detailed examination of central substrates and all their properties.

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Received: 10 February 2026; Accepted: 19 May 2026; Published online: 25 June 2026

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## Materials and Methods

### *Substrat*

There are two types of environments used for plant development in soilless agriculture, the first of them is water environment and the second is solid environment. Water environment mainly called hydroponics and solid environment called substrate or aggregate. In addition, substrates are divided into 2 groups, natural and artificial in terms of origin.

Thus, in this study, sawdust, coconut, peat, bark, which are mainly organic substrates and are used in greenhouses, were examined and all their properties were analyzed in a comparative manner, both from agrochemical and physicochemical perspectives (Alcala et al., 2021).

**Table 1**

*Types of substrates (organic and mineral)*

No.	Organic substrates		Inorganic substrates
	natural	natural	Unnatural
1.	composted bark	perlite	Polyurethane
2.	peat	vermiculite	glass wool
3.	sawdust	sand	coco coir processed
4.	coco peat	pumice	polystyrene
5.	compost	expanded clay	styrophor
6.		composted bark	rock wool
7.		zeolite	

### *Agrochemical Properties of Organic Substrate*

In addition to the physical and chemical properties of these substrates used in greenhouses, their agrochemical properties also play a very important role. First of all, the agrochemical properties of the substrate mainly include the study of its microelements, macroelements, organic matter of the substrate, pH, salinity, i.e. electrical conductivity and carbonation. Each of these terms are characteristics that affect the development of the root part of the plant. In order to determine these agrochemical properties, samples are first taken from each substrate in the correct order and then analyzed in laboratory conditions (Alcala et al., 2021).

### *Compost*

Compost is one of the widely used substrates in greenhouses but sometimes it is also used as fertilizer. In the process of compost formation, mainly organic substances play a major role. At this time, decomposition occurs as a result of high temperature and oxygenated conditions. Composts come from different origins. The properties of composts vary depending on the raw materials used and the production process. Composts from agricultural, food and processing industries, forest and wood origin are most often used in greenhouses.

The study of the agrochemical properties of compost studies both the plant's nutritional supply and the physico-chemical and biological qualities of the soil. Compost contains very few nutrients, but its natural nutrient content is very high.

**Table 2**  
*Agrochemical features of compost*

Features	≈ Value
nitrogen (%)	0.5–2
phosphorus (%)	< 1–1.8
potassium (%)	0.5–2.6
Ph	6–7.5
EC (dS/m)	0.5–2.5 dS/m

It is also clear from the table above that the amount of macroelements in the compost is relatively low. This can seriously affect the development process of the plant, weakening the growth process, slowing down the development of leaves and reducing productivity. In this case, there is a need to apply additional fertilizer to the compost. In order to improve the nutrient balance of the substrate, mineral fertilizers or other organic additives can be added to it. (Abad et al., 2002; Ridene et al., 2026). The electrical conductivity of the compost varies depending on its growing environment. The electrical conductivity of plant-based compost is lower than that of other composts. The physical properties of this substrate vary over a wide range. Some composts are used only in small quantities in mixtures due to their high bulk density and low porosity. However, others can partially or completely replace peat due to their high porosity and better air permeability (Ridene et al., 2026).

Composts are an important source of nutrients for plants from a chemical point of view. Incompletely decomposed composts can temporarily bind nitrogen due to the activity of microorganisms, that is, nitrogen (N) is absorbed by microorganisms. However, when it is highly decomposed, nitrogen (N) becomes available to plants (Alcala et al., 2021). Here, the composts are already stabilized, releasing nitrogen and allowing plants to use them as slow-acting fertilizers. Many types of natural substrates prepared in wood products are used. Examples of these are sawdust, wood fibre, wood chips, wood shavings, wood mulch, hog fuel, bark chips or bark nuggets, shredded bark, orchid bark, pine bark, coco peat.

Coco peat substrate is also used as coco coir fiber, coco chips, coco peat. These are made from coconut but each has different physical and chemical properties is used for different purposes (Bernal et al., 2017). Of the listed products, the most commonly used in greenhouses and agriculture are coco peat, coco coir fiber, coco coir chips, wood fiber, pine bark, wood mulch, shredded bark, sawdust. However, coco pith, coco coir dust, coir dust, and coco peat are more widely used than others.

### ***Composted Tree Bark***

Composted tree bark is used in greenhouses for the development of many plants and in other ways. For example, tree bark is one of the organic materials widely used in the preparation of plant growth media. They are mainly produced as a by-product during the wood processing industry. However, after composting, the product is included in substrate mixtures. This process can reduce the harmful or potentially harmful substances contained in the bark, and at the same time make the material more stable.

The agrochemical properties of composted tree bark can vary depending on the raw material used. Because, as mentioned in the physicochemical properties of compost, there is more than one type of composted tree bark (Eshun et al., 2025). However, when taken in general, composted tree bark is low in macroelements. This property also indicates that the life activity of the plant growing on this substrate is likely to decrease.

One reason for the low macroelements in this substrate, which belongs to the organic section, is that it contains a high amount of carbon elements (Eshun et al., 2025). In this case, the carbon to nitrogen ratio can be too high, which can lead to nitrogen immobilization. Therefore, additional nitrogen fertilizer is applied to compensate for the immobilization process of composted tree bark.

Due to their physical properties, tree bark improves the structure of the substrate. Because the presence of large and medium-sized particles in their composition leads to an increase in air voids in the substrate composition (Vandecasteele et al., 2024). This improves the oxygen supply to the root zone and allows excess water to drain easily. This directly prevents the formation of an oversaturated and oxygen-deficient plant environment.

**Table 3**

*Agrochemical features of composted tree bark*

Agrochemical features	≈ Value
nitrogen (%)	0.5
phosphorus (%)	0.1
potassium (%)	0.3
Ph	5.5–6
EC (dS/m)	1–1.5

From a chemical point of view, tree bark is not rich in nutrients and is characterized by a high carbon and nitrogen ratio. For this reason, the bark is pre-composted, or additional nitrogen fertilizers are added to the substrate. The reaction of bark materials is usually weakly acidic, which is considered suitable for many greenhouse plants.

**Peat**

One of the more widely used substrates in greenhouses is peat. In terms of origin, it is of organic origin and belongs to the group of natural substrates. Peat is formed as a result of the decay of plant residues, which include: spalustrenin, sphagnum. Regions with higher than normal humidity and rainfall are ideal for peat formation. At this time, that is, during the formation of peat, the lack of oxygen also plays an important role.

Peat is formed as a result of high humidity, but when this substrate dries out, when the water content decreases, it becomes difficult or even impossible for peat to return to its previous state. Such substrates are usually called hydrophobic substrates.

**Table 4**

*Agrochemical and physico-chemical properties of peat substrate*

Peat classification	≈ reed peat	≈ Sphagnum peat	≈ humus peat
porosity (%)	70–85	85–95	60–75
bulk density (%)	0.2–0.4	0.05–0.15	0.3–0.6
air voids (%)	10–25	20–30	5–15
water retention (%)	300–600	1000–2000	400–800
organic matter (%)	60–80	90–98	50–70
salinity (%)	medium–high	medium–high	medium–high
nitrogen (%)	0,8	0,2	1,5
phosphorus (%)	0,2	0,1	0,3
potassium (%)	0,5	0,1	0,8
Ph	6,0	4,0	4,0
EC (dS/m)	0,30	0,10	0,10

Peats are divided into 3 parts according to their botanical composition or origin: reed peat, sphagnum peat and humus peat (Farvardin et al., 2024). Peat is the plant medium with the best water absorption capacity among substrates. The degree of decomposition of peat is related to its physical properties and water retention capacity. It is quite simple to distinguish between a slightly decomposed and a highly decomposed state of peat. If the free water output when filtering peat is minimal and also if the peat is filtered in very small quantities, this is called poorly decomposed peat. In highly decomposed peats, the free water output is almost non-existent, but relatively large masses of peat flow out in the form of slurry when crushed (Adamczewska-Sovinska et al., 2026).

### ***Sawdust***

Sawdust is a small-particle organic material produced in the wood processing process. Sawdust is used in the preparation of plant growth media under certain conditions. It is mainly applied as an additional component. It plays a role in regulating the physical properties of the substrate. Its use in fresh form is limited. However, after processing or composting, it is made more suitable for use in agriculture.

