BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2023. Vol. **20**(2), p. 729-734

Toxic Effect Metabolites of Micromycetes Spread In Azerbaijan

Sevda M. Muradova[1,2]* and Sabiya M. Jabrailzade[1,2]

[1]Azerbaijan State Pedagogical University, Department of Biology and its Teaching Methodology, Az1000, Azerbaijan, Baku, U.Hajibaili 68.
[2]Institute of Microbiology of the Ministry of Science and Education of the Republic of Azerbaijan, Laboratory of Biologically Active Substances, Az1004, Azerbaijan, Baku, Mikhail Mushfik 103

http://dx.doi.org/10.13005/bbra/3127

(Received: 04 April 2023; accepted: 27 June 2023)

In the research conducted, endogenous and exogenous metabolites of fungi were studied according to their toxic activity in different areas of Azerbaijan. It became clear that among the 46 species of fungi isolated from different areas, there are species with strong, medium and weak toxic activity, as well as species without toxic activity. 26,7% have strong, 56.6% have medium, and 10% have weak phytotoxic activity, but 6.7% do not have such an phytotoxic activity. None of the fungi belonging to the division *Basidiomycota* have either strong or moderate phytotoxic activity, only 50% of the remaining fungi have weak phytotoxic activity. All the fungi belonging to the *Mucormycota* division have a weak phytotoxic activity. Differences in the phytotoxic activity of fungi belonging to different taxonomic groups are related to the nature of their struggle in the environment they live in. So that, because the struggle for food of xylotrophic macromycetes takes place under conditions of weaker competition, their phytotoxic activity is characterized by relatively low indicators.

Keywords: Competition, Different biotopes, Endogenous and Exogenous metabolites, Fungi, Phytotoxic Activity.

Fungi as a permanent component of the heterotrophic block of any ecosystem with organic matter, take an actively participate in all ecological processes (destruction, production, regulation and indication) occurring in nature and indication occurring in nature and is one of the groups represented by numerous species of living things on Earth. Although the number of species of fungi currently known to science is around 120,000, there is no doubt that their actual number in nature is many times greater than those known to science. It is worth noting that the number of species of fungi is assumed to be up to 3.8 million[1]. Fungi, which play an active role in the realization of important functions in the biotopes where they are spread, first of all, the process of soil formation, mineralization of organic residues, enrichment of soils with biologically active substances, etc. at the same time cause various pathologies in living beings, including humans. Fungi are always in the center of attention either because of their useful properties[2] or of the dangerous[3] consequences of the pathologies they cause[4].

*Corresponding author E-mail: azmbi@mail.ru

Fungi do not only use the substrates they inhabit to satisfy their nutritional needs, at the same time, they also enrich them with metabolites formed as a result of life activities. Among these metabolites, there are both useful[5], and dangerous[3] ones for other living things, and those with a toxic effect are among the research objects in focus in recent times. Thus, mycotoxins, which have a toxic effect, are synthesized as secondary metabolites in fungi and serves to improve their adaptation to the environment[6] . The fact that these substances have a negative effect on human health is one of the facts known today. Because most of these mycotoxins have mutagenic, carcinogenic and other negative effects[7-8]. It is no coincidence that, the permissible concentration limits for many mycotoxins are already being determined, and among them there are those that are dangerous for the human organizm at any concentration[9]. All mentioned allows us to note that the evaluation of the toxigenicity of fungi is one of the important research directions in modern times.

The richness of the nature of the Republic of Azerbaijan can also be applied to its mycobiota, as the studies conducted so far, the distribution of fungi, including toxigens, in different biotopes of Azerbaijan has been determined[10-12]. As in the world[13-14], also in Azerbaijan the presence of the difference between recorded fungi as well as their toxigenic species, and the actual number of species in nature is one of the realities accepted by everyone. On the other side, the role of natural climate-soil conditions, flora and fauna of the environment in the formation of this or that characteristic of the fungi, also the differences of metabolites synthesized by different strains belonging to the same species, at least in terms of quantitative indicators[2,10] has been confirmed in the conducted studies. On the one hand, this or that feature changes at the strain level, and on the other hand, the studied fungi make up only a small part of the species known to science allows us to confidently say that mushrooms are interesting objects of both scientific and practical research.
Therefore, the purpose of the presented work is devoted to the assessment of phytotoxic activity of exogenous and endogenous metabolites synthesized by fungi isolated from ecosystems related to soil, water and plants of Azerbaijan.

