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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

TOXIC METABOLITES OF MICROMYCETES DISTRIBUTED IN DIFFERENT BIOTOPES

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INTRODUCTION

Relevance and degree of the completion of the topic. Fungi, as a permanent component of the heterotrophic block of any ecosystem with organic matter, actively participate in all ecological processes (destruction, production, regulation and indication) occurring in nature, and are one of the groups of living organisms on Earth represented by numerous species. Although "*the number of species of fungi known to science is around 150,000*"[1], at present, there is one point that no one doubts, their actual number in nature is many times greater than what is known to science. It should be noted that "*the number of species of fungi was assumed to be between 2.2 and 3.8 million*"[2], however, the application of modern methods allows us to state that "*this number is more than 5 million*"[3]. They perform important functions in the biotopes where they are distributed, primarily they participate in the realization of processes such as soil formation, mineralization of organic residues, enrichment of soils with biologically active substances, etc., at the same time "*they cause various pathologies in living beings, including humans*"[4]. Thus, fungi are always in the spotlight either because of their useful properties or because of the dangerous consequences of the pathologies they cause.

Fungi use the substrates they inhabit not only to meet their nutritional needs but also to enrich them with metabolites produced as a result of their life activities. Among these metabolites, some

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[1] Cheek, M., Nic, L.E., Kirk, P., et al. New scientific discoveries: Plants and fungi.//Plant. People Planet, 2020, v.2, -p. 371–388.

[2] Hawksworth DL, Lücking R. Fungal Diversity Revisited: 2.2 to 3.8 Million Species. Microbiol Spectr. 2017 Jul;5(4). doi: 10.1128/microbiolspec.

[3] Baldrian, P.; Vetrovský, T.; Lepinay, C.; Kohout, P. High-throughput sequencing view on the magnitude of global fungal diversity//Fungal Divers. 2022, 114, 539–547.

[4] Li, D.-W. Biology of Microfungi; Springer: Berlin/Heidelberg, Germany, 2016, 631p

are useful for other living things, as well as those that are dangerous. The latter having toxic effects are the focus of recent research. Thus, those with a toxic effect, that is, mycotoxins, are synthesized as secondary metabolites in fungi and serve to increase their ability to adapt to the environment. Many studies confirmed that these types of compounds, which are important for the fungi that synthesize them, limit the life activity of other organisms, or even completely destroy them. Today, these substances are known to have a negative effect on human health. Because, most of these mycotoxins have mutagenic, carcinogenic, and other negative effects. It is natural that the permissible concentration limits for many mycotoxins have already been found, and some of them are dangerous for the human body at any concentration. All of the above allows us to note that the evaluation of the toxigenicity of fungi is currently one of the important research directions.

The richness of the nature of the Republic of Azerbaijan can also be related to its mycobiota, as the research conducted so far has found the distribution of different taxonomic groups of fungi in different biotopes of Azerbaijan, including species that are new to science. "*Studies aimed at the determination of toxigenic fungi among them, clarifying a number of issues related to their species composition, distribution patterns, and toxic activity*"[5] were also conducted. Like all over the world, there is a difference between the number of fungi registered in Azerbaijan, as well as their toxigenic species, and the actual number of species in nature.

On the other hand, the natural climate-soil conditions, flora and fauna of the environment play a role in the formation of certain characteristics of fungi. The difference in the effects of the metabolites synthesized by different strains of the same species, at

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[5] Baxşaliyeva, K.F. Azərbaycanda yayılan toksigen göbələklərin ekobioloji xüsusiyyətləri:/b.e.d. dissertasiyasının avtoreferatı/-Bakı, 2017, -43s.

least according to quantitative indicators, was confirmed by various studies. On one hand, such a change of a certain feature at the strain level, and on the other hand, the fact that the studied fungi are a small part of the species known to science, allows us to confirm that fungi are of both scientific and practical importance.

Purpose and tasks of the research. The presented work aimed to assess the nature of the toxic activity and chemical composition of fungi isolated from ecosystems related to soil, water, and plants of Azerbaijan and the exogenous and endogenous metabolites synthesized by them.

To achieve the set goal, it was considered appropriate to implement the following tasks:

- Transferring fungi isolated from different (soil, water, and plant) ecosystems of Azerbaijan into pure culture and determination of species composition;
- Choosing the method for the separation of endogenous and exogenous metabolites of fungi isolated from different ecosystems of Azerbaijan and obtaining appropriate tools;
- Evaluation of exogenous and endogenous metabolites obtained from fungi isolated from different ecosystems of Azerbaijan according to phytotoxic activity;
- Evaluation of exogenous and endogenous metabolites obtained from fungi isolated from different ecosystems of Azerbaijan according to zootoxic activity;
- Determination of the chemical nature of exogenous and endogenous metabolites of fungi.