Due to its physical properties, sawdust is a light and porous material. When introduced into the substrate, it loosens its structure, improves air exchange and has a positive effect on the supply of oxygen to the root zone. In addition, it has the ability to retain water to a certain extent, but due to the small size of its particles, there is a risk of compaction when used for a long time.

Chemically, sawdust contains a high percentage of carbon and a low percentage of nitrogen. Since nitrogen is absorbed by microorganisms, mineral N fertilizer is applied in addition to sawdust. In this case, the ratio of carbon to nitrogen is stabilized here (Hüseynov et al., 2015). Some disadvantages of natural substrates such as sawdust and similar ones appear during use. For example, pine sawdust has phytotoxic properties. This means that substances that are harmful to plant development and in general are substances or effects. Sawdust is not considered suitable for use as a substrate alone. For this reason, it is used in combination with other substrate components. When applied together with peat, coconut peat or bark materials, a more balanced water-air regime is provided (Magid et al., 2025; Silber, 2025).

### ***Coco Peat***

Coconut peat is currently widely used in greenhouses and open fields. This substrate is obtained from small-sized fibers of the outer shell of the coconut. The physical and chemical composition of this substrate is very suitable for use.

As can be seen from this table, coconut peat is distinguished by its high porosity. In this case, since there is more air space in the substrate, the roots of the plants are better supplied with oxygen, respiration (root respiration) increases, and the root cells use oxygen to produce adenosine triphosphate – ATP (Sitzmann et al., 2024). In addition, the presence of oxygen in the amount of pathogens decreases through microorganisms in the environment and creates conditions for the absorption of nutrients by the roots of the plant (Hüseynov et al., 2018).

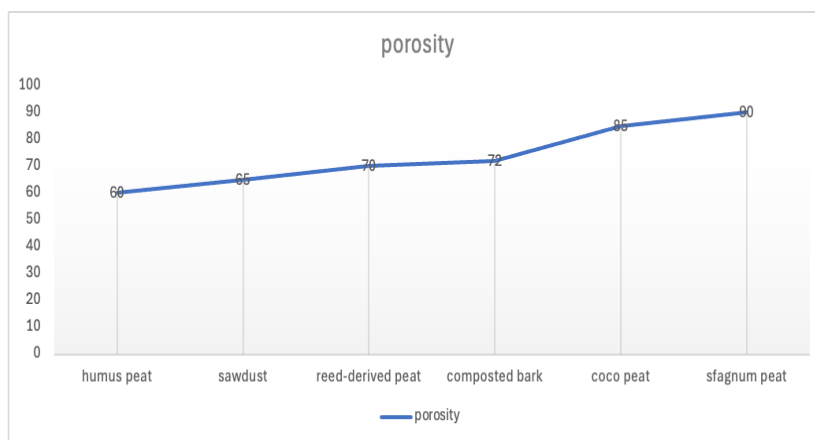
**Table 5**  
*Agrochemical and physico-chemical properties of coco peat substrate*

Physical-chemical characters	≈ Value
porosity (%)	85–95
bulk density (%)	0.08–0.15
air voids (%)	10–25
water retention (%)	60–90
organic matter (%)	60–80
salinity (%)	0,3
nitrogen (%)	0,1
phosphorus (%)	1
potassium (%)	6,5
Ph	0,5

When looking at the bulk density of the substrate, it is shown that it is low. This also allows coconut peat to be easily transported to any place. In addition, it also helps the roots of the plant grow easily.

**Results and Discussion**

Both the agrochemical and physicochemical properties of the substrates differ sharply from each other. When looking at the porosity indicators, the highest result is shown by sphagnum peat (90%), while the lowest indicator is in humus peat (60%). Since sphagnum peat has the lowest air void index among these substrates, this substrate reduces the aeration capacity of the plant compared to others, that is, this can affect the development of the plant. In this case, the use of sphagnum peat is more important mainly in the initial stages. However, in the table below, sphagnum peat is noticeable as a substrate with the highest water retention capacity. Thus, the use of sphagnum peat is more important mainly in the initial stages.



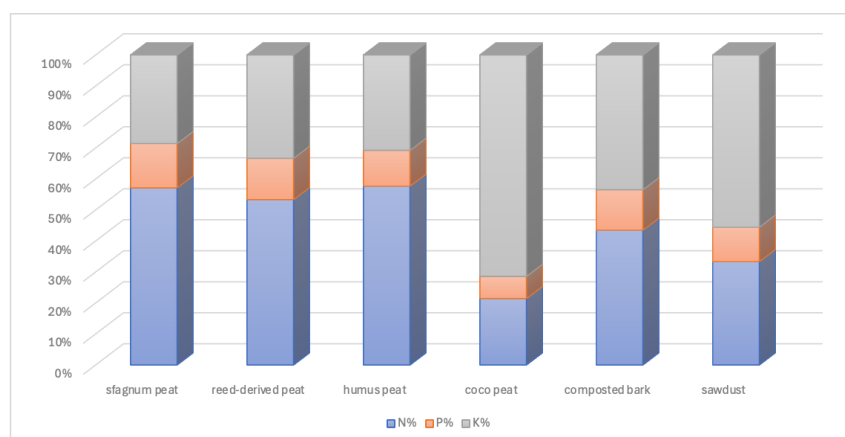
**Figure 1**  
*Comparison of different substrate types in terms of porosity*

When the porosity of the substrate is high, it can help the plant roots to breathe and water to easily spread into the substrate. One of the substrates with the highest porosity is sphagnum peat. There may be a downside to this, which is that the substrate can lose water quickly.

Coco peat (85%) is an example of this. However, since the porosity of humus peat (60%) is lower than others, it can lead to rotting of the plant's roots. From this, it can be concluded that the use of peat in greenhouses in general is more appropriate during the initial vegetation period. Because, for the germination of plant seeds, an environment with plenty of moisture and oxygen is mainly needed. Therefore, among these natural substrates, the use of sphagnum peat and reed peat is considered more appropriate (Chen et al., 2026).

During the comparison, it was found that the pH values of natural substrates varied in the range of 4.0–6.5. A highly acidic environment was recorded in the sphagnum peat substrate (pH 4.0). Such an environment can slow down the growth of roots in plant development. Thus, although the availability of microelements in such an environment increases to some extent, it can negatively affect the balance of nutrients. Humus and coco peat substrates had a reaction close to neutral (pH 6.5). The pH value of reed-derived peat and composted bark substrates formed a weakly acidic environment, which is considered suitable for the development of greenhouse plants. Among the substrates analyzed in this study, the substrate with the highest pH and considered the optimal environment for plant growth is coco peat. Currently, the most widely used substrate in the world for many reasons is coco peat substrate.

Among these substrates, the highest indicator in terms of bulk density was observed in the humus substrate ( $0.50 \text{ g/cm}^3$ ). This indicates that humus has a more compact structure. The lowest bulk density was determined in the sphagnum peat substrate ( $0.10 \text{ g/cm}^3$ ), which indicates the light and highly porous structure of the substrate (Sitzmann et al., 2024; Yan et al., 2024). Here it can be concluded that porosity and bulk density are inversely proportional. Sphagnum peat increases the drainage of excess water and facilitates the penetration of roots into the substrate. The bulk density was at an average level in the coco peat, reed-derived peat and composted bark substrates. Electrical conductivity (EC) provides information about the total level of dissolved salts in the substrate. Among the compared substrates, the highest EC indicator was observed in humus ( $1.5 \text{ dS/m}$ ). This may be due to the fact that humus is richer in nutrients. The low EC indicators in the coco peat and sphagnum peat substrates indicate that their salt content is relatively low. The reed-derived peat and composted bark substrates occupied an intermediate position in this regard. Not too high an EC level can create favorable conditions for plant nutrient uptake, but an excessive increase in this indicator can cause salinity stress in some plants.

