BIOLOGICAL SCIENCES

COMPARATIVE ASSESSMENT OF THE PHYTOTOXIC AND ZOOTOXIC ACTIVITIES OF ENDOGENOUS AND EXOGENOUS METABOLITES PRODUCED BY FUNGI ISOLATED FROM DIFFERENT ECOSYSTEMS OF AZERBAIJAN

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Abstract

This study evaluated the phytotoxic activity of endogenous and exogenous metabolites produced by fungi isolated from different ecosystems in Azerbaijan. A total of 46 fungal species belonging to the phyla Ascomycota, Basidiomycota, and Mucormycota were investigated to determine the toxicity of their metabolites. The results revealed considerable variation in phytotoxic activity among the studied fungi. Of all species examined, 26.7% exhibited strong phytotoxic activity, 56.6% moderate activity, 10.0% weak activity, while 6.7% showed no detectable phytotoxic effect. All fungi with strong phytotoxic activity belonged to the phylum Ascomycota. In contrast, none of the Basidiomycota species demonstrated either strong or moderate phytotoxicity; 50% exhibited weak activity, whereas the remaining species showed no phytotoxic effects. All representatives of the phylum Mucormycota displayed weak phytotoxic activity. Comparative analysis further demonstrated that exogenous metabolites generally exhibited higher phytotoxicity than endogenous metabolites. The observed differences among taxonomic groups appear to reflect their ecological adaptations and competitive strategies in natural habitats. Xylotrophic macromycetes, which colonize relatively stable woody substrates with lower levels of biological competition, exhibited substantially lower phytotoxic activity than soil- and plant-associated micromycetes. These findings provide new insights into the ecological significance of fungal metabolites and demonstrate that phytotoxicity is closely associated with fungal taxonomy, ecological niche, and adaptive strategies.

Keywords: fungi, endogenous metabolites, exogenous metabolites, phytotoxic activity, ecological adaptation, Azerbaijan

Introduction

Fungi constitute one of the most diverse and ecologically important groups of organisms on Earth, playing indispensable roles in ecosystem functioning. As major components of the heterotrophic compartment of terrestrial and aquatic ecosystems, fungi contribute to the decomposition of organic matter, nutrient cycling, soil formation, and the regulation of ecological processes. Although approximately 120,000 fungal species have been formally described, recent estimates suggest that their global diversity may reach 2.2–3.8 million species, highlighting the vast extent of undescribed fungal biodiversity[1]. In addition to their ecological significance, fungi synthesize a wide range of primary and secondary metabolites that influence interactions with other organisms and determine their adaptation to diverse environmental conditions (22-27).

Among fungal secondary metabolites, mycotoxins represent one of the most intensively studied groups because of their ecological and biological significance. These compounds enhance fungal competitiveness by inhibiting the growth of competing microorganisms and plants while also contributing to successful colonization of ecological niches. However, many mycotoxins are harmful to animals and humans due to their mutagenic, carcinogenic, hepatotoxic, nephrotoxic, and immunotoxic properties. Consequently, evaluating the toxic potential of fungal metabolites has become an important research area in environmental microbiology, mycology, agriculture, and food safety.

The Republic of Azerbaijan is characterized by diverse climatic zones and ecosystems that support a rich and insufficiently explored fungal diversity. Previous investigations have documented numerous toxigenic fungal species in soils, freshwater habitats, and plant-associated communities across different regions of the country[5–7]. Nevertheless, current knowledge remains limited regarding the comparative phytotoxic activity of endogenous and exogenous metabolites produced by fungi belonging to different taxonomic groups and ecological habitats. Moreover, the influence of ecological adaptation and habitat characteristics on fungal phytotoxicity has received relatively little attention.

Differences in environmental conditions, substrate availability, and biological competition are expected to affect both the qualitative and quantitative composition of fungal metabolites. Consequently, fungi occupying different ecological niches may exhibit distinct levels of phytotoxic activity, reflecting their adaptive strategies and ecological functions. Comparative investigations of metabolite toxicity among fungi from different habitats therefore provide valuable insights into fungal ecology and the biological significance of secondary metabolites.

The present study aimed to evaluate and compare the phytotoxic activity of endogenous and exogenous metabolites produced by fungi isolated from soil, water, and plant ecosystems of Azerbaijan and to determine how phytotoxicity varies among different taxonomic groups in relation to their ecological adaptation.