Research methods. Both classic and currently widely used methods in mycobiological research were used during the implementation of the tasks set to achieve the goal of the research. The purity of the reagents used for the analysis and the accuracy of the devices were such as in similar microbiological studies. In order to obtain quantitative results in research, all the experiments were carried out with at least 4 replicates, the obtained results were processed statistically, and the formula S/M = P≤ 0.05 was used as the basis for the degree of integrity.

Main points presented to the defense of the dissertation:

- Among the factors affecting the similarities and differences of the species composition of the fungi distributed in different biotopes, natural climate-soil conditions of the biotopes in which they are distributed also play a role;
- Difference in the phytotoxic activity of fungi is a feature related not only to their taxonomic affiliation but also to the biological characteristics of each species;
- Although the zootoxic activity is observed to be relatively high in fungi whose endogenous and exogenous metabolites have strong phytotoxic activity, this situation cannot be noted as a linear dependence;
- Endogenous and exogenous metabolites with toxic effects synthesized by fungi do not have the ability to precipitate under the influence of organic solvents, which is the basis for their non-protein nature.

The scientific novelty of the research. As a result of the analysis of samples taken from soil, water, and plants in the Greater Caucasus regions of Azerbaijan, 76 strains of 50 species of the *Ascomycota* (65.2%), *Bazidiomycota* (26.1%), and *Mucoromycota* (8.7%) phyla belonging to true fungi (Mycota or Fungi) were isolated and their collection was created and 16 of the cultures included in the collection belonged to macromycetes, and 60 to micromycetes.

During the evaluation of micro- and macromycete fungi recorded in the studies based on the degradation of polymer-containing plant waste, it became clear that only 10 of the macromycetes belonging to the *Basidiomycota* phylum have the qualitatively determined activity of enzymes such as laccase, peroxidase, and tyrosinase, which are considered to be involved in lignin biodegradation. All these enzymes are causative agents of white rot under natural conditions. The remaining 10 fungi have no activity of these enzymes, that is, they are brown rot agents. Cultures belonging to the remaining 26 species have the activity of one or sometimes two of the mentioned enzymes, which suggests them being agents of soft rot.

Exogenous and endogenous metabolites synthesized by fungi have phytotoxic activity, but they differ from each other according to their taxonomic affiliation and level of phytotoxic activity. Thus, 26.7% of the fungi belonging to the *Ascomycota* phylum have strong (reducing seed germination capacity by more than 40%), 56.6% -moderate (between 20-40%), 10% - weak (more than 20%), and 6.7% have no phytotoxic activity. None of the fungi belonging to the phylum *Basidiomycota* have either strong or moderate phytotoxic activity, only 50% of the remaining fungi have weak phytotoxic activity, 50% do not have such a characteristic, and some (*Cerrena unicolor*, *P.ostreatus* and *L.sulphureus*) even cause stimulation (4-7%). All fungi belonging to the *Zygomycota* phylum have weak phytotoxic activity.

During the comparative study of the phytotoxic activity of fungi of the same species isolated from different biotopes, it was found that the difference between the strains of the same species isolated from soil and plants is greater than in water. This trend is related to the fact that the fungal colonization of soil and plants differs from that in water because the aquatic environment is transient for the fungi recorded in the studies i.e., they are migrants of the aquatic environment. Sometimes, the phytotoxic activity is close to those isolated from plants, and sometimes to those isolated from the soil, which allows us to assume from which environment they fall into the water.

It was found that the endogenous and exogenous metabolites of the recorded fungi also have zootoxic activity. The number of infusers 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 and by 1.05-1.65 times due to the effect of endogenous metabolites. Although both endogenous and exogenous metabolites of fungi with weak phytotoxic activity do not significantly decrease the viability of infusers, no stimulation occurs either. Exogenous as well as endogenous metabolites obtained from xylotrophic macromycetes alone lead mainly to the increase in the growth ability of infusors. Thus, a different effect is observed between fungi with a fixed place of residence and those capable of inhabiting soil, plants, and water.

