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RESEARCH ARTICLE

Species Composition and Ecological Roles of Mycobiota in Selected Medicinal Plants of Azerbaijan

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Abstract: About 30 species of medicinal and aromatic plants included in the flora of Azerbaijan have been studied for their mycobiota and the phytotoxic activity of the fungal species involved in their formation. It became clear that 79 fungal species were involved in the formation of the mycobiota of the studied plants, and they, in addition to participating in the formation of the mycobiota of the studied plants in various combinations, also differ from each other in terms of plant-fungal relationships and phytotoxic activity. So, this varies depending on the plant; for example, while one species participates in the formation of the mycobiota of some plants (*Mentha piperita*), in another (Trifolium pratense), it constitutes 17 species. Of the recorded fungi, 18 were identified as biotrophs, 26 as hemibiotrophs, 16 as necrotrophs, 10 as endophytes, and the remaining 9 species were of unknown status. Thus, unlike fungi from the genus *Alternaria* (A.alternata) and *Fusarium* (F.gibbosum, F. oxysporium, F.solani and F.verticillioides), which have the highest phytotoxic activity (reduction in the germination capacity of plant seeds to 40.5-60.0%), this phytotoxic activity is not generally observed in fungi from the genus *Trichoderma* (Trichoderma asperellum, T.citrinoviride, T.hamatum, T.harzianum, T.koningii and T.viridii), and some of them, especially those identified by molecular-genetic methods as Trichoderma citrinoviride AEF-2024 and T. harzianum AEF-2024, show significant stimulation. In this case, the germination capacity of plant seeds increases to 2.5-13.6%.

Key words: Medicinal and Aromatic Plants**,** Fungal Biota, Phytotoxic Activity, Stimulation

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Introduction

As it is known, the autotrophic nutrition of plants has determined that they play an important role in the life of other living things, including microorganisms. It should be noted that the role of plants is irreplaceable in meeting the needs of all living things, including plants themselves, for oxygen and primary organic matter [1]. Therefore, the study of plants in various aspects has long been one of the tasks in the focus of attention of both theorists and practitioners, and it retains its relevance even today.

The fact that almost all plants are important as bioresources and characterized by a wide variety has also led to their systematization in different forms, and currently, both natural (phylogenetic) and artificial systems are used in the systematization of plants. One of the indicators used in the grouping of plants is compounds with pharmacological activity. Currently, the number of medicinal and aromatic plant species corresponding to this characteristic in the world flora is around 50000-80000 [2], of which about 1547 species [3] are distributed in the territory of the Republic of Azerbaijan.

Although these plants have been studied to some extent in various aspects, including botanical and mycological [3-5] aspects, not enough attention has been paid to the research on how the colonization of fungi on plants affects the biological activity of these fungi. Conducting these studies is even more important in the current conditions of global problems. So, since problems such as global climate change, desertification, loss of biodiversity, and others cover all areas, the changes caused by them first manifest themselves in living things and their biological activity. In this regard, it is worth noting only one fact that climate is considered the main factor in the distribution of plants and related organisms on Earth, and currently, one of the main directions of bioecological research, more precisely, the most important, is dedicated to the study of the impact of climate-related changes on numerous ecosystems [6]. In general, it should be noted that climate change has multiple effects on both the ecosystem and the communities and living organisms that inhabit it, and in the most pessimistic and most optimistic scenarios predicted in this regard, it is noted that the main negative impact of climate change will manifest itself in relation to biodiversity. In addition, the result of climate change will be observed differently at the local and global scales, which will make it necessary to approach the problems observed at those levels in the same way.

As is known, various species of pathogens, including bacteria, fungi, and viruses, cause diseases [7]. In plants, it is extremely important to correctly diagnose these diseases on site and in time, as well as predict the damage they can cause. The key to addressing these issues is to clearly understand how diseases caused by various pathogens interact and respond to biotic and abiotic factors. It is theoretically known that climate change will lead to changes in the interactions between pathogens and their hosts, which will also inevitably lead to the emergence of new ways of spreading diseases. In this regard, the assessment of plants according to their fungal biota in a given region, the prediction of possible changes in plant-fungus relationships, and the clarification of the role of individual groups, primarily endophytes, in this process are of great importance [8, 9]. Thus, the Republic of Azerbaijan has rich flora and fauna, soil and climatic conditions (http://www.eco.gov.az), which have created conditions for the formation of ecologically diverse biotopes in a relatively small area. Under such conditions, clarifying plant-fungal relationships, primarily those related to endophytes, may be relevant in terms of developing scientific and practical foundations for using endophytes to increase plant productivity, as well as their use as bioherbicides [10]. Because the adaptation of plants to the beneficial metabolites synthesized by endophytes, i.e., the "secret concubine" [11], is the primary guarantee for the effectiveness of the process. Therefore, there is a need for research aimed at clarifying these issues.