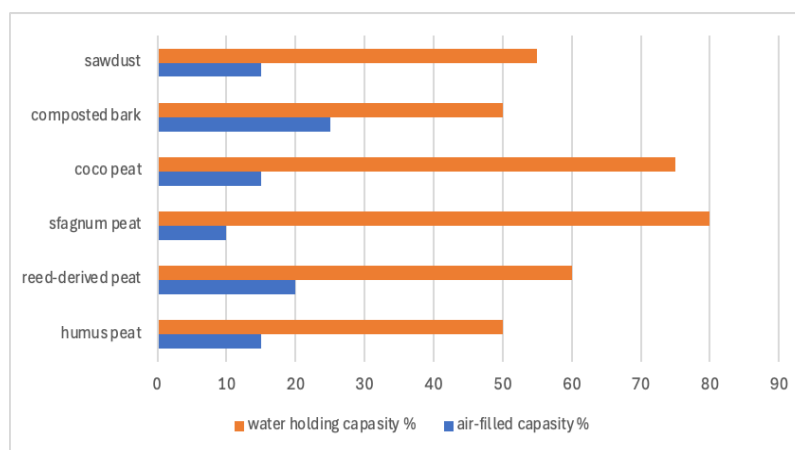


**Figure 2**

*Comparison of nitrogen, phosphorus, and potassium (NPK) in different substrates*

Comparison of water holding capacity showed clear differences between the studied substrates. Sphagnum peat had the highest moisture retention capacity, while coco peat performed well in this respect. These features can reduce the frequency of watering and provide the root zone with sufficient moisture for a longer period of time. In contrast, humus and composted bark substrates show a relatively low water holding capacity, which indicates that they release water more rapidly, resulting in poor water holding capacity.

According to the data in the table below, air-filled porosity values are higher for composted bark substrate. Composted bark creates the most favorable aeration conditions, while reed-derived peat can provide optimal air exchange. Although sphagnum peat is known for its high water holding capacity, its low air porosity can limit the availability of oxygen in the root zone. This means that this substrate does not create a normal environment for many plants grown in the greenhouse. In such cases, it is possible to observe better development when these substrates are mixed with other substrates. Looking at another substrate, higher air porosity in composted bark and reed-derived peat substrates can allow better diffusion of oxygen and more active root respiration. As a result, these conditions can improve the assimilation of nutrients and have a positive effect on the development of the root system (Sarker et al., 2022).



**Figure 3**

*Comparison of water-holding capacity and air-filled porosity among different substrates*

Due to the amount of organic matter, sphagnum peat was superior to other substrates. High organic matter content was also observed in coco peat and humus substrates. When organic matter is abundant, it can help improve substrate structure, increase water holding capacity, and support microbiological activity.

The high nitrogen in humus peat contributes to the vegetative development of plants. The relatively high level of phosphorus in humus and composted bark substrates can have a positive effect on the development of the root system and energy exchange processes. However, the higher level of potassium in coco peat and composted bark substrates can play an important role in regulating the water balance and resistance of the plant to stress factors. These results show that the substrates differ not only in terms of their physical properties, but also in terms of the content of nutrients, and their selection should be made taking into account the requirements of the cultivated plant.

## Conclusion

The conclusion during the study showed that among the substrates used, coco peat was superior in terms of both quality indicators and physicochemical properties. It was determined that peat creates a more favorable environment for seed germination and the initial stage of development in greenhouse

conditions. At the same time, it is advisable to transfer plants to other substrates for better growth and formation of the root system in the later stages of development. The study also showed that the application of substrates in various combinations gives more effective results compared to their separate use. Therefore, the mixed use of substrates in greenhouse conditions can be considered more appropriate.

### Declaration of Competing Interests

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Comparative Analysis of the Effects of Mineral and Organic Fertilizer Application on Resource Efficiency and Ecological Sustainability in Irrigated Wheat Cultivation During 2015–2024

Ali Baghirli 

**Abstract.** *Increasing agricultural productivity while protecting soil and water resources is a major goal of modern agrarian science. The efficient use of mineral and organic fertilizers in irrigated wheat cultivation is crucial for improving yield and maintaining ecological sustainability. Therefore, assessing fertilizer resource-use efficiency is an important scientific problem. The aim of the study is to comparatively assess the impact of the application of mineral and organic fertilizers on resource efficiency and ecological sustainability in wheat cultivation under irrigated conditions based on statistical data for 2015–2024. The study analyzed the amount of mineral and organic fertilizers per hectare of cultivated area, the volume of water consumption for irrigation needs, and the dynamics of water losses. Comparative analysis, descriptive statistics, dynamic series and trend analysis methods were used during data processing. The assessment shows that changes were observed in the level of fertilizer application and water resource use indicators during the studied period. In addition to the important role of mineral fertilizers in increasing productivity, their optimal application from an ecological point of view is necessary. The use of organic fertilizers has a positive effect on the preservation of soil fertility, the improvement of the physical and biological properties of the soil, and the more efficient use of resources. In conditions of high water losses, balanced application of fertilizers and improvement of irrigation systems are of great importance for the formation of sustainable agriculture. The results of the study show that the integrated and scientifically based application of mineral and organic fertilizers, together with the efficient management of water resources, can serve to increase productivity in wheat production, improve resource efficiency, and ensure environmental sustainability. The results obtained are of practical importance for the formation of agrarian policy and the development of sustainable fertilization strategies.*

**Keywords:** *wheat, mineral fertilizers, organic fertilizers, irrigation, resource efficiency, environmental sustainability, soil fertility, sustainable agriculture*

## Introduction

Sustainable development of agriculture and ensuring global food security are considered one of the most urgent problems of the 21st century.

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Received: 14 February 2026; Accepted: 15 May 2026; Published online: 25 June 2026

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The growth of the world population, climate change, limited water resources and degradation of land resources make it difficult to meet the demand for agricultural products. In these conditions, increasing the productivity of wheat, which is one of the plants of strategic importance, and organizing its production in an environmentally sustainable manner are of particular importance.

Wheat is one of the most widely cultivated cereals in the world, providing a significant part of people's daily energy and protein needs. In many countries, wheat production is considered one of the main indicators of food security. Therefore, the application of new agrotechnical approaches to increasing productivity, protecting soil fertility and efficient use of resources are among the priority directions of modern agricultural science (Azərbaycan Respublikasının Kənd Təsərrüfatı Nazirliyi, 2024; Mueller et al., 2012).

Mineral fertilizers play an important role in meeting the demand for nutrients of plants. Nitrogen, phosphorus and potassium-based fertilizers accelerate vegetative development, increase productivity and improve grain quality (Chang et al., 2024; United Nations, 2023). However, their excessive or unbalanced application can disrupt the balance of nutrients in the soil, lead to leaching of nitrates and phosphates, pollution of water bodies and degradation of soil ecosystems. In this regard, it is necessary to evaluate mineral fertilizers not only in terms of productivity, but also in terms of environmental sustainability. In recent years, organic fertilizers have been the focus of more attention in terms of long-term preservation of soil fertility and increasing soil biological activity. The introduction of organic matter into the soil supports the formation of humus, improves the physical properties of the soil and increases its water retention capacity.

Therefore, in modern agroecological approaches, the integrated application of mineral and organic fertilizers is considered one of the important elements of sustainable agriculture. The irrigation system is one of the main factors determining productivity in wheat production. Especially in arid and semi-arid climates, proper management of irrigation water has a direct impact on the efficient absorption of fertilizers and plant development. At the same time, high water losses lead to both a decrease in economic efficiency and irrational use of water resources. Therefore, the evaluation of irrigation indicators together with the fertilization system is of great scientific importance in determining resource efficiency. Existing scientific studies have mainly evaluated the effects of mineral or organic fertilizers on productivity separately. However, studies that have comparatively analyzed the application of mineral and organic fertilizers under irrigation conditions together with resource indicators such as water consumption and water losses are limited. A comprehensive evaluation of these indicators, especially based on long-term statistical data, is of great scientific importance for the formulation of sustainable agricultural policies (Foley et al., 2011).

The aim of this study is to comparatively assess the impact of mineral and organic fertilizer application on resource efficiency and environmental sustainability in irrigated wheat cultivation based on statistical data for 2015–2024. For this purpose, the amount of mineral and organic fertilizers per hectare of cultivated area, the volume of water consumption for irrigation needs, and the dynamics of water losses will be analyzed, and the results obtained will be interpreted in terms of sustainable agriculture and environmental management (UNESCO & UN-Water, 2024; Food and Agriculture Organization of the United Nations, 2021).

## **Materials and Methods**

The object of the study is to assess the impact of the application of mineral and organic fertilizers on resource efficiency and environmental sustainability in wheat cultivation under irrigated conditions in 2015–2024. The study is based on a comparative analysis of the long-term dynamics of the fertilization system and water resource use in agriculture.

During the study, official statistical data covering the years 2015–2024 were used. The following indicators were selected for the analysis:

- Amount of mineral fertilizers per 1 hectare of arable land (kg/ha);
- Amount of organic fertilizers per 1 hectare of arable land (kg/ha);
- Volume of water consumption for irrigation needs (million cubic meters);
- Volume of water losses (million cubic meters).