The study of the chemical nature of the toxic metabolites synthesized by fungi revealed neither phytotoxic nor zootoxic activity in both endogenous and exogenous metabolites precipitated by organic solvents. Both toxic and stimulatory phenomena are mainly observed only in the fraction that does not precipitate with organic solvents, which indicates a non-protein nature of the metabolites.

The scientific and practical significance of the work. The obtained results are actual materials that serve to expand on the effect, chemical nature of endogenous and exogenous metabolites of fungi distributed in the territory of the Republic of Azerbaijan.

The obtained results are important in terms of determining the effect of endogenous and exogenous metabolites synthesized by fungi and can be used as a database.

Publications, approbation, and application of the research. 13 scientific works related to the topic of the dissertation have been published. The materials of the dissertation were presented at the International scientific conference on "Actual problems of modern natural sciences" (Ganja, 2017), at the V International scientific-practical conference on "Advanced scientific-technical and social-humanitarian projects in modern science" (Moscow, Russia, 2022), at the XLIV-XLV International scientific-practical conference on "Natural sciences and medicine: theory and practice" (Russia, Novosibirsk, 2022), at the Republican scientific conference on "New trends and innovations: development prospects of microbiology in Azerbaijan" (Baku, 2022).

The name of the institution where the dissertation was carried out. The dissertation work was carried out in the laboratories of Biologically active substances and Microbiological biotechnology of the Institute of Microbiology of the Ministry of Science and Education of the Republic of Azerbaijan.

Structure and volume of the dissertation. The dissertation consists of Introduction, Literature review (Chapter I), Materials and Methods (Chapter II), Experimental part (Chapters III and IV), Conclusions, Results, Practical recommendations, and List of used literature. All these are 147 pages in total, the total number of characters is 220,300.

CHAPTER I

DISTRIBUTION OF TOXIGENIC FUNGI IN NATURE AND GENERAL CHARACTERISTICS OF THE BIOSYNTHETIC PROPERTIES

In section 1.1 of the dissertation, the general characteristics of the species of toxigenic fungi distributed in nature, in section 1.2, toxic metabolites of toxigenic fungi are given, and in section 1.3, the information about the species of toxigenic fungi distributed in Azerbaijan and their toxic activity is analyzed according to the purpose of the work.

CHAPTER II
MATERIALS AND METHODS

Section 2.1 of the dissertation provides a general characterization of the researched areas, that is, different areas of the Greater Caucasus, including the Guba-Khachmaz, Sheki-Zagatala, and Absheron-Khizi Economic Regions.

From the data in section 2.2 of the dissertation, it is clear that for taking samples, obtaining pure cultures from them, determining the species composition, separating endogenous and exogenous metabolites, determining their toxigenicity and the suitability of fungi for bioconversion, "methods known"[6,7,8] in microbiology, mycology, and biotechnology were used. Thus, the planned route method was used for taking samples from the studied areas. The samples were passportized on the spot and delivered to the laboratory no later than 8-16 hours for conducting relevant analyses. For the isolation of micromycetes, a 10% extract was prepared from the samples, transferred to standard nutrient media (SNM) by dilution and cultivated at a temperature of 26-28^{0}C. Thinning of the formed colonies (transferring a visually similar colony to a new nutrient medium) was carried out from time to time and the process was continued until pure cultures of the fungi present there were obtained.

To determine the species composition of the obtained pure cultures, their cultural-morphological and some physiological characteristics were determined, and identification was carried out based on "known determinants"[9,10,11], and as a result, the taxonomic affiliation of the specific strain was found.

For the isolation of macromycetes, the fruiting body (FB) inhabiting the trees in the forests located in the study area was

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[6]Методы экспериментальной микологии/Под. ред. Билай В.И. -Киев: Наукова думка, 1982,500с.

[7] Методы исследования углеводов / под ред. А.Я. Хорлина - М.: Мир, 1975, -135 с.

[8] Нетрусов, А.И. и др. Практикум по микробиологии.-М.:Издательский центр «Академия», 2005, 608с.

[9] Билай, В.И. Определитель токсинообразующих микромицетов./ В.И.Билай, З.А. Курбацкая. - Киев: «Наукова думка», -1990, -236 с.

[10] Саттон, Д.Определитель патогенных и условно-патогенных грибов / Саттон Д., Фотергилл А., Риналди М. -М.: Мир, 2001, 486с.

[11] Kirk, P. M. Dictionary of the fungi/ P. M. Kirk, P. F.Cannon, D. W. Minter [et al.]. - UK, -2008, -747 p.

used. Agarized malt juice (AMJ, 2-4^{0}B) was used as a nutrient medium for the isolation of these fungi.