Taking this into account, the aim of the presented study was to investigate the species composition of fungi inhabiting essential oil plants, the nature of the pathologies they cause, and the effect of differences in ecological conditions on their phytotoxic activity.

Materials and Methods

General Characteristics of the Research Areas and Sampled Plants

The research was conducted in the Kur-Araz lowland in the Greater and Lesser Caucasus, as well as in the territories of the Lankaran-Astara zone of the Republic of Azerbaijan. The mentioned areas are characterized by both similar and different indicators due to their natural soil-climatic conditions, flora, and fauna. This diversity is also observed within the borders of economic regions located within the same geomorphological unit. For example, the Lankaran-Astara zone is the region of the country with the highest rainfall (1200-1800 mm per year), while the Kur-Araz lowland is the region with the lowest (200-400 mm). Most of the country's irrigated land is located in the Kur-Araz lowland. The Absheron part of the Greater Caucasus has a dry climate, while the Lankaran-Astara zone has a humid subtropical climate. The Greater Caucasus ranges from -28 m below sea level (Absheron Peninsula) to 4466 m (Bazarduzu). All this determines the formation of a specific ecological environment in a particular region.

Samples for the study were taken from the above-ground organs of medicinal plants that are distributed in the mentioned areas and are included in the natural and cultural flora of the Republic of Azerbaijan, and are likely to be fungi. Sampling was carried out according to the planned route method and was carried out from plants such as Albizzia julibrissin Durazz., Astrodaucus orientalis (L.) Drude., Betula pendula Roth., Calendula officinalis L., Coriandrum L., Crataegus pentagyna L. Cucurbito pero L., Foeniculum vulqare Mill., Daucus carota L., Eupatorium cannabinum L., Fraxinus excelsior L., Juniperus communis L., Lamium album L. Laurus nobilis L., Leonurus cardiaca L., Melissa oficinalis L., Mentha piperita L., Myrtus communis L., Ocimum basilicum L., Pimpinella anisum L., Pinus sylvestris L. Polygonum hydropiper L., Rosa gallica L., Rosmarinus officinalis L. Sambulus nigra L. Senecio vulgaris L., Tanacetum vulqare L., Thea sinensis L., Trifolium pratense L., Viola tricolor L. et al. During the research, at least 10 samples were taken from each plant species. The selection of these plants is due to the fact that most of them grow in all of the research areas, and their reserves are sufficient. On the other hand, they are all medicinally important and contain biologically active substances with various effects, including essential oils in varying amounts, and are widely used in folk medicine. In this regard, the assessment of the mycobiota of plants by species composition is important for the development of principles of mycological safety of their use, as well as for the development of scientific and practical foundations for the use of fungi adapted to live in them as BAS producers.

Isolation of Fungi from Samples and Determination of Species Composition

Isolation of pure cultures of fungi from the samples and determination of their species composition were carried out on agarized nutrient media (Agarized malt juice, Saburo agar, etc.) according to classical mycological methods. For this purpose, the samples taken were transferred to the nutrient medium either directly or after washing with sterile water. The inoculated nutrient medium was kept in a thermostat at a temperature of 280 °C for 3-5 days and transferred to a new nutrient medium based on the visual differences of the colonies formed, and the process was continued until a pure culture was obtained [12, 13]. The purity of the cultures obtained was monitored using a microscope (OMAX 40X-2500X LED Digital Lab Trinocular Compound Microscope with USB Camera) with a magnification of up to 2500 times. The species composition of the obtained pure cultures was determined based on the macroscopic and microscopic characteristics of the colonies they formed, based on known determinants and atlases [14-16].