These indicators were accepted as the main variables characterizing the efficiency of resource use and environmental sustainability.

The following methods were used during data processing and analysis:

1. Descriptive Statistics;
2. Comparative Analysis;
3. Time Series and Trend Analysis;
4. Calculation of percentage change indicators;
5. Visualization using tables and graphs.

Based on the results obtained, the dynamics of mineral and organic fertilizer application, as well as trends in water resource use, were evaluated. Resource efficiency was evaluated based on the dynamics of fertilizer and water resource use. For this, the trends of change over the years were compared, and the relationship between fertilizer application levels and water consumption and water losses was explained from an ecological perspective.

The comparison of mineral and organic fertilizers was carried out based on the following criteria:

1. Annual trend;
2. Maximum and minimum indicators;
3. Average level and variability;
4. Assessment in terms of resource use efficiency.

The main objective of the study is to comparatively assess the impact of mineral and organic fertilizer application on resource efficiency and environmental sustainability in irrigated wheat cultivation based on statistical data for 2015–2024.

To achieve this goal, the following tasks were set:

1. To analyze the dynamics of mineral fertilizer application;
2. To evaluate the dynamics of organic fertilizer application;
3. To study the changes in water consumption and water losses for irrigation needs;
4. To conduct a comparative analysis between indicators;
5. To put forward scientifically based recommendations for sustainable agriculture.

In this study, the assessment was carried out according to the following conceptual scheme:

1. Mineral fertilizers (kg/ha);
2. Organic fertilizers (kg/ha);
3. Water consumption for irrigation needs (million m<sup>3</sup>);
4. Volume of water losses (million m<sup>3</sup>);
5. Assessment of resource efficiency and environmental sustainability.

## Results and Discussion

**Table 1**

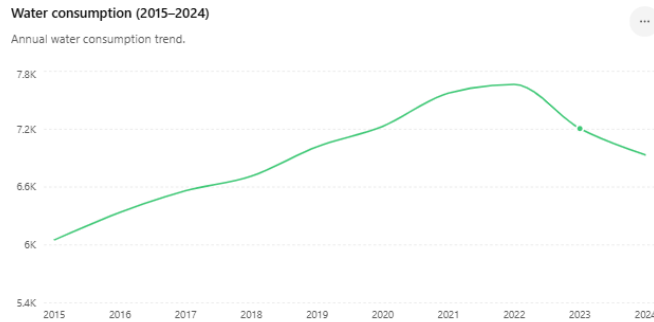
*Main indicators for the period 2015–2024*

Year	Water Consumption (million m <sup>3</sup> )	Water Losses (million m <sup>3</sup> )	Mineral Fertilizer (kg/ha)	Organic Fertilizer (kg/ha)
2015	6050.9	3718.3	23	738
2016	6336.5	3680.0	44	722
2017	6561.5	3627.6	63	703
2018	6712.5	3643.0	72	666
2019	7018.7	3755.3	82	669
2020	7229.8	3267.8	81	700
2021	7574.6	3216.5	47	694
2022	7664.4	3313.5	65	700
2023	7206.7	3034.4	65	731
2024	6933.3	3615.6	55	653

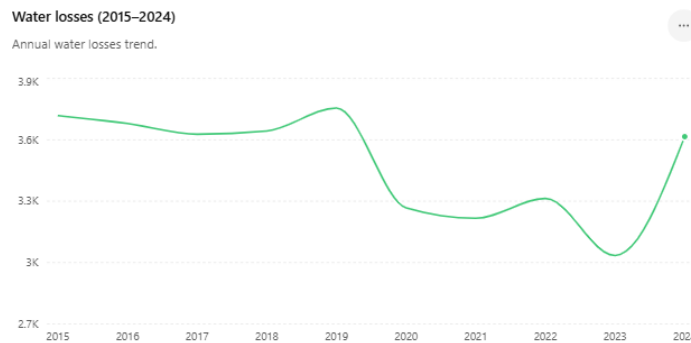
During 2015–2024, a general upward trend was observed in the volume of water used for irrigation needs (Tab. 1). While water consumption at the beginning of the period was 6,050.9 million cubic meters, it reached a maximum level of 7,664.4 million cubic meters in 2022. A certain decrease was observed in the next two years, and in 2024 this indicator was 6,933.3 million cubic meters. This dynamics not only indicates an increase in water demand in irrigated agriculture, but also reveals the importance of more efficient management of water resources (Azərbaycan Respublikasının Dövlət Statistika Komitəsi, 2025). The analysis shows that the volume of water losses in 2015–2024 varied in the range of 3,034.4–3,755.3 million cubic meters. The highest indicator was recorded in 2019 (3,755.3 million cubic meters), and the lowest indicator was recorded in 2023 (3,034.4 million cubic meters). Although a certain downward trend in water losses was observed during the period, the increase in the indicator again to 3,615.6 million cubic meters in 2024 indicates the importance of modernizing irrigation systems and improving water management. The amount of mineral fertilizers per hectare of cultivated area showed significant changes during the studied period. While this indicator was 23 kg/ha in 2015, it increased to 82 kg/ha in 2019. In 2020, the indicator remained at a high level (81 kg/ha), but decreased to 47 kg/ha in 2021.

Although an increase was observed again in subsequent years, 55 kg/ha was recorded in 2024. These results reflect the impact of changing policies and production conditions on the application of mineral fertilizers over the years. The amount of organic fertilizers per hectare of cultivated area was 738 kg/ha in 2015. A gradual decrease was observed in subsequent years, reaching 666 kg/ha in 2018. In 2020–2023, the indicator stabilized in the range of 694–731 kg/ha, and in 2024 it was 653 kg/ha. The changes observed in the application of organic fertilizers are of particular importance in terms of long-term protection of soil fertility. The study shows that a rapid increase in the application of mineral fertilizers was observed during 2015–2019, while variable dynamics were recorded in subsequent years. In contrast, the application of organic fertilizers was more stable and accompanied by relatively small changes. The increase in the application level of mineral fertilizers in years when

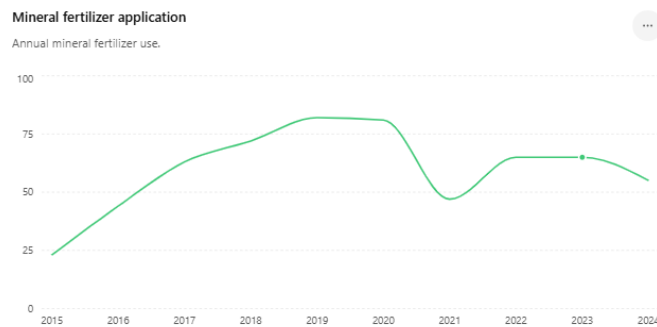
water consumption increased indicates that they are more widely used in intensive agricultural systems (Shehzadi et al., 2017). On the other hand, the application of high volumes of organic fertilizers has significant advantages in terms of improving the physical and biological properties of the soil (Shi et al., 2024). Overall, the results show that the application of not only mineral or only organic fertilizers, but their scientifically justified and balanced combination is a more appropriate approach to increasing resource efficiency and ensuring ecological sustainability.



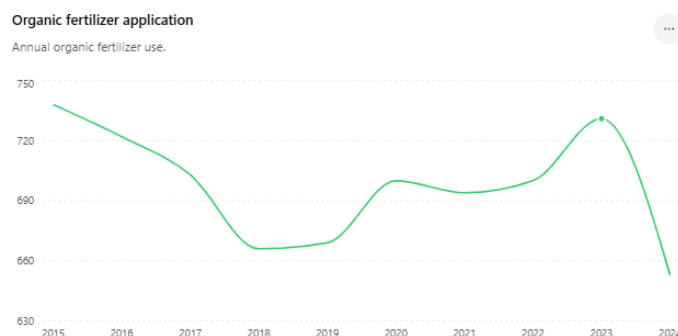
**Figure 1**  
*Water consumption (2015-2024)*



**Figure 2**  
*Water losses (2015-2024)*



**Figure 3**  
*Mineral fertilizer application*



**Figure 4**  
*Organic fertilizer application*

### ***Dynamics of Water Consumption***

Diagram 1 presents the dynamics of water consumption for 2015–2024. The analysis shows that water consumption showed an overall upward trend during the study period, but a relative decrease was observed in recent years. While water consumption in 2015 was 6050.9 million m<sup>3</sup>, this indicator reached 7664.4 million m<sup>3</sup> in 2022, reaching the maximum level for the study period. Thus, water consumption increased by approximately 26.7% over seven years. This increase can be explained by the expansion of arable land, the introduction of intensive farming systems, and the increase in water demand during the vegetation period. However, the decrease in the indicator to 7206.7 million m<sup>3</sup> in 2023 and to 6933.3 million m<sup>3</sup> in 2024 indicates a weakening of the previous upward trend. This dynamics can be attributed to more efficient management of water resources, improved irrigation technologies, or changes in climate conditions. Overall, Figure 1 shows that water demand in agriculture remains high in the long term, but optimization trends have been emerging in recent years (Food and Agriculture Organization of the United Nations, 2022; Azərbaycan Respublikasının Ekologiya və Təbii Sərvətlər Nazirliyi, 2024).