For obtaining pure cultures of micromycetes, AMJ (4^{0}B), agarized Czapek medium, Sabouraud agar, potato and rice agars were used. Their preparation for planting was carried out according to standard approaches accepted in microbiology.

For the isolation of endogenous and exogenous metabolites specific to fungi, the diluted Czapek medium was used, cultivation was carried out under deep cultivation conditions at 28^{0}C for 5 days. As a source of exogenous metabolites, the culture solution (CS) obtained during the cultivation of fungi in diluted nutrient media for 5 days was used, and as a source of endogenous metabolites, the biomass formed by the fungus (i.e. vegetative mycelium - VM) under the same condition was used. The biomass obtained during the use of the latter is separated from the CS and washed several times with phosphate buffer (pH=7.0). Then, 50 ml of the buffer is added to it again, and the cell structure is destroyed in a tissue grinder 3 times for 3 minutes and centrifuged (10 min, 5000 rpm), the obtained supernatant is used as a source of endogenous metabolites.

Toxigenicity was assessed based on the effect on the germination capacity of some plant seeds and on the viability of the infusor (*Paramaecium caudatum* Ehren).

To find out whether the exogenous and endogenous metabolites are of a protein nature or not, the known methods using organic solvents in the obtained CM and extraction solution (ES) were applied, and in this case, acetone was used.

"*Qualitative reactions*"[12] were used to determine the activity of oxidizing enzymes suitable for the bioconversion of fungi.

In the conducted studies, all experiments were mainly carried out in 4 repetitions, the obtained results were "*statistically*

[12] Бухало, А.С. Высшие съедобные базидиомицеты в. чистой культуре/ А.С. Бухало. -Киев: Наукова думка, -1988, -144с.

processed"[13], and the maximum deviation limit of 5% (i.e., m/M≤0.05) was accepted for reliability.

CHAPTER III
ISOLATION OF FUNGI FROM DIFFERENT BIOTOPES OF AZERBAIJAN, DETERMINATION OF SPECIES COMPOSITION AND CREATION OF A COLLECTION

3.1. Isolation of fungi distributed in different biotopes, determination of species composition and creation of a collection of cultures

A slightly different approach was applied to isolate the pure culture of fungi from the studied areas and to create a collection consisting of them, using samples taken from biotopes that are globally the same (Great Caucasus) and locally different.

As a result of the analysis of nearly 500 samples taken from soil, water, and plants in different parts of the researched areas during 2016-2022, a total of 80 pure cultures were isolated, 18 of which were isolated from the FB of macromycetes, and the remaining 62 belonged to micromycetes.

When determining the taxonomic affiliation of these cultures, it became clear that all of them belong to 50 species of true fungi (Mycota or Fungi) (Fig. 3.1).

Recorded fungi are characterized by a wide diversity, so 3 of the 34 species belonging to sac fungi (*Aureobasidium pullulans*, *Candida alpicans* and *Nectria cinnabarina*) are telemorphs, and

[13] Методы статистической обработки медицинских данных: Методические рекомендации для ординаторов и аспирантов медицинских учебных заведений, научных работников /А.Г. Кочетов, О.В. Лянг., В.П. Масенко, и др. – М.: РКНПК, -2012. -42 с.

Table 3.1.

Distribution of recorded fungal species by phyla

Mycormycota	Ascomycota	Bazidiomycota
Mucor himalis, M.mucedo, M.plumbers, Rhisopus stolonifer	*Alternaria alternata, A.chrysantemi, Aspergillus flavus, A.fumigatus, A.niger, A.ochraceus, A.terreus, A.versicolor, Botrytis cinerea, Aureobasidium pullulans,Candida alpicans, Cladosporium cladosporioides, C.herbarum, Fusarium dimerum, F.gibbosum, F.moniliforme, F.oxysporum, F.solani, Nectria cinnabarina, Penicillium chrysogenum, P.citrinum, P.cyclopium, P.expansum, P.janthinellum, P.notatum, P.purpurogenum, Thielaviopsis basicola, Trichoderma atroviride, T.harzianum, T.viride, Trichotecum rosea, Verticillium dahile* və *V.lateritium, Ulocladum artrum*	*Bjerkandera adusta, Cerrena unicolar, Fomes fomentarius, Fomitopsis pinicola, Ganoderma lipsiense, Inonotus hispidus, Laetiporus sulphureus, Phellinus igniarus, Pleurotus ostreatus, Polyporus agariceus, Trametes hirsuta, T.versicolor,*
4	34	12

the remaining 31 belong to anamorphs. The diversity of macromycetes manifests itself both in the color of decay they produce under natural conditions, in their hyphal systems, and ectotrophic relationships.