During the molecular genetic identification of fungi belonging to the genus Trichoderma, a method based on determining the sequence of the ITS gene and comparing it with the corresponding reference strains [17, 18] in GenBank [19] was used.

When grouping the pathologies caused by fungi recorded in the studies according to their manifestation, the methods and approaches used by various authors [20, 21], as well as our previous studies [5, 22, 23], were used.

Determination of Phytotoxic Activity of Fungi

During the research, the phytotoxic activity of individual fungal species isolated from plants was evaluated for their effect on the germination of seeds of plants such as tomato, melon, and peas, using the following approach, which was applied in our previous works [3, 24]. According to the mentioned approach, a specific number (100-150) of seeds of each plant were used, and for the determination of phytotoxic activity, the Culture Solution (CS) was separated from the biomass formed by the fungi after cultivation in liquid Chapek medium for 5 days, and filtration was used. The plant seeds used were soaked in the same CS for 24 hours. The control variant used sterile Chapek medium. The seeds were then placed on moistened filter paper and allowed to germinate at room temperature (20-220C) for 7 days. The phytotoxic activity of the fungi (in %) was also determined according to the formula $P = (n/N) \times 100$, where P is the phytotoxic activity, n is the number of non-germinating seeds, and N is the total number of seeds collected.

Statistical Processing of the Obtained Results

During the research, all experiments were performed in at least 4 replicates, and all quantitative data were statistically processed [25], and the results that met the formula $\sigma/X0 \leq 0.05$ were used in the article. In this formula, σ -is the mean square deviation, and X0 is the average value of the replicates.

Results

Determination of the Species Composition of the Mycobiota of the Sampled Plants

In the course of the research, it was clear from the results of the analysis of about 200 samples taken from the medicinal plants spread in the research areas according to the fungal biota that, despite the presence of phytocomponents with antimicrobial activity, the studied plants are also suitable for the spread of fungi and the continuation of their life activities. Thus, in studies conducted in 2020-2024, it was determined that 79 species participate in the formation of fungal biota of about 30 species of plants growing in different areas and differing in their life forms (grasses, shrubs, and trees), the quantitative characteristics of the data about them are given in Table 1. As can be seen, 52 (Alternaria alternata, A. chrysanthemi). A.radicuina, A.solani, Ascochyta betae, Asc. iridis, Asc. leonuri, Asc. foeniclina, Asc. vulgaris, Aspergillus flavus, A.niger, A.ustus, Aureobasidium pullulans, Botrytis cinerea, Cercospora anethi, Cladosporium cladosporioides, C.herbarum, C.transchelii, Colletotrichum circinans, C.ocmi, C.panicola, C.valerianae, Fusarium dimerum, F.gibbosum, F.oxysporium, F.solani, F.verticillioides, Gliocladium roseum, Penicillium chrysogenum, P.citrinum, P.cyclopium, P.janthinellum, P.notatum, Phoma anethi, Ph.betae, Ph.capsisi, Ph.dauci, Ph.medicaginis, Septoria carotae, S.menthae, S. salviae-pratensis, S.sojae, Thielaviopsis basicola, Trichoderma asperellum, T.citrinoviride, T.hamatum, T.harzianum, T. koningii, T.viridii, Trichotectum roseum, Verticillium alboartum, V.dahile and V.lateritium) species of recorded fungi belong to anamorphs of sac fungi, 9 (Chaetomium globosum, Erysiphe cichoracearum, E.communis, E.umbelliferarum, E.trifolii, Monilia fructigena, Sclerotina graminearum, S.sclerotiorum and Sphaerotheca pannosa) species to telemorphs, 7 (Absidia ramose, Actinomucor elegans, Mucor hiemalis, M.mucedo. M.plumbeus, M.rasemosus and Rhizopus stolonifera) species to Mucoromyceta and 11 (Aecidium foeniculi, Puccinia artemisiae, P.iridis, P.matricariae, P.mentha, Rhizoctonia solani, Tuphula trifolii, Uromyces striatus and Ustilaga zeae) species to Bazidiomycota. In addition, the distribution of the specific fungi species was also recorded in grass, bushes, and trees, such as Alternaria alternata, Monilia fructigena, Rhizoctonia solani, and Sclerotinia sclerotiorum.