MATERIALS AND METHODS

Fungal isolation and identification

The study was conducted during 2024–2025 using fungal isolates obtained from soil, water, and plant samples collected in different regions of the Greater Caucasus, including the Guba–Khachmaz and Absheron Economic Regions of Azerbaijan. Fungal isolation, purification, species identification, and the assessment of their suitability for bioconversion were performed using standard microbiological, mycological, and biotechnological methods described previously (15–16, 42-44).

Preparation of endogenous and exogenous metabolites

The phytotoxic and zootoxic activities of fungal metabolites were evaluated using both exogenous and endogenous metabolites. Exogenous metabolites were represented by culture filtrates (CF) obtained after cultivating fungal isolates for five days in liquid Czapek or glucose–peptone medium. Endogenous metabolites were obtained from the fungal biomass (vegetative mycelium, VM).

Following cultivation, the fungal biomass was separated from the culture filtrate and washed several times with neutral phosphate buffer (pH 7.0). Subsequently, 50 mL of phosphate buffer was added to the biomass, after which the cells were disrupted using a tissue grinder. The homogenate was centrifuged three times at 5,000 rpm for 10 min, and the resulting supernatant was collected as the source of endogenous metabolites.

Evaluation of phytotoxic and zootoxic activity

Phytotoxic activity was assessed by determining the effects of fungal metabolites on the germination of wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.) seeds using established bioassay methods. Zootoxic activity was evaluated using the ciliate *Paramecium caudatum* Ehrenberg as the test organism following standard protocols reported in previous studies.

Each experiment was performed in at least four independent replicates. The obtained data were statistically analyzed, and the results are presented as mean values. Statistical significance was evaluated using appropriate statistical methods at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Approximately 500 soil, water, and plant samples were collected from different locations within the study area during 2024–2025. From these samples, 76 pure fungal isolates were obtained, including 60 micromycetes and 16 macromycetes. Based on their cultural and morphological characteristics, these isolates were identified as belonging to 46 fungal species. Of the identified species, 65.2% belonged to the phylum Ascomycota, 26.1% to Basidiomycota, and 8.7% to Mucoromycota.

The phytotoxic activity of fungal metabolites varied considerably among the investigated taxa. Strong phytotoxic activity, defined as more than 40% inhibition of seed germination, was observed exclusively among micromycetes. Moderate phytotoxic activity (20–40% inhibition) was also recorded primarily in species belonging to Ascomycota, whereas Basidiomycota species did not exhibit either strong or moderate phytotoxic effects. Weak phytotoxic activity (less than 20% inhibition) was detected in several representatives of Basidiomycota and all representatives of Mucoromycota.

Overall, 26.7% of the Ascomycota species exhibited strong phytotoxic activity, 56.6% moderate activity, 10.0% weak activity, and only 6.7% showed no detectable phytotoxic effect. In contrast, none of the Basidiomycota species demonstrated strong or moderate phytotoxicity; half of the species exhibited weak activity, whereas the remaining species showed no phytotoxic effects. All Mucoromycota species exhibited weak phytotoxic activity.

Interestingly, culture filtrates obtained from the xylotrophic fungi *Cerrena unicolor*, *Pleurotus ostreatus*, and *Laetiporus sulphureus* did not inhibit seed germination. Instead, they stimulated wheat seed germination by approximately 4–7% compared with the untreated control, suggesting the presence of metabolites with growth-promoting properties.

Comparison of endogenous and exogenous metabolites demonstrated that exogenous metabolites consistently exhibited higher phytotoxic activity. The number of fungal species showing strong phytotoxicity decreased from eight to six when endogenous metabolites were evaluated. Likewise, the overall phytotoxicity index decreased from 44.4–59.7% for exogenous metabolites to 40.4–54.4% for endogenous metabolites, indicating that extracellular metabolites contribute more substantially to fungal phytotoxicity. Similar findings have been reported for fungal secondary metabolites by Keller (18) and Vasilenko et al. (5).