Fungi are carriers of both useful and harmful properties in terms of practical needs, and for this reason, it was considered appropriate to evaluate the fungi recorded in the studies from these aspects.

Although the obtaining of biologically active substances for various purposes is a useful sign of fungi, in solving these issues, it is important that they also use materials that are formed during the use of green biomass formed in the process of photosynthesis and in many cases are not suitable for use in the initial form. Taking this into account, the fungal cultures recorded in the study were evaluated for this purpose, more specifically, in terms of their suitability for the bioconversion of lignocellulosic substrates with a complex polymer composition. For this reason, it was carried out according to the method based on the qualitative determination of the synthesis of oxidases involved in the degradation of lignin in the vegetative growth phase of the fungi recorded in the research. According to the obtained results, only 9 species of fungi have the activity of all three mentioned enzymes (Table 3.2). As seen, some fungi do not have the activity of any of the enzymes, and the number of species corresponding to this characteristic is equal to 10.

From the fungi presented in the table, those with the activity of all 3 enzymes belong to the *Bazidiomycota* phylum and cause white rot under natural conditions. Thus, the enzyme systems include hydrolases along with oxidases. This allows them to more intensively break down polymer compounds of lignocellulosic substrates. In short, the use of fungi matching this characteristic for bioconversion can allow more efficient waste utilization. This was proven by the results of our joint research. Thus, fungi, such as *Bjerkandera adusta, Pleurotus ostreatus, Trametes hirsuta, T.versicolor,* isolated during the research, actively degrading the complex polymers contained in lignocellulosic plant waste, enrich

Table 3.2.

Evaluation of recorded fungi according to oxidase activity (for 24 hours).

Fungi	Lac-case	Peroxi-dase	Tyrosi-nase
B.adusta, C.unicolar, F.fomentarius, G.lipsiense, Ph.igniarus, P.ostreatus, P. agariceus, T.hirsuta, T.versicolor	+	+	+
A.alternata, A.flavus, A.fumigatus, A.niger, A.ochraceus, C. herbarum	+	-	-
Th.basicola,	-	+	-
A.terreus, A.versicolor, P.chrysogenum, P.citrinum, P.cyclopium, P.expansum, P.janthinellum, P.notatum, V.dahile, V.lateritium	-	-	+
C.cladosporioides,	+	+	-
Botrytis cinerea, Fusarium dimerum, F.gibbosum, F.moniliforme, F.oxysporum, F.solani, Penicillium purpurogenum, ,	-	+	+
T.atroviride, T.harzianum, T.viride, T.rosea	+	-	+
A.chrysantemi, A.terreus, A.versicolor, F.pinicola, Inonotus hispidus, L.sulphureus, M.hiemalis, M.mucedo, M.plumbers, Rh.nigricans	-	-	-

them with protein and other biologically active substances. This opens up wide prospects for using them in obtaining feed and food products. Thus, during the cultivation of the mentioned fungi in wastes such as straw and vine prunings produced in the country's agrarian sector, at least a 2-fold increase in the amount of proteins in its content, the decrease in the amount of lignin and cellulose by 30-35% for 10 days, and the increase in the amount of soluble sugars were also confirmed in our research. At the same time, promising cultures such as active producers of hydrolase and phenoloxidase are among these fungi.

3.2.An annotated list of fungi recorded in studies

In microbiological and mycological studies conducted in various scientific centers, as a rule, information about microorganisms, primarily micro- and macromycetes, is given in an annotated form, and there is no uniform approach in compiling this list. So, some are satisfied by indicating the name of the fungus currently given on the official website of the IMA, the registration number of the author, and the substrate from which it was isolated. At the same time, information about ecotrophic relationships of fungi, hyphal system, distribution in the world and in the researched country, and what features are in focus are also added to some of the mentioned information. Taking into account the above, we considered it appropriate to compile the annotated list of fungi recorded in our studies by indicating the current legitimate name of the fungus, the author, the registration number in the IMA, the place of isolation and the substrates where it was found.