Table 1: Characterization of fungi recorded on plants in studies according to their taxonomic structure

Life forms of the plants sampled	Dikarya		Mucoromyceta	Total
	Ascomycota	Bazidiomycota		
Grass	41	1	4	46
Bush	29	3	3	35
Tree	14	10	2	26
Total	61	11	7	79

Recorded fungi are characterized by diversity both according to their ecotrophic relationships (saprotroph, endophyte, and hemibiotroph), their distribution on individual plants, and combinations of participation in the formation of the mycobiota of a specific plant.

The registered fungi are diverse in terms of the nature of the pathologies they cause; thus, 18 species of registered fungi are biotrophs, 26 are hemibiotrophs, 16 are necrotrophs, and 10 are endophytes. These groups of fungi have different ecological functions, and hemibiotrophs are considered more dangerous due to the damage they cause to plants [26, 27]. Thus, members of this group can more easily meet their nutritional needs than others, which means that they adapt to the environment more easily than others. The remaining 9 species (Absidia ramose, Actinomucor elegans, Mucor hiemalis, M. mucedo, M. plumbeus, M.rasemosus, Penicillium janthinellum, P. notatum, and Verticillium lateritium) belong to any of the mentioned groups, but this is not confirmed by either our studies or literature data, i.e., the available information is insufficient to classify them into any of the mentioned groups. Thus, neither our studies nor the analysis of literature data confirmed that these fungi caused any pathology in the plants studied. Although these fungi include entomopathogens such as M.hiemalis [28], as well as species such as Penicillium janthinellum, which has attracted attention as an active producer of secondary metabolites [29]. For this reason, it is important to mark the status of those fungi as unknown and to comprehensively study them in this direction in future studies to clarify the issue.

Phytotoxic Activity of Fungi Involved in the Formation of the Mycobiota of the Studied Plants

At the next stage of research, the same fungi species found on the same plant under different environmental conditions, or the same fungi species distributed on different plants under different environmental conditions, were evaluated for phytotoxic activity. As mentioned, this is based on the influence of the culture solution, i.e. exogenous metabolites, on the germination of plant seeds (tomato, melons, peas etc.) (within 5-7 days) (Table 2) and on the growth of stems (vegetative organs according to their morphometric measurements - for a period of 40 days) (Fig. 1) after cultivating the fungi in liquid Czapek medium for 5 days. From the results of the research conducted in this regard, it became clear that 15 of the 25 evaluated fungi species had phytotoxic activity to one degree.

A B

Fig. 1: General view of the growth of seedlings obtained from the same number of tomato plant seeds, but prepared in different ways

A - From seeds sown according to the currently used approach (control)

B - From seeds sown additionally soaked for 14 hours with means from the fungus T. harzianum AEF-2024 (B)

Table 2: Phytotoxic activity of some fungi recorded in studies

Degree (%) of phytotoxic activity (FTA)	Number of plant seeds used, pcs.	FTA (according to the number of non-germinating seeds, %)			Tested Fungal Strains
		Tomato	Melon	Peas	
Weak (FTA≤10)	400(4x100)	3,5-8,6	4,3-9,1	2,1-7,4	Cladosporium cladosporioides, Mucor hiemalis, M.mucedo. Rhizopus stolonifera
Average (10>FTA<40)		14,5-37,6	16,4-38,1	12,3-34,5	Aspergillus flavus, Botrytis cinerea, Cladosporium herbarum Penicillium chrysogenum, P.cyclopium, Verticillium alboartum, V.dahile
Strong (FTA>40)		42,1-59,0	45,4-60,0	40,5-53,6	Alternaria alternata, F. oxysporium, F.solani, F. verticillioides
Stimulation activity		2,5-13,5	2,9-14,0	3,2-13,6	Gliocladium roseum, Trichoderma citrinoviride, T.harzianum,T. koningii
Those do not show FTA		0	0	0	Actinomucor elegans, Aureobasidium pullulans, Chaetomium globosum, Cladosporium transchelii, Mucor plumbeus, M.rasemosus