### ***Dynamics of Water Losses***

Figure 2 reflects the change in water losses over the years. The data show that although there are certain fluctuations in the indicator, the general trend is characterized by a decreasing trend. In 2015, water losses amounted to 3718.3 million m<sup>3</sup>, and in 2019 they increased to 3755.3 million m<sup>3</sup>, reaching the highest indicator for the period. However, a significant decrease occurred in subsequent years, and in 2023, water losses amounted to 3034.4 million m<sup>3</sup>, which is the minimum indicator for the study period. The increase in water losses to 3615.6 million m<sup>3</sup> in 2024 indicates that the previous downward trend is not completely stable. This change can be explained by the technical condition of irrigation networks, hydrometeorological conditions and differences in water management systems (Farouk et al., 2024).

The results show that although some progress has been made in reducing water losses over the past decade, additional measures are needed to ensure sustainable and sustainable results (DSK, 2025; Intergovernmental Panel on Climate Change, 2023).

### ***Dynamics of Mineral Fertilizer Application***

Analysis of Diagram 3 shows that there have been sharp changes in the level of mineral fertilizer application. In 2015, the amount of mineral fertilizer per hectare of cultivated area was 23 kg/ha. In subsequent years, this indicator has consistently increased and reached 82 kg/ha in 2019. Although the indicator remained at a high level (81 kg/ha) in 2020, a significant decrease was observed in 2021 to 47 kg/ha. Then, stabilization at 65 kg/ha was recorded in 2022–2023, and a decrease to 55 kg/ha in 2024. This dynamics shows that the application of mineral fertilizers can be affected not only by

agrotechnical factors, but also by economic conditions, price changes in the fertilizer market, and state support mechanisms. At the same time, in the long term, a general upward trend in the application of mineral fertilizers is observed, which is consistent with the development of intensive agricultural production (DSK, 2025).

### ***Dynamics of the Application of Organic Fertilizers***

Diagram 4 shows that there is a relatively stable dynamics in the application of organic fertilizers. In 2015, the indicator was 738 kg/ha, some decreases were observed in subsequent years, and in 2018 it decreased to 666 kg/ha. In 2020–2022, the indicator remained stable in the range of approximately 694–700 kg/ha, and increased to 731 kg/ha in 2023. However, in 2024, the application of organic fertilizers decreased to the lowest level in the studied period, at 653 kg/ha. Compared to mineral fertilizers, the amplitude of change of organic fertilizers was lower, which indicates that their application depends more on the resource supply of farms, livestock products, and long-term soil fertility strategies. In general, although the stability of the application of organic fertilizers is assessed positively in terms of preserving the soil organic matter reserve, the decrease observed in the last year can be considered a trend that requires attention in terms of ecological sustainability (Lal, 2020).

### **Conclusion**

Overall conclusion for the section: A comprehensive analysis of Diagrams 1–4 shows that:

1. Water consumption has shown a long-term increase and a relative decrease in recent years;
2. Although water losses have generally shown a decreasing trend, fluctuations have continued over the years;
3. The application of mineral fertilizers has shown intense variability and the maximum level was recorded in 2019;
4. The application of organic fertilizers has been more stable, but a minimum indicator was observed in 2024.

The conducted analyses have shown that significant dynamic changes were observed in water consumption, water losses and fertilizer application levels during 2015–2024. The research results indicate that there is an interaction between the efficient use of water resources and the optimization of the fertilization system, and these factors play an important role in the sustainability of agricultural production. Therefore, the improvement of irrigation technologies and the application of scientifically based fertilization strategies should be considered priority areas in terms of increasing the productivity of the agricultural sector and ensuring environmental sustainability. In future studies, the integration of productivity, soil properties and climatic factors will allow for more complete scientific results.

### **Declaration of Competing Interests**

The author declares that there are no conflicts of interest regarding the publication of this study.

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## Tree Species Growing within the Turyanchay State Nature Reserve

Rana Suleymanova 

**Abstract.** *This research paper examines the main tree species found within the reserve, their biological characteristics, ecological significance and role in nature conservation. The reserve is considered one of Azerbaijan's areas of rich biodiversity, where various forest ecosystems have developed. The area is home to widespread populations of oak (*Quercus*), hornbeam (*Carpinus*), ash (*Fraxinus*), juniper (*Juniperus*), elm (*Ulmus*), plane tree (*Platanus*), pomegranate (*Punica granatum*), alder (*Alnus*), poplar (*Populus*), silverberry (*Elaeagnus*), willow (*Salix*) and other valuable tree species are widespread. These trees play an important role in protecting the soil from erosion, regulating the climate and improving living conditions for organisms. The study analysed the reserve's floral diversity, the characteristics of its forest cover, and the distribution ranges of individual tree species. It was found that human impact, illegal logging, climate change and natural factors are leading to a decline in certain tree species. Therefore, the protection of the reserve, the strengthening of environmental monitoring and the conservation of biodiversity are of particular importance. The study concluded that the Turyanchay State Nature Reserve is one of the key natural areas for the conservation of rare and valuable tree species. Preserving the existing vegetation cover is of great importance for protecting natural resources for future generations and ensuring ecological balance.*

**Keywords:** *Turyanchay State Nature Reserve, tree species, forest ecosystem, biodiversity, flora*

### Introduction

The Turyanchay State Nature Reserve represents a significant protected natural area within the borders of Azerbaijan, characterised by a wealth of natural resources and biodiversity. As stated in the materials published by the Ministry of Ecology and Natural Resources of the Republic of Azerbaijan (Ministry of Ecology and Natural Resources, 2020), general information regarding the ecological significance of nature reserves and national parks is also available. Hajiyev (2018) assertion that the ecological characteristics of Azerbaijan's forests play a fundamental role in the conservation of biodiversity is supported by extensive research.

This nature reserve occupies a significant position within the country's ecological system, playing a substantial role in the conservation of various plant and animal species. The topography of the reserve is characterised by predominantly foothill and wooded areas, resulting in a diverse arboreal flora. The tree species present in the Turyanchay State Nature Reserve are of significant ecological importance, playing a crucial role in shaping the natural landscape and maintaining ecological balance (Aliyeva, 2020).

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Received: 5 February 2026; Accepted: 13 May 2026; Published online: 25 June 2026

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In the present age, environmental protection and the sustainable utilisation of natural resources are regarded as some of the most pressing issues on a global scale. It is evident that issues such as climate change, human impact, deforestation and soil erosion are having a detrimental effect on natural ecosystems. From this standpoint, state nature reserves are of particular importance for the conservation of biodiversity, the preservation of rare plant species and the maintenance of ecological stability (Aliyev et al., 2023).

The Turyanchay State Nature Reserve, as one of these important natural areas, contributes to the conservation of Azerbaijan's forest resources (Mammadov & Khalilov, 2015). The reserve's flora is exceptionally rich; it is home to such valuable tree species as oak (*Quercus*), hornbeam (*Carpinus*), ash (*Fraxinus*), juniper (*Juniperus*), elm (*Ulmus*), plane tree (*Platanus*), pine (*Pinus*) and others. These tree species play a vital role in maintaining soil fertility, purifying the air, ensuring oxygen balance and providing habitats for various living organisms. It has been proven that forest cover plays a pivotal role in the mitigation of natural disasters, including but not limited to mudslides, landslides and erosion.

The study of tree species found in the Turyanchay State Nature Reserve is of great importance from both scientific and practical perspectives. The objective of the present study is to identify the reserve's floristic characteristics, to study the distribution ranges of tree species, and to develop measures for their conservation. The study of the biological characteristics of tree species is of great significance for future progress in the fields of reforestation and nature conservation.