Evaluation of zootoxic activity using *Paramecium caudatum* revealed a pattern similar to that observed for phytotoxicity. Exogenous metabolites from fungi exhibiting strong or moderate phytotoxicity reduced the viability of the test organism by approximately 1.2–2.0-fold relative to the control, whereas endogenous metabolites reduced viability by 1.05–1.65-fold. In contrast, metabolites produced by fungi with weak phytotoxic activity had little or no adverse effect on the survival of *P. caudatum*. Furthermore, metabolites produced by xylotrophic macromycetes frequently stimulated the growth of the protozoan rather than inhibiting it.

The observed differences in phytotoxic and zootoxic activities among fungal taxa are likely associated with their ecological adaptation and competitive strategies (6,18). Soil- and water-associated micromycetes inhabit environments characterized by intense competition for nutrients and space, favouring the production of biologically active secondary metabolites that enhance competitive ability (2,6). In contrast, xylotrophic macromycetes colonize relatively stable woody substrates where interspecific competition is generally less intense. Consequently, these fungi appear to invest less in the production of toxic metabolites, which explains their comparatively low phytotoxic activity.

The consistently higher toxicity of exogenous metabolites further supports the ecological role of extracellular secondary metabolites in mediating interactions between fungi and other organisms (5,6,18). These metabolites may suppress competing microorganisms and influence surrounding biota, whereas endogenous metabolites primarily participate in intracellular metabolic processes necessary for fungal growth and development. Similar ecological functions of fungal secondary metabolites have been widely reported in previous studies (5,6,18), supporting the findings of the present investigation.

CONCLUSION

This study demonstrated that endogenous and exogenous metabolites produced by fungi isolated from different ecosystems of Azerbaijan differ significantly in their phytotoxic and zootoxic activities. Exogenous metabolites consistently exhibited stronger biological activity than endogenous metabolites, indicating that extracellular secondary metabolites play a more important role in fungal interactions with other organisms.

The results also revealed that phytotoxic activity varied among fungal taxonomic groups. Species belonging to the phylum Ascomycota showed the highest phytotoxic activity, whereas Basidiomycota and Mucoromycota exhibited predominantly weak or no phytotoxic effects. In particular, xylotrophic macromycetes displayed substantially lower toxicity than micromycetes.

The observed differences are likely associated with ecological adaptation and habitat-specific competitive strategies. Fungi inhabiting soil and water environments experience stronger competition for resources, which may promote the production of biologically active secondary metabolites. In contrast, xylotrophic macromycetes colonize relatively stable woody substrates and therefore exhibit lower levels of phytotoxic activity.

Overall, the findings improve our understanding of the ecological functions of fungal metabolites and demonstrate that metabolite toxicity is closely related to fungal taxonomy, ecological niche, and adaptive strategies. These results provide a useful basis for future studies on fungal secondary metabolites and their potential applications in biotechnology, agriculture, and environmental management.

References:

1. Hawksworth, D.L. and Lücking, R., 2017. Fungal diversity revisited: 2.2 to 3.8 million species. Microbiol Spectr, 5:79–95

2. Frljak, J. Mulabecirovic, A., Ýsakovic, S. et al., 2021. Biological Active Components of Selected Medical Fungi. Open Journal of Preventive Medicine, 11:9-22.

3. Jain, A.. Sarsaiya, S, Wu, Q. et al., 2019. A review of plant leaf fungal diseases and its environment speciation. Bioengineered., 10(1):409-424.

4. Vasilenko, A., Ivanushkina, N., Kochkina, G., Ozerskaya, S., 2022. Fungi in Microbial Culture Collections and Their Metabolites. Diversity, 14, 507. https://doi.org/10.3390/ d14070507

5. Avalos, J. and Limón, M.C., 2022. Fungal Secondary Metabolism. Encyclopedia, 2(1):

6. Akbari, P., Braber, S., Varasteh, S. et al., 2017. The intestinal barrier as an emerging target in the toxicological assessment of mycotoxins. Arch Toxicol, 91:1007–1029.

7. Awuchi, C.G., Ondari, E.N., Ogbonna, C.U. et al., 2021. Mycotoxins Affecting Animals, Foods, Humans, and Plants: Types, Occurrence, Toxicities, Action Mechanisms, Prevention, and Detoxification Strategies-A Revisit. Foods, 3;10(6):1279. doi: 10.3390/foods10061279.

8. Logrieco, A., Mule, G., Moretti, A., Bottalico, A., 2002. Toxigenic Fusarium species and mycotoxins associated with maize ear rot in Europe // European Journal of Plant Pathology, 108:597–609.

