



DETERMINATION OF MEDICINAL PLANTS' MICROBIOMES WITH SEASONAL ACTIVITY IN NAKHCHIVAN REGION, AZERBAIJAN

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SUMMARY

The concerned research aimed to investigate the influence of medicinal plants of different families on the number and composition of micromycetes and the phytotoxic activity of the soil. It also sought to evaluate the use of antimicrobial activity of total extracts obtained from raw materials of some medicinal plants in folk medicines. The results revealed essential oils and aqueous extracts obtained from the medicinal plants native to Nakhchivan, Azerbaijan, exhibited antimicrobial effects of varying degrees. Studies on the dynamics of the soil and root microbiota of medicinal and essential oil plants depending on the different seasons and locations in the Nakhchivan Region were insufficient. For the first time, composition and spatiotemporal patterns of microbial communities, as well as their seasonal (spring, summer, and fall) and microbiome domain specificity (bacteria and fungi), have gained studies. The β -diversity analysis revealed considerable differences in microbial communities.

Keywords: Vegetation, medicinal plants, phytocenoses, soil micromycetes, antimicrobial effects, oil content, fungal species

Key findings: The principal coordinate analysis (PCoA) based on distance to the rhizosphere bacterial communities of the studied *Allium* plant species showed the two principal components explaining 45.6% and 4.4% of the total variation for bacteria and 14.9% and 7.2% of the total variation for fungi, respectively.

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INTRODUCTION

In the present era, the mycoflora of essential oils and medicinal plants has undergone no more than 20% research; therefore, it is necessary to continue mycological studies of fungi in these groups of plants (Akbarova and Mammadova, 2024). Medicinal plants contain a wide range of microorganisms with diverse individual properties and significant differences for qualitative aspects. The plants with microbial backgrounds could result from several influences caused by sources of living and non-living nature, with microbial pollutants simply transmitted through the air and soil (Akhundova *et al.*, 2025; Amanova *et al.*, 2025). Medicinal plant materials (MPM), which provide the basis for herbal preparations, have more natural contaminations of microflora than synthetic drugs and were the most likely carriers of microbial spores and fungal conidia. Microbial contamination of plants primarily depends on the existing environment (soil, air, and water) (Allahverdi *et al.*, 2026). Various bacteria, fungi, yeasts, and viruses can exist on and within plants, along with considerable contamination from rodents and insects (Gurbanov and Huseynova, 2022; Bunyatova *et al.*, 2025).

Inevitably, the plants' microbiological background primarily depends on numerous environmental factors, which have a significant impact on the overall quality of MPM and the preparations based on medicinal plant materials. Therefore, assessing the risks of microbial contamination of medicinal plants has become an important subject in the establishment of a modern Hazard Analysis and Critical Control Point (HACCP) system. By cultivating medicinal plant materials on specialized farms, the plants may face the problems of rapidly declining plant productivity. Seed germination, growth, and development rates appeared to be lower than in natural ecosystems, and therefore, the medicinal plants cannot be grown in one place for a long time (Ismayil *et al.*, 2025; Ismayilova *et al.*, 2025).

A pressing environmental issue is elucidating the mechanisms regulating the functions of the 'soil-microbial community-plant' system. The studies typically focus on the effect of certain plants on others (allelopathy) and the development of phytotoxicity in the soil beneath plants; however, insufficient data prevails on the impact of plants on the soil microbial community (Ibrahimova and Imanov, 2025). It is also typical for the volume of living plant excretions into the soil (rhizoexudates) is to be around 30%–40% of the total volume of photosynthetic products (Mammadova *et al.*, 2024; Ismayil *et al.*, 2025). The root exudates' composition significantly varied among the plants with different families. For example, the sugars and organic acids predominate in cereals; meanwhile, amino acids and amides predominate in legume crops. Several studies have been progressive on the influence of plants on the composition and structure of the soil microbial community. However, an analysis of the direction and degree of expression of the effect of plants with different families has not received many studies (Mammadova *et al.*, 2025).