The primary objective of this study is to examine the principal tree species growing within the Turyanchay State Nature Reserve, to determine their ecological significance, and to analyse their role in the conservation of biodiversity. The study encompassed a thorough investigation into the reserve's natural and geographical characteristics, the characteristics of the forest cover, and the distribution of various tree species. Furthermore, the significance of safeguarding natural resources and the measures being implemented in the domain of forest conservation were contemplated.

The Turyanchay State Nature Reserve is therefore considered to be one of Azerbaijan's most significant natural areas, boasting a rich variety of plant life. The conservation of the tree species found in this region is of paramount importance, not only in terms of maintaining the ecological balance, but also with a view to ensuring the preservation of a healthy natural environment for future generations.

According to Ismayilov (2019), Azerbaijan's nature reserves play a vital role in the region's ecological balance. This perspective is further substantiated by Mammadov and Khalilov (2015), who acknowledges the country's notable plant biodiversity.

The Turyanchay State Nature Reserve is a protected natural area of significant importance, distinguished by its unique natural and geographical conditions. The topography of the reserve is characterised by a predominance of foothills and low- to mid-mountain zones, resulting in a diverse array of landforms, climatic conditions and soil types. The area's topography is intricate, giving rise to diverse vegetation cover and a rich variety of flora.

The prevailing climatic conditions in the regions encompassing the nature reserve are characterised by a temperate and arid climate. Given the variation in rainfall and temperature levels with altitude, favourable conditions have developed here for the growth of various trees and shrubs. The presence of elevated levels of humidity, particularly within forested regions, is a crucial factor in ensuring the optimal growth of trees. The role of the Turyanchay River and other minor water sources in preserving the area's ecological system is also of significance (Huseynov, 2017).

The nature reserve is characterised by a predominantly mountainous topography, with forestry and grey-brown soil types predominating. These soils are characterised by a high mineral content, which is conducive to the growth of various tree species. The forest cover not only preserves soil fertility but also helps to regulate water resources and prevent erosion. Forests located on mountain slopes have been shown to significantly mitigate the negative effects of heavy rainfall and wind (Jones et al., 2022). Mammadov and Khalilov (2015) posit that the preservation of forest ecosystems is a prerequisite for ecological stability.

The Turyanchay State Nature Reserve is also home to a variety of animal species. The area exhibits favourable natural conditions, providing a conducive environment for the proliferation of mammals, birds, reptiles, and insects. The presence of tree and shrub vegetation is of paramount importance for the sustenance, shelter and reproduction of the animals. From this standpoint, the reserve's forest ecosystem is significant not only for the flora, but also for the conservation of the fauna and the biota in its entirety. As asserted by Safarov (2016), the forest ecosystems of Azerbaijan are intimately associated with the region's climatic and soil characteristics.

The tree species present within the nature reserve play a pivotal role in shaping the natural landscape. Trees such as oak and hornbeam are key components of the forest ecosystem, playing a crucial role in its stability and resilience. Their longevity is a key factor in maintaining the ecological balance of the forest. Conversely, Juniper (*Juniperus*) demonstrates resilience to drought conditions and is prevalent in alpine regions. It is evident that species such as plane trees (*Platanus*) and elms (*Ulmus*) are of significance due to two factors: firstly, their aesthetic appeal, and secondly, their ecological benefits.

In the contemporary era, the impact of human activity on natural ecosystems is gradually escalating. The ongoing and detrimental impact of illegal logging, the expansion of grazing land, fires and climate change on forest cover is well-documented. Consequently, the populations of certain tree species are in decline, and the ecological balance is being disturbed. For this reason, the protection of nature reserves and the conservation of natural resources are considered to be among the primary responsibilities of the state and society at large.

The conservation measures implemented in the Turyanchay State Nature Reserve are aimed at preserving biodiversity and restoring forest resources. The implementation of environmental monitoring within the reserve is of paramount importance, as it facilitates the study of the condition of tree species. Furthermore, the development of special programmes for the protection of rare plants is essential. Concurrently, a range of initiatives are being undertaken to enhance public cognisance of environmental concerns. These activities have been shown to engender a greater sense of responsibility among people regarding nature conservation.

## **Materials and Methods**

This study utilised an array of research methodologies in order to investigate the tree species present within the Turyanchay State Nature Reserve. A dual approach encompassing theoretical and practical methodologies was adopted during the research process. The obtained data underwent a comparative analysis. The primary objective of the methodology was to ascertain the reserve's floristic diversity, the distribution patterns of tree species and their ecological significance.

A comprehensive study of the flora of Azerbaijan was conducted by the ANAS (Azerbaijan National Academy of Sciences, 2011). In the initial stage of the research, a detailed examination of the scientific literature, including articles, monographs, research reports and official environmental data relevant to the subject, was conducted. Pursuant to the theoretical information obtained, a comprehensive database was compiled on the floristic diversity, forest cover and biological

characteristics of tree species in the Turyanchay State Nature Reserve. In the course of the literature review, particular attention was paid to scientific sources relating to the flora and forestry resources of Azerbaijan.

The observational method was the primary approach employed in the course of the study. An investigation was conducted on the natural habitats of the tree species found within the reserve. The study encompassed the examination of the species' ranges, density, and ecological condition. During the course of the observations, the following factors were taken into account: tree height, trunk structure, distribution patterns and the degree of adaptation to the environment. This method yielded practical data on the tree species growing in various parts of the reserve. The study also employed a comparative analysis method. A comparison was made between the ecological characteristics of various tree species, their resilience to natural conditions, and the role they play in the ecosystem. Concurrently, an analysis was conducted of the floristic composition of various forest areas, with the objective of identifying both the similarities and differences between them. This approach facilitated more precise assessment of the significance of tree species from the perspective of biodiversity.

The study also employed statistical analysis. A comprehensive analysis of existing statistical data concerning the reserve's forest areas, the distribution of tree species, and biodiversity was conducted. The results obtained were presented in tabular form and summarised. The application of statistical analysis facilitated the determination of the conservation status of various tree species and the identification of pertinent environmental issues.

The ecological approach adopted in this research proved to be of significant importance. This method was utilised to investigate the relationship between tree species and their environment, as well as their impact on soil, water and climatic conditions. The analysis encompassed the contribution of trees to the protection of soil against erosion, the purification of atmospheric air, and the formation of habitats for living organisms. Concurrently, the impact of anthropogenic factors on the forest ecosystem was examined.

Following the conclusion of the study, the data obtained were summarised and the results subjected to a systematic analysis. The methodological approaches employed in this study enabled the identification of the biological and ecological characteristics of tree species found in the Turyanchay State Nature Reserve, thus justifying the need for their conservation. This methodology is of great importance for a more in-depth study of the reserve's floristic diversity in the future, as well as for conducting new scientific research in the field of forest conservation.

## Results and Discussion

The tree species present within the Turyanchay State Nature Reserve are indicative of the biodiversity and resilience of the region's forest ecosystems. The reserve is dominated mainly by tree species such as oak, hornbeam, maple, ash and linden. These species play a pivotal role in the prevention of soil erosion, the maintenance of the water balance, and the formation of a natural habitat for animals. The presence of diverse climatic and topographical conditions within the reserve has contributed to an increase in the variety of tree species. The forest cover of the reserve is of particular importance in maintaining ecological balance and in the conservation of rare and relict plant species.

The flora of the Turyanchay State Nature Reserve has been found to be exceptionally rich and diverse. The plant species present within the reserve are indicative of the natural flora of the southern slopes of the Greater Caucasus. The vegetation of this region is characterised by a preponderance of broad-leaved species, a phenomenon that is contingent upon the prevailing climatic conditions, soil characteristics and topography of the region. A wide variety of tree species is present in the area, including oak (*Quercus*), hornbeam (*Carpinus*), eastern beech (*Fagus sylvatica*), maple (*Acer*), ash

(*Fraxinus*), linden (*Tilia*) and elm (*Ulmus*). In addition to these, the biodiversity of the reserve is enriched by the presence of various shrubs and herbaceous plants.

The reserve's flora also includes relict and endemic plant species, which further enhances its scientific and ecological significance. The development of different plant communities across the various altitudinal zones is a well-documented phenomenon. In the low-altitude zones, drought-resistant shrubs and sparse woodlands are common, while in the higher and more humid zones, dense broad-leaved forests predominate. This plant diversity ensures the stability of the ecosystem and creates conditions for the preservation of natural connections between living organisms (Jorge et al., 2026).