9. Cheek, M., Nic Lughadha, E., Kirk, P. et al., 2020. New scientific discoveries: Plants and fungi. PLANT. People Planet. 2:371–388.

10. Netrusov, A.I., Egorova, L.M., Zakharchuk M.A. et al., 2005. Workshop on microbiology. - Moscow: Publishing Center “Academy”, 608.

11. Kochetov, A.G., Lang, O.V., Masenko, V.P. al., 2012. Methods of statistical processing of medical data: Guidelines for residents and graduate students of medical schools, scientists. - M .: RKNPK, 42.

12. Keller, N.P., 2019. Fungal secondary metabolism: regulation, function and drug discovery.// Nat Rev Microbiol., 17(3):167-180.

13. Jabrayilzade, S., & Aslanova, S. SOME DISEASES OF APPLE PLANTS OCCURRING IN THE TALYSH ZONE (AZERBAIJAN).

14. Jabrayilzade, S., & Aslanova, S. SEPTORIA DISEASE OF APPLE PLANTS OCCURRING IN THE TALYSH ZONE.

15. Aslanova, S. (2023). New Locations of Some Plant Species in the Mountain Part of Yardimli, Lerik and Astara Districts (Azerbaijan). Bulletin of Science and Practice, 9(1), 55-59.

16. Aslanova, S. (2023). Phytocoenological Characteristics and Importance of Vegetation on the Territory of Lerik District (Azerbaijan).

17. Гурбанов, Э., Асланов, С., & Сафарова, А. (2024). КЛИМАТИЧЕСКИЕ УСЛОВИЯ ГОРНОЙ ЧАСТИ ТАЛЫША. *German International Journal of Modern Science/Deutsche Internationale Zeitschrift für Zeitgenössische Wissenschaft*, (90).

18. Aslanova, S. (2019). Flora and vegetation of the mountainous part of Lankaran. *Printing house ADMIU*.

19. Yusifova, A., Asadova, B., & Aslanova, S. (2024). SPECIES COMPOSITION AND RESOURCES OF CULTIVATED AND WILD FORAGE PLANTS IN AZERBAIJAN. *German International Journal of Modern Science/Deutsche Internationale Zeitschrift für Zeitgenössische Wissenschaft*, (84).

20. Aslanova, S. (2023). Petrosimonieta brachiatae and Suaedeta confusae formations distributed in the territory of Azerbaijan. *AS-Proceedings*, *1*(5), 3.

21. Yusifova, A., Asadova, B., & Aslanova, S. (2024). Plants, Human Health And Civilization. *Norwegian Journal of Development of the International Science*, *136*, 6-10.

22. Aslanova, S. (2024). Subalpine Meadow Vegetation of Talish Highlands of Azerbaijan. *Бюллетень науки и практики*, *10*(2), 38-46.

23. Gurbanov, E., & Aslanova, S. (2025). Study of the Area, Environmental Conditions of the" Binagadineft" NGCI Mines in the Absheron Peninsula of the Republic of Azerbaijan, and Study of Vegetation-Soil

Cover Contaminated by Oil and Oil Products. Advanced Studies in Biology, 17(1), 11-17.

24. Yusifova, A., Asadova, B., & Aslanova, S. (2025). Evaluation of Phytopathogenic Fungi According to the Degree of Danger. *Advanced Studies in Biology*, *17*(1), 27-36.

25. Yusifova, A., Aslanova, S., & Asadova, B. (2024). Mycology of Fodder Plants in Different Areas of Azerbaijan the Results of Studies Devoted to the Evaluation. *Бюллетень науки и практики*, *10*(10), 49-54.

26. Jabraylzadeh, S., Aslanova, S., & Ismayilova, G. (2024). GENERAL CHARACTERISTICS OF PHYTOPATHOGENIC SPECIES OF FUNGAL BIOTA OF SOME MEDICINAL PLANTS FOUND IN THE FLORA OF TALYSH (AZERBAIJAN). *German International Journal of Modern Science/Deutsche Internationale Zeitschrift für Zeitgenössische Wissenschaft*, (84).

27. Balakhanova, G., & Aslanova, S. (2025). The main causes of pathogenic diseases in soil ecosystems by microfungi. *Nature & Science*, *7*(4), 28-32.