Another, including 3 (F. oxysporium, F. solani and F. verticillioides) species belonging to the Fusarium genus differed from the others, i.e., due to their influence, the ability of seeds to germinate decreases by 40-60%, and the growth of stems decreases by 40-47%. In the remaining 12 species of fungi, similar indicators respectively varied between 2,1-38,1% and 3-25%. Of the 25 fungal species, 7 had no phytotoxic activity, that is, neither negative nor positive effects were observed from their action. Three species of the genus Trichoderma (Trichoderma citrinoviride, T.hamatum, T. harzianum, and T.koningii) and one species of the genus Gliocladium (G. roseum) had a stimulating effect on both the germination of plant seeds and the growth of fruit and berry seedlings. As a result, seed germination increased by 7-14%, and seedling growth by 5-12%. This increase was maximally observed in the fungi Trichoderma citrinoviride and T. harzianum, the species affiliation of which was also determined by molecular genetics. Both of those identified by classical mycological methods as Trichoderma citrinoviride and T.harzianum were confirmed by molecular genetic methods, more precisely, the nucleotide sequence of their ITS gene matches the sequence of the corresponding species in GenBank (tab. 3). The most active types of exogenous metabolites causing phytostimulation were named as Trichoderma citrinoviride AEF-2024 and T. harzianum AEF-2024. In the future, they were considered promising for the purchase of biopreparations that stimulate the growth of seedlings of agricultural plants, including fruits and berries.

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Discussion

Because plants play an important role in the lives of living things and have an irreplaceable role in providing aerobic organisms with oxygen, they have always been and continue to be the subject of research in one aspect or another. It can be safely stated that this will continue in the future. Thus, plants, which synthesize organic matter for themselves and other living things, also have various relationships with other living things. Fungi are another group of organisms that plants are related to in various aspects [30]. The interactions of plants with fungi, especially their phytopathogenic species, have always been the focus of researchers' attention. [31, 32]. Although various organisms cause diseases in plants, fungi stand out from other disease-causing organisms both in the number of pathologies they cause and the extent of damage they cause [33]. On the other side, the vast majority of fungi are mesophiles in their relationship to temperature, and unlike bacteria, there are no true thermophiles among them. For this reason, how global climate change affects fungi, including phytopathogens, is of interest from both scientific and practical perspectives. In order to clarify these, a number of issues must be clarified, primarily the species composition of pathogenic fungi, their development cycle, their relationship to the substrate, their resistance to the effects of abiotic environmental factors, their interactions with other organisms in the environment they live in, and so on. Although fungi are characterized by low numbers in ecosystems compared to bacteria, the ecological functions they perform in various ecosystems are extremely broad and fundamental [34]. The fact that environmental conditions play a role in the observation of this or that characteristic of mushrooms has been reflected in various studies [35] conducted at various scientific centers, but the essence of this issue has not been fully clarified. In our studies, we do not find clearly expressed specific signs arising from differences in ecological conditions in the species composition of fungi, the pathologies they cause, and the

Table 3: Determining the species composition of active producers based on nucleotide sequence

Fragment sequence of İTS	BLAST		
	Accession number	**Species name**	**Similarity, %**
CTCCGTTGGTGAACCAGCGGAgGGATCATTACCGAGTTTACAACTCCCAA ACCCAATGTGAACGTTACCAATCTGTTGCCTCGGCGGGATTCTCTGCCCC GGGCGCGTCGCAGCCCCGGATCCCATGGCGCCCGCCGGAGGACCAACT CAAACTCTTTTTTTCTCTCCGTCGCGGCCTACGTCGCGGCTCTGTTTTATT TTTGCTCTGAGCCTTTCTCGGCGACCCTAGCGGGCGTCTCGAAAATGAAT CAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA GCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC TTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGA GCGTCATTTCAACCCTCGAACCCCTCCGGGGGGTCGGCGTTGGGGATCG GCCCCTCACCGGGCCGCCCCCGAAATACAGTGGCGGTCTCGCCGCAGC CTCTCCTGCGCAGTAGTTTGCACACTCGCACCGGGAGCGCGGCGCGGCC ACAGCCGTAAAACACCCCAAACTCTGAAATGTGACCT	KY764860.1	Trichoderma citrinoviride	99.83
TTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCA ATGTGAACGTTACCAAACTGTTGCCTCGGCGGGATCTCTGCCCCGGGTGC GTCGCAGCCCCGGACCAAGGCGCCCGCCGGAGGACCAACCAAAACTCTT ATTGTATACCCCCTCGCGGGTTTTTTTATAATCTGAGCCTTCTCGGCGCCT CTCGTAGGCGTTTCGAAAATGAATCAAAACTTTCAACAACGGATCTCTTGG TTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTG CAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGT ATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTC CGGGGGGTCGGCGTTGGGGATCGGCCCTCCCTtAGCGGGGTGGCCGTCT CCGAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTG CACACTCGCATCGGGAGCGCGGCGCGTCCACAGCCGTTAAACACCCAAC TTCTGAAATGTTGACCTCGGATCAGGTAGGAATACCCGCT	MH865865.1	T.harzianum	99.66