The flora of the Turyanchay State Nature Reserve plays a vital role in the conservation of soil, the preservation of water resources and the maintenance of the climate balance. The presence of vegetation has been demonstrated to have several key benefits, including the protection of soil from erosion, the purification of air, and the maintenance of biodiversity. The reserve is notable for its diverse array of medicinal, fodder and ornamental plants, which play a crucial role in preserving the biodiversity of the region. The preservation of this area's botanical wealth is contingent on the limitation of human impact and the implementation of conservation measures. Consequently, the Turyanchay State Nature Reserve is regarded as one of the primary centres of natural vegetation in Azerbaijan, playing a pivotal role in the conservation of rare plant species.

The reserve's vegetation is also characterised by seasonal changes. During the spring and summer months, the area is characterised by a diverse array of herbaceous plants, flowering species, and various wild berry bushes. The closure (increase in density) of the vegetation cover at this time helps to maintain soil moisture levels and creates conditions conducive to the formation of a favourable food source for many species of living organisms. However, during the autumn months, the process of leaf fall in broad-leaved trees results in an augmentation of organic matter within the soil, thereby playing a crucial role in the enrichment of the humus layer (Aryal et al., 2022).

Hajiyev (2018) posits that vegetation performs a pivotal function in the preservation of biodiversity. It is evident that the flora of the Turyanchay State Nature Reserve functions as a natural defence against climate change. Forests have been shown to have a beneficial effect on the concentration of carbon dioxide in the atmosphere, in addition to playing a regulatory role in air temperature and maintaining the oxygen balance. These characteristics indicate that the reserve is an important area not only on a regional scale but also in broader ecological terms (Saha et al., 2026). Talibov (2014) research analyses the current state and trends in changes to plant species. The implementation of conservation measures and environmental monitoring within the reserve is instrumental in ensuring the preservation of rare and endangered plant species. The key measures that must be implemented to ensure the sustainability of the reserve's vegetation cover are as follows: the prevention of illegal logging, the minimisation of the risk of fire, and the support of natural reforestation processes. Consequently, the Turyanchay State Nature Reserve is of considerable ecological importance in terms of the conservation of Azerbaijan's natural resources.

## Conclusion

The study has established that the Turyanchay State Nature Reserve is home to a rich variety of flora, and that the tree species found there play a vital role in the conservation of the ecosystem. The oak, hornbeam, ash, juniper, elm, plane tree and other tree species that are widely represented in the reserve have a significant impact not only on the formation of the natural landscape, but also on the preservation of biodiversity. These tree species are of particular importance for maintaining soil fertility, preventing erosion, purifying the air and preserving the climatic balance. The study revealed that the reserve's forest cover creates a favourable habitat for various species of living organisms. The arboreal vegetation in this area provides a series of ecological conditions that are indispensable for

the feeding, settlement and reproduction of animals. The stability of the forest ecosystem is pivotal in ensuring the sustainable development of both flora and fauna. From this standpoint, the Turyanchay State Nature Reserve is of significant importance for the conservation of Azerbaijan's natural heritage.

The research conducted indicates that the Turyanchay State Nature Reserve is a significant natural asset in Azerbaijan. The conservation of the tree species present in this reserve is of strategic importance. The conservation of the local forest ecosystems is of paramount importance in maintaining the health of the environment, mitigating the negative effects of climate change, and ensuring the sustainability of biodiversity. Consequently, the investigation and conservation of the vegetation within the Turyanchay State Nature Reserve, with a particular focus on its tree species, provide a significant foundation for future scientific research. The ongoing implementation of strategies focused on the conservation of the reserve's natural resources is of paramount importance in ensuring the environmental security of Azerbaijan and safeguarding its natural heritage for posterity.

### Declaration of Competing Interests

The author declares that they have no competing interests.

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## Optimization of the Synthesis Parameters of (E)-4-(Dimethylamino)-N-Benzylideneaniline

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Pari Huseynova<sup>2\*</sup> , Bahruz Mammadov<sup>3</sup> 

**Abstract.** Schiff bases containing azomethine functional groups continue to attract considerable interest due to their structural diversity and broad applicability in coordination chemistry, organic synthesis, and functional material design. In the present study, (E)-4-(dimethylamino)-N-benzylideneaniline was synthesized through the condensation reaction of 4-dimethylaminobenzaldehyde with aniline in ethanol medium under mild acidic conditions. The influence of major synthesis parameters, including reaction temperature, reaction time, and catalyst amount, on product yield and crystallization behavior was systematically investigated. The reaction progress was visually monitored through changes in solution color and crystal formation. Experimental results demonstrated that the synthesis efficiency strongly depended on reaction conditions. Moderate temperatures promoted effective azomethine bond formation, whereas excessive heating reduced product quality and yield. The optimal synthesis conditions were determined to be 50 °C reaction temperature, 35 minutes reaction time, and the addition of two drops of acetic acid catalyst. Under these conditions, the target Schiff base was obtained as an intense yellow crystalline compound with high yield. The study confirms that mild reaction conditions provide an efficient and experimentally simple approach for the synthesis of conjugated azomethine derivatives.

**Keywords:** schiff base, azomethine, condensation reaction, synthesis optimization, dimethylaminobenzaldehyde, aniline

### Introduction

Schiff bases represent an important class of organic compounds containing the azomethine (-CH=N) functional group formed by condensation reactions between primary amines and carbonyl compounds. Due to the presence of electron-rich nitrogen atoms and conjugated  $\pi$ -electron systems, these compounds have attracted continuous attention in coordination chemistry, catalysis, medicinal chemistry, optical materials, and analytical applications (Huseynova et al., 2025).

The structural and physicochemical properties of Schiff bases strongly depend on the nature of substituent groups attached to the aromatic system. Electron-donating substituents such as dimethylamino groups significantly influence electron delocalization within the molecule and affect the stability, color, and crystallization behavior of the resulting azomethine derivatives.

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Received: 8 February 2026; Accepted: 11 May 2026; Published online: 25 June 2026

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Aromatic Schiff bases containing donor substituents are particularly interesting because of their extended conjugation systems and enhanced electronic interactions (Rajanna et al., 2025). Among substituted azomethine compounds, Schiff bases derived from 4-dimethylamino benzaldehyde have gained increasing attention because the dimethylamino group substantially modifies the electronic distribution of the aromatic framework (Waziri et al., 2023). The electron-releasing nature of this substituent increases electron density within the conjugated system and promotes stabilization of the imine functionality. Such electronic effects may alter molecular polarity, intermolecular interactions, and reactivity toward electrophilic or coordination processes (Fejfarova et al., 2010).

Structurally, these compounds generally adopt the thermodynamically favorable E-configuration around the azomethine bond (Awad et al., 2020). The spatial arrangement associated with the E-isomer contributes to molecular stability by reducing steric repulsion between aromatic fragments and improving  $\pi$ -conjugation throughout the molecular skeleton. Extended conjugation is also responsible for the intense coloration commonly observed in dimethylamino-substituted Schiff bases (Soliz et al., 2024).

In recent years, Schiff bases containing dimethylamino substituents have been investigated for a wide variety of applications. Numerous studies have demonstrated that such compounds and their metal complexes may exhibit antibacterial, antifungal, antioxidant, and anticancer activities (Ahmed et al., 2020). In many cases, coordination of Schiff bases with transition metal ions enhances biological efficiency due to modifications in lipophilicity, electronic structure, and metal-assisted transport processes (You et al., 2004).

Beyond medicinal applications, conjugated Schiff bases are also considered promising functional materials (Ali et al., 2022). Their ability to coordinate with metal ions enables their utilization in sensor technology, electrochemical systems, and corrosion inhibition studies. The presence of donor nitrogen atoms and aromatic  $\pi$ -systems facilitates adsorption on metallic surfaces and contributes to the formation of stable coordination environments (Trzesowska-Kruszynska, 2010). Additionally, intermolecular interactions such as  $\pi$ - $\pi$  stacking and weak hydrogen bonding may influence crystal packing, solubility, and physicochemical stability (Khalaji et al., 2011).