Medicinal plants significantly accumulate the biologically active substances, such as hormones, alkaloids, glycosides, essential oils, tannins, phenols, and flavonoids, which provide their therapeutic effect. Plants of the Lamiaceae family (*Thymus*, *Salvia*, *Hyssopus*, and *Leonurus*) accumulate essential oils (*Thymolum* [C₁₀H₁₄O]; *Mentha* [C₁₀H₂₀O], *Carvacrol* [C₁₀H₁₄O], *Linalool* [C₁₀H₁₈O], *Geraniol* [C₁₀H₁₈O], *Salvinum* [KCl]), and *Glycosidum* (C₁₅H₁₆O₉) have the pronounced antibiotic and fungicidal effects. Plants of the Rosaceae family (*Sanguisorba* and *Potentilla*) produce the glycosides (tannica and catechinum), which have antiseptic properties. Flavonoids from bergenia (arbutin and isocoumarin [natural organic compound and lactone, characterized as a structural isomer of coumarin {3,4-benzo-2-pyrone}]) and alkaloids from comfrey (lasiocarpine and cynoglossine) provide these plants with anti-

inflammatory properties. In the root exudation process by plants during their lifetime, these compounds undergo active release into the soil and can also change the number and composition of the microbial community, including the complex of soil micromycetes (Nasirova *et al.*, 2026).

Essential oils obtained from medicinal plants have found wide application in numerous medicines, while the use of distilled waters is rare. However, if detecting pharmacological activity, distilled waters could be applicable as per the principles of rational use of plant materials (particularly when adhering to green chemistry principles and focusing on polar compounds). To this end, we studied the antimicrobial effect of distilled waters. The antimicrobial activity of essential oils is popular due to their aromatic and terpenoid structure. Plants' pure essential oils (EOs) are not very convenient herbal remedies and are primarily beneficial in topical dosage forms. Furthermore, pure essential oils proved very unstable during storage; the utilization of such preparations causes several significant drawbacks, such as irritancy, allergic reactions, and gastrointestinal disorders (Macnunlu *et al.*, 2025; Mammadova *et al.*, 2026; Nasirova *et al.*, 2026).

Therefore, various phytoextraction preparations obtained through combined extracts from medicinal plant materials are currently being actively developed and researched. The phytoextraction preparations are beneficial in terms of cost-effectiveness and rational use of plant materials, allowing for getting the maximum yield of biologically active substances. Biochemical studies revealed the rate of spore germination had significant reductions in solutions of phenolic substances, as well as ascorbic acid. The rational use and restoration of natural resources is not possible without understanding the interactions between organisms and their existing environment. Therefore, all the mycological and phytopathological studies were also essential for a comprehensive study of biocenoses and determining the relationships between the plants, fungi, and other organisms (Shirinova *et al.*, 2025; Rzayeva *et al.*, 2026).

MATERIALS AND METHODS

Natural biotopes with Kastnozems under a grass-forb association and soil without plants served as controls. The counting of micromycetes continued by plating on Czapek agar medium (pH 4.5). Species identification of micromycetes hazardous to human health proceeded using identification guides. Soil phytotoxicity determination used biotests (radish plant test), expressed as a percentage of inhibition of seed germination and seedling root growth on soil plates compared with growing on moistened filter paper. Soil acidity testing ensued potentiometrically in an aqueous extract. Data for three growing seasons (2023–2025), with contrasting hydrothermal conditions presented. Soil sample collection occurred during the budding-flowering phenophase (second 10-day period of June), when plant root exudation was the greatest (Nasirova *et al.*, 2026).

The raw material collection of studied plants transpired in 2023–2025 from steppe and meadow phytocenoses in Nakhchivan, Azerbaijan: the aboveground parts during the flowering phase and the underground parts after fruiting. The underground parts (rhizomes with roots) were the choice raw materials for the study, as this was the part where the essential oil has full concentration in the plant. Essential oil and aqueous extract from the raw material of an official medicinal plant, the antimicrobial activity of which was already known, served as comparison drugs in the study of antimicrobial properties. The raw materials sustained air-drying at 25 °C–35 °C to an air-dry state. Obtaining aqueous extracts from the plant materials ensued under aseptic conditions using a standard method, evaporated to dryness, and added to the nutrient medium at doses of 3, 6, 12, and 24 mg/ml. Essential oils obtained entailed hydrodistillation from air-dried raw materials for three hours. The collection of distillation waters also took place during the essential oil extraction process.