CHAPTER IV
GENERAL CHARACTERISTICS OF ENDOGENOUS AND EXOGENEOUS METABOLITES OF TOXIGENIC FUNGI AND DETERMINATION OF THEIR CHEMICAL NATURE

4.1. Evaluation of endogenous and exogenous metabolites of fungi recorded in studies according to toxic activity

The metabolites synthesized by fungi are different in terms of purpose, place of secretion, effect, and chemical nature. Elucidation of each of the mentioned features separately or in general is one of the important issues from both scientific and practical points of view for making the activities of fungi manageable, eliminating their negative features, and effectively using their positive features. Taking this into account, these issues were also clarified during the research.

During the determination of the phytotoxic activity of exogenous and endogenous metabolites obtained from fungi, it became clear that among micromycetes, there are also enough species with the strong phytotoxic activity of exogenous metabolites (reducing the seed germination capacity by more than 40%). However, macromycetes are not among fungi with this level of activity. In some cases, even stimulation of the process is observed (Table 4.1). A large group of fungi, which does not include *Basidiomycete* species, have moderate (reducing seed germination capacity by 20-40%) phytotoxic activity. Some of the *Zygomycete* species, as well as the *Basidiomycete* species, have weak (reducing seed germination capacity by less than 20%) toxic activity. Due to the effect of CS obtained from *Cerrena unicolor, P.ostreatus,* and *L.sulphureus* fungi, the germination capacity of wheat seeds does not decrease compared to the control, but can even increase by 4-7%.

The fungi with recorded phytotoxic activity are characterized according to their taxonomic affiliation. It was found that 26.7% of the fungi belonging to the *Ascomycota* phylum have strong, 56.6%-moderate, and 10%-weak phytotoxic activity. Whereas, 6.7% of them do not have phytotoxic activity. None of the fungi belonging to the phylum *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 fungi belonging to the *Zygomycota* phylum demonstrate weak phytotoxic activity.

Table 4.1

Evaluation of exogenous metabolites from fungi for their effect (*the number of ungerminated seeds, %*) on plant seed germination capacity

Indicator of phytotoxic activity	*Matching fungal species*	*The share of the total number (%)*
Strong (the number of ungerminated seeds is 44.4-59.7%)	*A.alternata, F.solani, F.gibbosum, F.moniliforme, F.oxysporum, P.chrysogenum, P.cyclopium, V.dahile*	*17.4*
Moderate (the number of ungerminated seeds is between 25.0 and 39.2%)	*A.chrysantemi, A.flavus, A.fumigatus, A.niger, A.terreus, A.ochraceus, A.versicolor, B.cinerea, C.cladosporioides, C.herbarum, P.citrinum, P.expansum, P.janthinellum, P.notatum, P.purpurogenum, Th.basicola, T.rosea*	*36.9*
Weak (the number of ungerminated seeds is between 1.5 and 19.1%)	*F.dimerum, F.fomentarius, F.pinicola, C.unicolar, G.lipsiense, I. hispidus, M.hiemalis, M.mucedo, M.plumbers, Ph.igniarus, Rh.stolonifer, T.viride, V.lateritium*	*28.3*
Stimulation effect (0-10%)	*B. adusta, L.sulphureus, P.ostreatus, P.agariceus, T.hirsuta, T.versicolor, T.atroviride, T.harzianum*	*17.4*

It is necessary to mention one point about the phytotoxic activity of the fungus, which is related to the taxonomic affiliation of the plant seeds used. Thus, the plant seeds used belong to both monocotyledons (wheat) and dicotyledons (beans). Exogenous metabolites synthesized by fungi do not depend on the taxonomic affiliation of plants. The same fungus indeed shows different activity in relation to wheat and beans, but the level of activity of some fungi is higher in beans, and the activity of others is higher in wheat. Thus, an unsystematic effect occurs.

It can be attributed to the fact that most of the fungi, whose toxicity has been studied, belong to polytrophs and their wider spread, as well as their synthesized metabolites serve this purpose. Besides, fungi are characterized as organisms with high adaptability, and the wider distribution of phytotoxic activity among anamorphs allows them to have a wider range of adaptation opportunities in the environment.

As mentioned, the micromycetes used in the research, more precisely those belonging to sac fungi, were isolated from different biotopes associated with soil, water, and plants. During the research, strains belonging to 34 species of telemorphs and anamorphs of true fungi were isolated from these sources, and strains belonging to the same species and isolated from different biotopes were also among them (Table 4.2). As seen, some fungi were distributed in all 3 sampled sources, some in 2, and some in 1 of them. The number of fungal species spread in all 3 sampled sources is equal to 7.