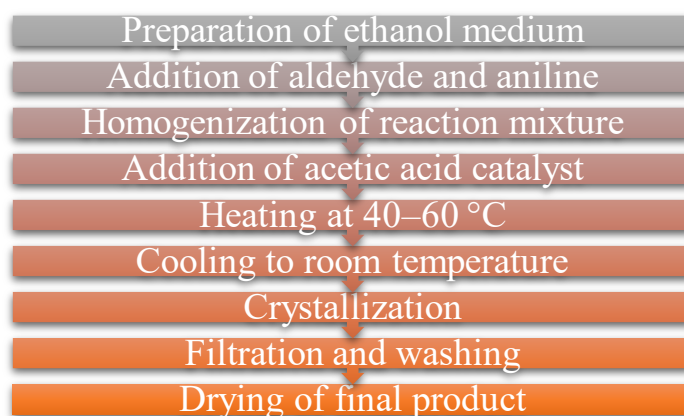
Despite the simplicity of Schiff base synthesis, optimization of experimental parameters remains critically important for obtaining products with high yield and desirable purity. Reaction temperature, catalyst concentration, solvent polarity, and reaction duration directly influence the rate of nucleophilic addition, dehydration efficiency, and subsequent crystallization behavior. Therefore, systematic evaluation of synthesis conditions is necessary for establishing reproducible and experimentally efficient synthetic methodologies (Ngoudjou et al., 2023).

In this study, (E)-4-(dimethylamino)-N-benzylideneaniline was synthesized from 4-dimethylaminobenzaldehyde and aniline in ethanol medium under mild acidic conditions. The effects of reaction temperature, reaction time, and catalyst amount on synthesis efficiency were comparatively investigated in order to determine the optimal experimental conditions for azomethine bond formation.

## Materials and Methods

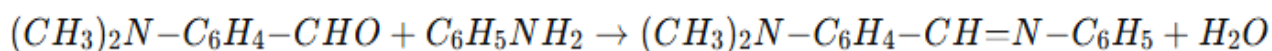
4-Dimethylaminobenzaldehyde, aniline, ethanol, and acetic acid were used without additional purification. All reagents were of analytical grade. The synthesis procedure was carried out using a magnetic stirrer, water bath, analytical balance, filtration apparatus, and drying oven.

### General Synthesis Procedure



Initially, 10–15 ml of ethanol was introduced into a reaction flask. Equimolar amounts of 4-dimethylaminobenzaldehyde and aniline were then added to the solvent medium. The mixture was stirred until a homogeneous solution was obtained. Subsequently, 2–3 drops of acetic acid were added as a catalyst to accelerate the condensation process. The reaction mixture was heated in a water bath at temperatures ranging from 40 to 60 °C for 30–40 minutes under continuous stirring. During heating, gradual color changes were observed, indicating the formation of the azomethine linkage. After completion of the reaction, the mixture was cooled to room temperature. Formation of a crystalline precipitate was observed during the cooling stage. In cases where precipitation was incomplete, a small quantity of distilled water was added to facilitate crystallization.

The obtained solid product was separated by filtration, washed with cold ethanol, and dried at 50–60°C. The synthesis reaction proceeded according to the following equation:



#### Optimization studies

The influence of three major synthesis parameters was investigated: Reaction temperature; Reaction time; Catalyst amount. The isolated product yield was calculated gravimetrically after drying.

### Results

#### Effect of Reaction Temperature

The effect of temperature on product formation was investigated under identical reaction conditions.



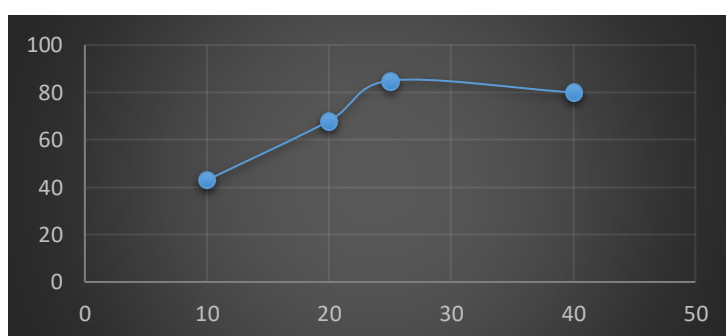
**Figure 1**  
*The process of obtaining a crystalline product*

Temperature (°C)	Reaction time (min)	Yield (%)	Product appearance
30	35	56	Pale yellow crystals
40	35	72	Yellow crystals
50	35	85	Intense yellow crystals
60	35	80	Dark yellow crystals

The results indicate that increasing the temperature from 30 °C to 50 °C significantly improved product yield. However, further increase in temperature slightly reduced the efficiency of the synthesis.

### ***Effect of Reaction Time***

The influence of reaction duration on product formation was also evaluated. The highest yield was obtained after 35 minutes of heating.



### ***Effect of Catalyst Amount***

The influence of catalyst concentration on the synthesis efficiency of (E)-4-(dimethylamino)-N-benzylideneaniline was investigated using different amounts of acetic acid under identical reaction conditions. The experimental results demonstrated that catalyst quantity significantly affected the formation of the azomethine linkage and the overall product yield.

When one drop of acetic acid was used, the isolated yield of the product reached 61%, indicating that the catalytic activation of the carbonyl group was relatively limited under these conditions. Increasing the catalyst amount to two drops considerably improved the reaction efficiency and resulted in the highest yield of 84%. This improvement can be attributed to enhanced protonation of the carbonyl oxygen, which facilitated nucleophilic attack by aniline and accelerated imine formation.

However, further increase of the catalyst amount to five drops led to a decrease in product yield to 73%. Excessive acidic conditions likely affected the crystallization process and promoted partial side reactions, resulting in lower isolation efficiency of the Schiff base product. The obtained results indicate that moderate catalyst concentration provides the most favorable conditions for efficient synthesis of the target azomethine derivative.

## **Discussion**

The obtained results demonstrate that synthesis efficiency is strongly dependent on reaction parameters. The condensation reaction between 4-dimethylaminobenzaldehyde and aniline proceeds through nucleophilic attack of the amine nitrogen on the carbonyl carbon atom, followed by dehydration and formation of the azomethine linkage. The dimethylamino substituent acts as an electron-donating group and increases electron density within the aromatic system. This electronic effect contributes to stabilization of the conjugated structure formed after condensation. As a result,

the synthesized Schiff base exhibits intense coloration associated with extended  $\pi$ -electron delocalization (Hassan & Yusoff, 2019).

Temperature was found to be one of the most influential factors affecting product formation. At lower temperatures, insufficient molecular collisions slowed down the condensation process, resulting in lower yields. Moderate heating accelerated azomethine bond formation and improved crystallization efficiency. However, excessive heating likely promoted secondary processes and reduced product purity.

Reaction duration also affected the synthesis outcome. Short reaction periods did not allow complete conversion of reactants, whereas prolonged heating slightly decreased yield, probably due to partial decomposition or side reactions. Acetic acid played an important catalytic role by activating the carbonyl group toward nucleophilic attack. Nevertheless, excessive catalyst concentration negatively influenced crystal formation, leading to reduced isolated yields.

Overall, the experimental results confirmed that mild synthetic conditions can efficiently produce conjugated Schiff base derivatives with relatively high yields and simple experimental procedures.

## Conclusion

(E)-4-(Dimethylamino)-N-benzylideneaniline was successfully synthesized through condensation of 4-dimethylaminobenzaldehyde with aniline in ethanol medium under acidic conditions. The influence of reaction temperature, reaction time, and catalyst amount on synthesis efficiency was systematically investigated. Optimal conditions were determined as 50 °C reaction temperature, 35 minutes reaction time, and two drops of acetic acid catalyst.

Under these conditions, the target Schiff base was obtained as an intense yellow crystalline product with high yield. The experimental findings demonstrated that careful optimization of reaction parameters significantly improves azomethine bond formation, crystallization efficiency, and overall synthetic performance.

The electronic influence of the dimethylamino substituent contributes to stabilization of the conjugated structure and may enhance the coordination ability and functional applicability of the synthesized compound. Owing to its conjugated molecular architecture and donor nitrogen center, the synthesized Schiff base may serve as a promising precursor for future investigations in coordination chemistry, functional materials, corrosion inhibition, and biologically active metal complexes.

## Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Signed: 19.06.2026

Online publication: 25.06.2026

Paper printing: 10.07.2026

Format: 60/84, 1/8

Stock issuance: 13, 25 p.s..

Order: 190

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It has been published in the printing house  
“ZANGAZURDA”

Address: 19 A.M.Sharifzade St.,  
Yasamal Dist., Baku, Azerbaijan

Phone: +994 50 209 59 68

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