In the antimicrobial activity assessment, the study used daily cultures of microorganisms of museum strains recommended by the state, *Staphylococcus*

aureus 6538-P and *Pseudomonas aeruginosa* K-453. The microbial load was 0.5 million cells/mL (based on the turbidity standard). The antimicrobial activity of the essential oil entailed studies using the disk diffusion method. For the study, the prepared suspension containing a standard number of viable bacterial cells underwent inoculation as a lawn on the surface of nutrient agar in Petri dishes. Sterile filter paper disks sustained coating with 0.01 ml of freshly distilled essential oil or distilled water. The disks with the preparations, as placed on the inoculation, had a distance of 2.5 cm from the center of the dish in a circle (five disks per dish) (Shirinova *et al.*, 2025; Allahverdi *et al.*, 2026).

RESULTS AND DISCUSSION

The concerned study focused on a complex of micromycetes in leached gray-brown soil under various phytocenoses. Soil samples collected came from the 0–20 cm layer during a third-year stationary microfield experiment on medicinal plants at the Nakchivan Region, Azerbaijan. The soil under the plants of 21 species belonging to 11 families received examinations: Lamiaceae (*Mentha piperita*, *Origanum vulgare*, *Hyssopus officinalis*, *Thymus serpyllum*, *Salvia officinalis*, *Scutellaria galericulata* L., *Melissa officinalis*, *Leonurus quinquelobatus*, and *Lavandula angustifolia*); Rosaceae (*Potentilla alba* and *Sanguisorba officinalis*); Asteraceae (*Artemisia annua*, *Artemisia dracunculus*, and *Echinacea purpurea*); Boraginaceae (*Symphytum officinale*); Umbelliferae (*Levisticum officinale*); Polygonaceae (*Rheum palmatum* L.); Valerianoideae (*Valeriana officinalis*); Rutaceae (*Rúta graveólens*); Scrophulariaceae (*Digitalis purpurea*); Saxifragaceae (*Bergenia crassifolia*); and Brassicaceae (*Brassica napus* L.).

In monoculture, all the medicinal plants suppressed the fungal growth to varying degrees compared with bare fallow, and the inhibition was being the most prominent during dry and hot seasons. A 2–7-fold reduction in soil

micromycete abundance was evident for the studied families, and the most significant reduction occurred under the families Lamiaceae, Rosaceae, and Asteraceae (for example, under *Echinacea purpurea*, the fungal primordia content decreased up to 10-fold in the soil). The dynamics of bacterial numbers and fungi during long-term storage showed characteristics of an initial phase of adaptation or decline, followed by a long-term stationary phase (LTSP). Here, population sizes reduce slowly over months or years, driven by resource exhaustion and intense competition. Upon entering storage, bacterial populations and fungi often experience a rapid decrease in numbers as they adjust to the absence of new nutrients and the accumulation of metabolic waste (Figures 1 and 2). Among the same plant family members, significant variations emerged for Lamiaceae (the least effect was notable for *Origanum vulgare*, while the utmost effect was noticeable for *Thymus serpyllum* and *Hyssopus officinalis*, which reduced the fungal profusion up to 20-fold in the soil). Previous studies also revealed growing medicinal plants in monoculture, unlike natural plant associations, led to increased soil phytotoxicity (Shirinova *et al.*, 2025; Rzaeva *et al.*, 2026).

Using the bioassay evaluation method, the most informative indicator of soil phytotoxic properties was the inhibition of root growth of the test plant seedling. Under the medicinal plants, the soil phytotoxicity increased by 1.5–5 times. However, the study observed it was most significant under plants of families Lamiaceae, Rosaceae, and Asteraceae (for *Sanguisorba officinalis*, the soil phytotoxicity was 32%; for *Scutellaria galericulata* L., 26%; and for *Echinacea purpurea*, 25%), which corresponds to the average level of soil phytotoxicity. Assessing the paradoxical contrary relationship between the indicators of soil biological activity (a decrease in the number of fungi and an increase in phytotoxicity), we placed several hypotheses: a direct influence of plant root exudates, a change in soil properties, and an interruption in the composition and structure of the fungal complex (Bakhshaliyeva *et al.*, 2019; Shirinova *et al.*, 2025).