28. Aslanova, S. (2021). Effect of Ecological Conditions on Change of Some Morphological Characteristics and Yield of Corn Hybrids. *Bulletin of Science and Practice*, *7*(8), 153-157.

29. Юсифова, А., Асланова, С., & Асадова, Б. (2025). ОЦЕНКА МИКОЛОГИЧЕСКОЙ БЕЗОПАСНОСТИ КОРМОВЫХ РАСТЕНИЙ, РАСПРОСТРАНЕННЫХ В НЕКОТОРЫХ РАЙОНАХ АЗЕРБАЙДЖАНА. *German International Journal of Modern Science/Deutsche Internationale Zeitschrift für Zeitgenössische Wissenschaft*, (99).

30. Jabrailzade, S., & Aslanova, S. (2024). Assessment of Pathologies Caused by Anamorphous Fungi Distributed in Some Areas of Azerbaijan According to the Degree of Hazard.

31. Yusifova, A., Asadova, B., & Aslanova, S. (2024). General Characteristics of Azerbaijan Forage Plants and Their Mycobiota and Mycological Safety Principles Applied During Use. *Бюллетень науки и практики*, *10*(8), 66-74.

32. Aslanova, S. (2026, June). Floristic and ecological analysis of the Stipetum–Festucosum formation in the mountainous areas of Lerik region. In International Anatolian Agriculture, Food, Environment and Biology Congress.

33. Aslanova, S. (2026, June). Ecological-geobotanical study of the Festuceta-Poaeltum-Thymusosum formation phytocenosis in the mountainous part of Lankaran. In International Anatolian Agriculture, Food, Environment and Biology Congress.

34. Bakshaliyeva, K. F., Namazov, N. R., Jabrailzade, S. M., Yusifova, A. A., & Rzaeva, A. L. (2020). Ecophysiological features of toxigenic fungi prevalent in different biotopes of Azerbaijan. *Biointerface Research in Applied Chemistry (Romania)*, *10*(6), 6773.

35. Muradov, P. Z., Gasimova, G. C., Namazov, N. R., Sultanova, N. H., & Jabrailzade, S. M. (2020). Comparatıve Study Of Mycobıota Of Some Relıct Plants Included To The Flora Of Azerbaıjan. *Journal of Complementary Medicine Research*, *11*(2), 227.

36. Muradova, S. M., & Jabrailzade, S. M. (2023). Toxıc effect metabolıtes of mıcromycetes spread in Azerbaıjan. Biosciences biotechnology research Asia, 20(2), 729.

37. Bakhshaliyeva, K. F., Muradov, P. Z., Ismayilova, G. E., Namazov, N. R., & Jabrailzade, S. M. (2017). Fungicidal activity of some medicinal plants of the flora of Azerbaijan. Modern Mycology in Russia, 7, 208-209.

38. Gasimova, G., Sultanova, N., Muradov, P., Jabrailzade, S., & Namazov, N. (2020). Prospective uses of relict trees in the urban landscaping of Azerbaijan for resistance to fungal disease. *Revista Cubana de Ciencias Forestales*, *8*(2), 231-240.

39. Aliyeva, G. R., Yusifova, A. A., Rzayeva, A. L., & Jabrailzade, S. M. (2019). Species composition of Trichoderma Pers. common for Technogenically violated Cenosis in the conditions of Azerbaijan. *International Journal of Advanced Research in Biological Sciences*.

40. Bakshaliyeva, K. F., Guliyeva, N. N., Jabrailzade, S. M., & Hashimova, P. M. (2019). Enzymatic activity of mikromycetes isolated from thermal water sources of Azerbaijan. *Int. J. Curr. Microbiol. App. Sci*, *8*(7), 2473-2477.

41. Джабраилзаде, С., & Асланова, С. (2024). ОЦЕНКА ПАТОЛОГИЙ, ВЫЗВАННЫХ АНАМОРФНЫМИ ГРИБАМИ, РАСПРОСТРАНЕННЫМИ В НЕКОТОРЫХ РАЙОНАХ АЗЕРБАЙДЖАНА ПО СТЕПЕНИ ОПАСНОСТИ. *Бюллетень науки и практики*, *10*(9), 17-22.