MATERIAL AND METHODS

Researches were carried out with fungi taken to the pure culture from samples of soil, water and plants in different areas of the Greater Caucasus, including Guba-Khachmaz and Absheron Economic Regions in 2015-2022. For the taking samples, obtaining pure cultures from them, determining the species composition, separating endogenous and exogenous metabolites, determining their toxigenicity, as well as to determine the suitability of fungi for bioconversion were used known methods currently used in microbiology, mycology, and biotechnology[5,15-16]. The toxigenicity of mushrooms was evaluated according to their exogenous and endogenous metabolites. As a source of exogenous metabolites were used culture solution (CS) obtained during cultivation of fungi in liquid culture medium (Chapek or glucose-peptone) for 5 days, but as a source of endogenous metabolites was used the biomass produced by fungi (i.e. vegetative mycelium - VM). After 5 days of cultivation of fungi in the mentioned liquid culture medium, the obtained biomass was separated from CS, and it was washed several times with phosphate buffer having a neutral pH, and 50 ml of buffer was again added to the biomass. The cell structure was disrupted and centrifuged in a tissue grinder 3 times for 3 minutes (10 min, 5000 cycles/ min), amd the supernatant from the obtained solution was used as a source of endogenous metabolites after fragmentation (disruption of the cell structure). Phytotoxic activity of metabolites on the germination ability of some plant (wheat and pea) seeds, and zootoxic activity on the viability of infusor (*Paramaecium caudatum* Ehren) were determined using known methods and approaches used in the work of researchers conducting research in this field.

In order to obtain quantitative results, the researches were carried out in at least 4 repetitions, and the obtained results were processed statistically[17].

RESULTS AND DISCUSSION

As a result of the analysis of about 500 of soil, water, and plant samples taken from different

parts of the researched areas, 76 pure fungal cultures were isolated, 16 of which belonged to macro- and 60 to micro-mycetes. During determining the taxonomic affiliation of these cultures according to cultural-morphological signs, it became clear that all of them belong to 46 species of true fungi (Mycota or Fungi). 65.2% of them belong to sac fungi (Ascomycota), 26.1% to Basidiomycota, and 8.7% to Mucoromycota.

In the next stage of research, the phytotoxic activity of the metabolites synthesized by the recorded fungi was studied. It became clear that among the micromycetes there are enough species with strong phytotoxic activity (reducing the germination of seeds by more than 40%), however, macromycetes are not included among them (tab. 1). As seen, a large group of fungi have Moderate (reducing seed germination by 20-40%) phytotoxic activity, which does not include species belonging to the *Basidiomycetes*. Some of the species belonging to the division *Mucormycota* and *Basidiomycota* have a weak (less than 20% reduction in seed germination) toxic activity. On the effect of CS obtained from the fungi *Cerrena unicolor, P.ostreatus* and *L.sulphureus* the germination capacity of wheat seeds not only decreases compared to the control, but can even increase by 4-7%. When characterized by taxonomic affiliation recorded fungi by phytotoxic activity became clear that 26.7% of fungi belonging to the *Ascomycota* department have strong, 56.6% moderate, 10% weak, and 6.7% no phytotoxic activity. None of the fungi belonging to the division *Basidiomycota* have either strong or moderate phytotoxic activity, 50% of the remaining fungi have weak phytotoxic activity, and 50% do not have such a characteristic. All the fungi belonging to the *Mucormycota* division have a weak phytotoxic activity.

During the study of phytotoxic activity of endogenous metabolites of fungi, it became clear that the obtained results are relatively weak compared to exogenous metabolites. This is manifested by the decrease in the number of species

Tabbe 1. Evaluation of CS obtained from fungi for the effect on germination of plant seeds

Indicator of phytotoxic activity (according to the number of seeds that did not germinate compared to the control, %)	Suitable fungi species	Share in the total number of recorded fungi (%)
Strong (more than 44.4-59.7% of non-germinating seeds)	*Alternaria alternata, Fusarium gibbosum, F. moniliforme, F. oxysporum, F. solani, Penicillium chrysogenum, P. cyclopium, Verticillium dahile*	17.4
Medium (the number of non-germinating seeds 25.0-39.2%)	*Alternaria chrysantemi, Aspergillus flavus, A. fumigatus, A. niger, A. ochraceus, A. terreus, A. versicolor, Botrytis cinerea, Cladosporium cladosporioides, C. herbarum, P. citrinum, P. expansum, P. janthinellum, P. notatum, P. purpurogenum, Thielaviopsis basicola, Trichotecum rosea*	36.9
Weak (the number of non-germinating seeds 1.5-19.1% seeds)	*Fusarium dimerum, Fomes fomentarius, Fomitopsis pinicola, Cerrena unicolar, Ganoderma lipsiense, Inonotus hispidus, Mucor hiemalis, M. mucedo, M. plumbers, Phellinus igniarus, Rhisopus stolonifer, Trichoderma viride, Verticillium lateritium.*	28.3
Stimulation effect (0-10%)	*Bjerkandera adusta, Laetiporus sulphureus, Pleurotus ostreatus, Polyporus agariceus, Trametes hirsuta, T. versicolor, Trichoderma atroviride, T. harzianum,*	17.4