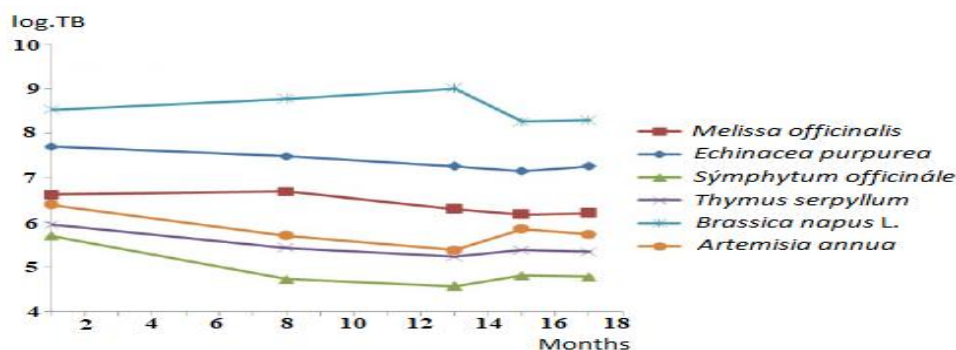


Figure 1. Dynamics of variations during long-term storage: Number of bacteria. Note: Log. - Logarithm; TB - Total bacteria number.

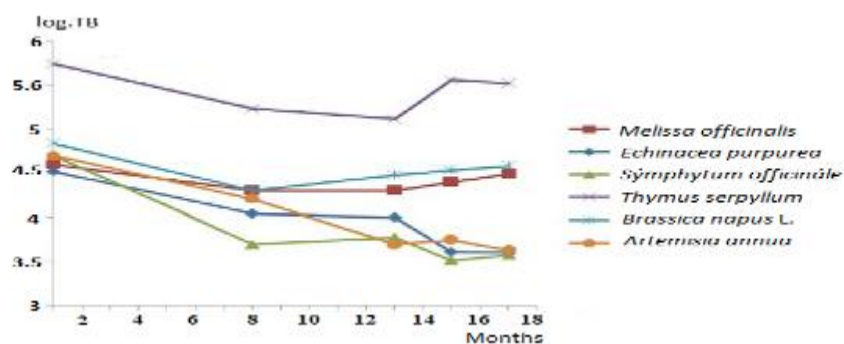


Figure 2. Dynamics of variations during long-term storage: Number of fungi. Note: Log. - Logarithm; TB - Total bacteria number.

For the first time, the study discovered that monocultures of the studied medicinal plants caused soil alkalization to varying degrees over three years. This effect may be due to the prevalence of biologically active phenolic compounds in plants' root exudates. In aqueous solution, these compounds dissociate to form phenyl anions, which, through anion exchange reactions, dislodge hydroxyl ions (OH^-) from the soil-absorbing complex. Soils with different types of medicinal plants commonly existed to contain not only typical leached fungal species of natural Kastnozems but also the species uncharacteristic for this soil: *A. ustus*, *A. clavatus*, *A. ochraceus*, *Penicillium funiculosum* Thom., *P. viridicatum*, *P. purpurogenum*, *P. velutinum*, *P. notatum*, *Gliocladium roseum*, *Fusarium nivale*, *F. oxysporum*, *Alternaria alternata* Ness., and

Talaromyces flavus (Bunyatova *et al.*, 2025; Verdiyeva *et al.*, 2025).

These fungal species are popular in producing mycotoxins with various chemical natures. The potential for mycotoxin contamination from the soil poses a possible risk of mycotoxin accumulation in medicinal plants grown as perennial crops in the same field. Thus, in the soil, the number of micromycetes under medicinal plants lessens. A non-specific fungicidal effect of root exudates of medicinal plants through alkalization of the soil solution succeeded in its discovery for the first time; however, no association was evident between the degree of pH change and the decrease in micromycete numbers (Akhundova *et al.*, 2025).