Phytotoxic activity of exogenous metabolites, but certain differences are observed both in the combination of fungi participating in the formation of the mycobiota of plants distributed in different zones, and in the quantitative indicators of their phytotoxic activity. Research conducted in various scientific centers in this direction has not clearly clarified the essence of the problem mentioned [36]. Considering that the number of species currently known to science is around 150,000, and the actual number of species in nature is estimated to be up to 12 million [37], it can be noted that the issue is an open and relevant problem for ongoing research. This idea alone clearly confirms the need to continue research in this direction.

On the other hand, a complete clarification of all forms of plant-fungal relationships and a complete understanding of their mechanisms are important issues in terms of expanding their use (primarily endophytes) for practical purposes.

Finally, it would be appropriate to touch on one issue, which is related to the future uses of the fungi Trichoderma citrinoviride AEF-2024 and T. harzianum AEF-202. As mentioned, these fungi can be used both as stimulants and biofungicides in the future, and such cases are encountered in world practice [38]. However, neither the production area based on the use of these fungi nor the cultivation technologies based on the use of these fungi are currently used in the Republic of Azerbaijan. If we take into account that this or that microorganism, including fungi, that is distributed in specific ecological conditions can perform [39, 40], its vital functions more effectively in those conditions, then the practical value of these fungi will increase somewhat and they will play an important role in the development of the agricultural sector in the country.

Conclusion

Thus, from the conducted studies became clear that fungi also participate in the formation of the biota of medicinal and aromatic plants included in the cultural and natural flora of Azerbaijan, but the species participating in the formation of the fungal biota differ from each other both in the nature of the fungal-plant relationship and in the phytotoxic activity of their exogenous metabolites. The studies conducted determined that 79 fungal species involved in the formation of the mycobiota of the studied plants differ from each other in terms of their plant-fungus relationships. Thus, 18 of the recorded fungi were biotrophs, 26 were hemibiotrophs, 17 were necrotrophs, and 10 were endophytes. Among these fungi, which perform various functions in the life of plants, hemibiotrophs are considered more dangerous from a practical point of view. The presence of species with strong phytotoxic activity, such as A.alternata, F. gibbosum, F. oxysporium, F. solani, and F. verticillioides, among these fungi is also a negative aspect. Nevertheless, the presence of endophytes among the recorded fungi can be considered a positive aspect of plant-fungus relationships. Because these fungi, especially those belonging to the genus Trichoderma, synthesize secondary metabolites with a diverse spectrum, which will allow them to obtain biopreparations that increase plant productivity and improve the phytosanitary condition of agrocenoses in the future. The fact that species of unknown status are also found among the recorded fungi suggests that it is appropriate to continue studying these fungi.

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Authors Contributions

Panah Z. Muradov: Provided strategic management and overall leadership of the study and ensured the scientific integrity ofthe study.

Konul F. Bakhshaliyeva and .Vafa Y. Hasanova: Conceptualization, methodology, visualization, formal analysis, experimental design, and writing, both in the original version and in reviewing and editing.

Nizami R. Namazov and Tarana S. Agayeva: Helped in data processing and formal analysis, ensuring consistency and reliability of experimental.

Sevda M. Muradova and Saadat F. Azadaliyeva: Writing, reviewing and editing and participation in the development ofthe final version and interpretation of the results.

Arzu H. Hasanova and Rafiga Z. Nagıyeva: Participated in the preparation of the laboratory experiment and contributed to its practical implementation.

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Ethics

The information in this study is to be found in the manuscript tables and figures. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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