Table 2. Evaluation of endogenous metabolites of fungi for their effect on plant seed germination

Indicator of phytotoxic activity (according to the number of seeds that did not germinate compared to the control, %)	Suitable fungi species	Share in the total number of recorded fungi (%)
Strong(more than 40.4-54.4% of ungerminated seeds)	*F. gibbosum, F. moniliforme*, *F. oxysporum, F. solani, Penicillium chrysogenum, P. cyclopium*	13
Medium((the number of ungerminating seeds is between 21.4-35.4%)	*Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, A. ochraceus, A. terreus, A. versicolor, Botrytis cinerea, Cladosporium cladosporioides, C. herbarum, Penicillium citrinum, P. expansum, P. janthinellum, P. notatum, P. purpurogenum, Thielaviopsis basicola, Verticillium dahile*	37
Weak(the number of ungerminating seeds is between 0.5-18.3%)	*Alternaria chrysantemi, Fusarium dimerum, Fomes fomentarius, Fomitopsis pinicola, Ganoderma lipsiense, Inonotus hispidus, Mucor hiemalis, M. mucedo, M. plumbers, Phellinus igniarus, Rhisopus nicricans, Trichoderma viride, Trichotecum rosea, vY Verticillium lateritium*	30.4
Stimulation effect (0-10%)	*Bjerkandera adusta, Cerrena unicolar, Laetiporus sulphureus, Pleurotus ostreatus, Polyporus agariceus, Trametes hirsuta, T. versicolor, Trichoderma atroviride, T. harzianum.*	19.6

Table 3. Effect of exogenous and endogenous metabolites of fungi on the viability of infusor

Suitable fungi species	Growth effect recorded due to the Effect of metabolites(times)	
	Endogenous	Exogenous
Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, A. ochraceus, A. terreus, A. versicolor, Botrytis cinerea, Cladosporium cladosporioides, C. herbarum, Fusarium gibbosum, F. moniliforme, F. oxysporum, F. solani, Penicillium chrysogenum, P. citrinum, P. cyclopium, P. expansum, P. janthinellum, P. notatum, P. purpurogenum, Thielaviopsis basicola, Verticillium dahile.	-(1.05-1.65)	-(1.2-2.0)
Alternaria chrysantemi, Fusarium dimerum, Mucor hiemalis, M. mucedo, M. plumbers, Phellinus igniarus, Rhisopus nicricans, Trichoderma viride, Trichotecum rosea, Verticillium lateritium.	-(1.01-1.10)	-(1.07-1.15)
Bjerkandera adusta, Cerrena unicolar, Fomes fomentarius, Fomitopsis pinicola, Ganoderma lipsiense, Inonotus hispidus, Laetiporus sulphureus, Pleurotus ostreatus, Polyporus agariceus, Trametes hirsuta, T. versicolor, Trichoderma atroviride, T. harzianum,	-1.02-(+1,10)	+(1.1-1.3)

Note: "-" is a decrease, "+" is an increase effect.

with strong toxic activity and the relatively low activity index of individual fungi species (tab. 2). For example, the number of species of fungi whose exogenous metabolites have strong toxic activity decreases from 8 to 6, and the total toxic activity decreases from 44.4 to 59.7% to 40.4 to 54.4%.

Similar situations were observed in other variants. It was clear from the results obtained during the evaluation of the endogenous and exogenous metabolites of the recorded fungi according to their zootoxic activity that the obtained result does not differ significantly from obtained during the study of phytotoxic activity, and the recorded differences are mainly quantitative. The noted differences are due to the biological properties of the fungi used, the level of effect of the metabolites they synthesize, as well as the biological properties of the infusor used as a test(tab. 3).