At the next stage of the research, strains of the same fungal species recorded in different biotopes were evaluated for their phytotoxic activity. According to the obtained results, despite their isolation from different sources, their phytotoxic activity is characterized by close indicators (Table 4.3). Although certain quantitative differences were observed in some fungal strains, this was not systematic, and no clearly expressed dependence on the

Table 4.2

Species composition of fungi isolated from different biotopes

Place of separation (number of species)	**Suitable species**
Soil(27)	*A.alternata, A.flavus, A.fumigatus, A. niger, A.ochraceus, A.terreus, A.versicolor, A.pullulans, C.cladosporioides, C.herbarum, F.gibbosum, F.moniliforme, F.oxysporum, F.solani, P.chrysogenum, P.citrinum, P.cyclopium, P.expansum, P.janthinellum, P.notatum, P.purpurogenum, T.atroviride, T.harzianum, T.viride, Ulocladum artrum, Verticillium dahile V.lateritium*
Water(15)	*A.alternata, A.fumigatus, A.niger, A.versicolor, A.pullulans, C.tropicalis, C.cladosporioides, F.oxysporum, P.citrinum, P.chrysogenum, P.cyclopium, P.notatum, S.brevicaulis, T.viride, U.artrum*
Plant(18)	*A.alternata, A.chrysantemi, A.niger, A.versicolor, B.cinerea, C.herbarum, F.dimerum, F.gibbosum, F.moniliforme, F.oxysporum, F.solani, N.cinnabarina, P.citrinum, P.chrysogenum P.cyclopium, Th.basicola, T.rosea, V.dahile*

fungi from soil, water, or plants, in general, was observed. For example, the phytotoxic activity of strains of the A.niger fungus isolated from different sources varies between 36.7-38.2% with wheat and 35.0-36.2% with peas. A similar situation was observed concerning other fungi. Nevertheless, the obtained results suggest, albeit weakly, that the difference between strains belonging to the same species isolated from soil and plants is greater than that from

Table 4.3

Phytotoxic activity of strains belonging to the same fungal species isolated from different sources

	Sources	The number of sampled seeds		The number of germinated seeds		Phytotoxic activity (%)	
		wheat	pea	wheat	pea	wheat	pea
A.alternata	1*	400	400	247	255	38.2	36.2
	2			249	260	37.7	35.0
	3			253	257	36.7	35.7
A.niger	1			303	312	25.8	22.0
	2			297	314	25.7	21.5
	3			294	319	26.5	20.2
A.versicolor	1			284	297	29.0	25.7
	2			290	302	27.5	24.5
	3			293	307	26.7	23.2
F.oxysporum	1			202	215	49.5	46.2
	2			200	214	50.0	46.5
	3			197	210	50.7	47.5
P.citrinum	1			237	252	40.7	37.0
	2			241	245	39.7	38.7
	3			245	241	38.7	39.7
P.chrysogenum	1			228	238	43.0	40.5
	2			224	236	44.0	41.0
	3			220	230	45.0	42.5
P.cyclopium	1			223	240	44.2	40.0
	2			220	236	45.0	41.0
	3			215	230	46.3	42.5

Note. *1- Soil, 2 – Water and 3 - Plant

water. We believe it is attributed to the different colonization of fungi in soil and plants compared to those in water, because the aquatic environment for the fungi recorded in the studies is transient, more precisely, fungi are migrants of the aquatic environment. The fact that the phytotoxic activity is sometimes relatively close to the fungi isolated from plants, and sometimes to those isolated from the soil, gives a certain reason to assume from which environment they fall into the water.

The study of the phytotoxic activity of endogenous metabolites of fungi showed that the results obtained are slightly different from those obtained with exogenous metabolites, and the total toxicity is relatively weaker, which is reflected in the decrease in the number of species with strong toxic activity and the activity of individual fungal species. For example, the number of species of fungi whose exogenous metabolites have strong toxic activity decreased from 8 to 6, and the total toxic activity decreased from 44.4-59.7% to 40.4-54.4%. Similar cases were observed in other variants. The evaluation of the endogenous and exogenous metabolites of the registered fungi according to zootoxic activity showed that the obtained results do not differ significantly from those obtained during the study of phytotoxic activity, and the recorded differences are mainly quantitative. The noted differences are due to the biological characteristics of the used fungi, the level of effect of the metabolites they synthesize, as well as the biological characteristics of the infusor used as a test (Table 4.4). Thus, in these cases, phytotoxicity increases or decreases viability or has no effect.