Furthermore, the reason for the increased phytotoxic activity of the soil while the

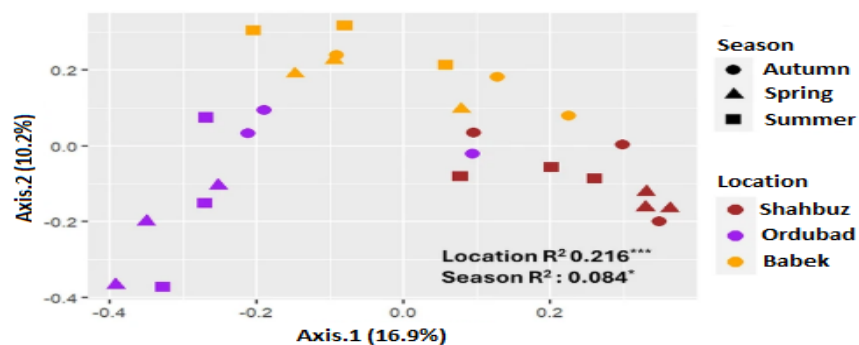


Figure 3. Study of spatiotemporal dynamics of soil and root microbiota in Nakhchivan biotopes: β -diversity analyses for bacterial communities across locations and seasons. Note: β -diversity is shown as Principal coordinate analysis (PCoA) based on dissimilarity measures. Significance test *** $P < 0.001$; ** $P < 0.01$; and * $P < 0.05$.

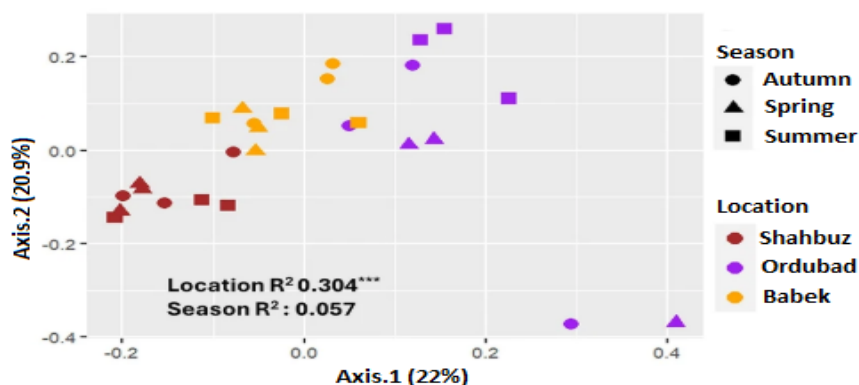


Figure 4. Study of spatiotemporal dynamics of soil and root microbiota in Nakhchivan biotopes: β -diversity analyses for fungal communities across locations and seasons. Note: β -diversity is shown as Principal coordinate analysis (PCoA) based on dissimilarity measures. Significance test *** $P < 0.001$; ** $P < 0.01$; and * $P < 0.05$.

inhibition of fungal growth happens is unclear. In soil, the discovery of toxigenic fungal species under perennial herbs, which are atypical for natural ecosystems, poses a potential risk of mycotoxin accumulation in medicinal plants. Therefore, growing medicinal plants in the same location for more than three years is unrecommendable. Special crop rotations are necessary. Essential oils obtained from medicinal plants have found wide application in medicines, while using distillation waters (collected in the apparatus' receiver after the separation of essential oil) was not typical. However, if detecting pharmacological activity, the distillation waters could be applicable, in

accordance with the principles of rational use of plant materials (Verdiyeva et al., 2025). Geographical variation between different biotopes is often a stronger driver of bacterial diversity than season, with specific edaphic factors, such as soil pH, nutrient availability, and salinity, dictating community structure. A study of spatiotemporal dynamics of soil and root microbiota in Nakhchivan biotopes comprised β -diversity analyses for fungal and bacterial communities across locations and seasons. Note: β -diversity appears as the principal coordinate analysis (PCoA) based on dissimilarity measures (Figures 3 and 4).

Table 1. Antimicrobial activity of essential oils and distilled waters from raw materials of studied plants in relation to the test culture.