As can be seen, in these cases, the impact effect is observed either by increasing, decreasing, or not affecting viability. The number of infusors losing their ability to live due to the effect of exogenous metabolites of fungi characterized by strong and moderate phytotoxic activity is reduced by 1.2-2.0 times compared to the initial sample, and by 1.05-1.65 times due to the effect of endogenous. Although, neither endogenous nor exogenous metabolites of fungi considered to have weak phytotoxic activity did not strongly reduce the viability of infusors, either no occurs stimulation. Exogenous as well as endogenous metabolites obtained from xylotrophic macromycetes alone lead to the observation of a growth effect in relation to the growth ability of infusors, ie. different effect is observed between fungi with a fixed habitat of residence and those that are universal in its distribution (those capable of inhabiting both soil, plants, and water). The reason for this, in our opinion, can be explained by the fact that the competition between the creatures that live in the soil and water and use it for food purposes is stronger. So that, the creatures spread there are characterized by a wide variety both according to their taxonomic affiliation and ecotrophic relations. Despite this vastness, nutrients in soil and water are not as rich as in plants, and therefore xylotrophic macromycetes do not need a powerful "weapon" in the struggle for survival. Thus, the number of fungi that settle on plants and live there permanently, both species and individuals is much less compared to soil and water. As a confirmation of we said, it also confirms that the toxic activity of exogenous metabolites is higher than that of endogenous metabolites. Thus, exogenous metabolites can also affect to the other living things[18], but endogenous metabolites are involved in the metabolism that going to continue the life activity of the living beings.

CONCLUSION

From the conducted studies, it became clear that endogenous and exogenous metabolites synthesized by fungi isolated from different biotypes and differing in taxonomic affiliation have different quantitative indicators due to their effects on plants and infusors. The formation of this difference is also influenced by the characteristics acquired by the fungi due to adaptation to the place where they live are also affected. For this reason, the toxic activity of micromycetes is higher than that of xylotrophic macromycetes, which is related to the fact that they live in conditions where the competition, more specifically, the struggle for existence, is tougher.

ACKNOWLEDGEMENT

The authors thank P.Z. Muradov, general director of the Institute of Microbiology of the Ministry of Science and Education of the Republic of Azerbaijan, corresponding member of ANAS, for providing them with laboratory equipment, chemicals and disposable items for conducting research.

Conflict of Interest

None.

Source of Funding

None.

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. Ibrahim, O. and Menkovska, M., 2019. The

Nature, Sources, Detections and Regulations of Mycotoxins That Contaminate Foods and Feeds Causing Health Hazards for Both Human and Animals. Journal of Agricultural Chemistry and Environment, 8:33-57.

4. 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.
5. 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
6. Avalos, J. and Limón, M.C., 2022. Fungal Secondary Metabolism. *Encyclopedia*, 2(1):1-13.
7. 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.
8. 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.
9. 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.
10. Bakshaliyeva, K.F. Namazov, N.R., Jabrailzade S.M. et al., 2020. Ecophysiological Features of Toxigenic Fungi Prevalent in Different Biotopes of Azerbaijan. Biointerface Research in Applied Chemistry, 10,6:6773 – 6782.
11. Bakhshaliyeva K., Namazov N., Hasanova A. et al., 2020b. Assessment of the prospects of studying and using mushrooms of Azerbaijan as effective producers of biologically active substances. Periódico Tchê Química, 17, 34:403-411.
12. Muradov, P.Z., Gasimova, G.Ch., Namazov N.R. et al., 2020. Comparatýve Study Of Mycobýota Of Some Relýct Plants Included To The Flora Of Azerbaýjan. Journal of Complementary Medicine Research, 11(2): 227-231.
13. Baldrian, P., Vetrovský, T., Lepinay, C., Kohout, P., 2022. High-throughput sequencing view on the magnitude of global fungal diversity. Fungal Divers, 114:539–547.
14. Cheek, M., Nic Lughadha, E., Kirk, P. et al., 2020. New scientific discoveries: Plants and fungi. PLANT. People Planet. 2:371–388.
15. Methods of experimental mycology, 1982 / ed. Bilay V.I. - Kyiv: - Naukova Dumka, -500.
16. Netrusov, A.I., Egorova, L.M., Zakharchuk M.A. et al., 2005. Workshop on microbiology. - Moscow: Publishing Center “Academy”, 608.
17. 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.
18. Keller, N.P., 2019. Fungal secondary metabolism: regulation, function and drug discovery.// Nat Rev Microbiol., 17(3):167-180.