Plants	Diameter of bactericidal (1) and bacteriostatic (2) zone (cm)			
	Essential oils		Distillation waters	
	1	2	1	2
Test culture of <i>Staphylococcus aureus</i>				
<i>Origanum vulgare</i>	1.3±0.25	1.7±0.12	1.3±0.18	1.9±0.15
<i>Mentha piperita</i>	1.5±0.38	1.8±0.15	0.9±0.19	0
<i>Lavandula angustifolia</i>	1.9±0.19	3.7±0.19*	0.8±0.18	1.5±0.30
<i>Melissa officinalis</i>	3.0±0.38*	3.3±0.26*	5.1±0.20*	6.1±0.28*
<i>Echinacea purpurea</i>	4.1±0.38*	4.5±0.33*	0.9±0.17	1.9±0.24
<i>Potentilla alba</i>	1.9±0.17	2.1±0.19	1.0±0.15	1.7±0.19
<i>Symphytum officinale</i>	4.5±0.19*	5.4±0.24*	0	0.7±0.12
<i>Thymus serpyllum</i>	5.2±0.23*	5.9±0.26*	1.1±0.21	2.2±0.19
<i>Artemisia annua</i>	1.7±0.13	6.0±0.14*	0	0
<i>Brassica napus</i> L.	3.6±0.23*	4.6±0.22*	1.6±0.22	2.6±0.17*
Test culture of <i>Pseudomonas aeruginosa</i>				
<i>Origanum vulgare</i>	0.7±0.15	0	0	0.7±0.14
<i>Mentha piperita</i>	0.9±0.19	0	0.7±0.23	1.0±0.16
<i>Lavandula angustifolia</i>	0	0	0	0.8±0.25
<i>Melissa officinalis</i>	2.9±0.20*	3.9±0.20*	0.9±0.20	1.1±0.25
<i>Echinacea purpurea</i>	1.3±0.22*	0	0	1.0±0.14
<i>Potentilla alba</i>	0	0	0.8±0.13	1.5±0.19*
<i>Symphytum officinale</i>	0.8±0.17	0.8±0.19	1.0±0.20	0
<i>Thymus serpyllum</i>	1.8±0.13*	0	1.7±0.17	2.1±0.16*
<i>Artemisia annua</i>	0.7±0.13	0	0	0
<i>Brassica napus</i> L.	3.7±0.23*	7.2±0.15*	1.3±0.19	3.4±0.19*

Note: 1 – zone of bactericidal action; 2 – zone of bacteriostatic action; * – difference from the antimicrobial activity of *Origanum vulgare* biologically active substances is reliable. Source: by author's (2023–2025 years)

For this purpose, a study continued on the antimicrobial effect of distillation waters (Table 1). In the experiment, distillation waters from the medicinal herb *O. vulgare* exhibited bactericidal activity against *Staphylococcus aureus* (1.3 ± 0.18 cm) and bacteriostatic activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (1.9 ± 0.15 and 0.7 ± 0.14 cm, respectively). The distillation waters of the studied species with antimicrobial activity against *P. aeruginosa* were less manifested. However, the bactericidal effect was notable in the distilled waters of six raw material samples, having the highest effect found for distilled waters of the species *Thymus serpyllum* (1.7 ± 0.17 cm).

The inoculations' incubation took 18–20 h at 36 °C. After incubation, zones of complete (bactericidal action) and partial (bacteriostatic action) growth inhibition, shaped like circles,

formed around the disks against a uniform bacterial lawn. The results recorded included measuring the diameter of the bactericidal and bacteriostatic zones. "Bactericidal" and "bacteriostatic" zones refer to distinct areas of inhibition observed in antimicrobial susceptibility tests, representing different actions of antibacterial agents on bacterial growth. The antimicrobial properties of aqueous extracts received assessment by dilution in nutrient broth at concentrations ranging from 3 to 24 mg/ml. Bacterial cultures sustained incubation at 36 °C for 24 h. Confirming the absence of microbial growth ensued from succeeding control cultures on nutrient agar. The minimum inhibitory concentration entailed a definition as the extract dose that completely inhibited the growth of the test microorganisms (Nazim and Oqtay, 2024; Mammadova *et al.*, 2025; Shirinova *et al.*, 2025).

CONCLUSIONS

No information is available on the biology and seasonal development of fungi on essential oils and medicinal plants, which complicates the solution to the problem of plant protection and obtaining high-quality harvests in industrial crops. The PCoA analysis based on distance to the rhizosphere bacterial communities of the studied *Allium* plant species showed an obvious tendency to cluster by sample type. It had two principal components explaining 45.6% and 4.4% of the total variation for bacteria and 14.9% and 7.2% of the total variation for fungi, respectively.

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