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 and by 1.05-1.65 times due to the effect of endogenous metabolites. Although neither endogenous nor exogenous metabolites of fungi with weak phytotoxic activity significantly decrease the viability of infusors, no stimulation occurs either. Only exogenous as well as endogenous metabolites from xylotrophic macromycetes lead to an increase in the growth ability of infusors. Thus, different effects are observed between fungi with a fixed habitat and those that are

Table 4.4

Evaluation of exogenous and endogenous metabolites from fungi for their effects on infusor viability

Appropriate fungal species	*Increased effect (fold) recorded due to exposure to metabolites*	
	endogenous	exogenous
A.alternata A. flavus, A.fumigatus, A.niger, A.ochraceus, A.terreus, A.versicolor, Botrytis cinerea, C.cladosporioides, C.herbarum, F.gibbosum, F.moniliforme, F.oxysporum, F.solani , P.chrysogenum, P.citrinum, P.cyclopium, P.expansum, P.janthinellum, P.notatum, P.purpurogenum, Th.basicola, Verticillium dahile	*-(1.05-1.65)*	*-(1.2-2.0)*
A.chrysantemi, F.dimerum, M.hiemalis, M.mucedo, M.plumbers, Ph.igniarus, Rh.nicricans, T.viride, Trichotecum rosea, V.lateritium	*-(1.01-1.10)*	*-(1.07-1.15)*
B.adusta, C.unicolar, F.fomentarius, F.pinicola, G.lipsiense, I.hispidus, L.sulphureus, P.ostreatus, P.agariceus, T. hirsuta, T.versicolor, T.atroviride, T.harzianum,	*-1.02-(+1.10)*	*+(1.1-1.3)*

Note: "-" – decrease, "+"- increase

universal in terms of distribution, i.e., capable of inhabiting soil, plants, and water. We suggest that the competition between the fungi and other living things that live in the soil and water and use them for food purposes is stronger. So, the living things that spread there are characterized by a wide variety both according to their taxonomic affiliation and ecotrophic relationships. Despite this vastness, soil, and water are not as rich in nutrients as plants. Therefore, xylotrophic macromycetes do not need a strong "weapon" in the struggle for survival. Because the number of species and individuals of fungi that settle and live permanently on plants is much less compared to those in soil and water. It is confirmed by the higher toxic activity of exogenous metabolites compared to endogenous metabolites. Thus, exogenous metabolites can also affect other living things, but endogenous metabolites are involved in the metabolism that occurs to continue the life activity of those living things.

4.2. Chemical nature of endogenous and exogenous metabolites of fungi

At the end of the research, it was clarified whether the metabolites synthesized by fungi and having toxic effects are proteins or polysaccharides. For this purpose, it was considered appropriate to study the 3 strains with the highest phytotoxic activity and zootoxic activity (*F.moniliforme*, *F.oxysporum*, *P.cyclopium*) and the sample with the lowest one, that is, with a high stimulation effect. For this purpose, the material obtained as a result of precipitation of CS and VM obtained from fungi in a 1:2 ratio of acetone (5000 rpm, 10 min) was used. So, as a result of this approach, the precipitated substances are mainly proteins and the ones remaining in the solution are mainly soluble polysaccharides and organic acids. The research showed no phytotoxic activity in the protein metabolites of the examined fungi in the obtained sediments (Table 4.5). Apparently, this is true for both VM and CS sediments.

Table 4.5

Phytotoxic and zootoxic activities of CS and VM in fungi

Appropriate fungal species	Recorded activity caused by the effects of metabolites (%)							
	In sediment				In solution			
	Phytotoxic		Zootoxic		Phytotoxic		Zootoxic	
	VM	CS	VM	CS	VM	CS	VM	CS
F. moniliforme	1,0	0	1,01	0	45,9	57,8	-1,20	-1,34
F.oxysporum	1,0	0	1,02	0	44,7	59,3	-1,12	-1,41
P.cyclopium	0,0	0	1,04	0	30,5	45,3	-1,08	-1,17
P.ostreatus	0,0	0	1,05	0	-7,8	-15,3	1,15	1,22

Almost the same situation was observed for the zootoxic activity. Significant toxic activity and stimulation were detected only in the non-precipitating part (Table 4.5). This allows us to note that the metabolites that cause either toxic activity or stimulation are not proteins, they are relatively light molecular compounds that do not precipitate under normal conditions due to the effect of organic precipitants.

FINAL ANALYSIS OF THE RESULTS OBTAINED FROM THE RESEARCH

Fungi, the main condition for the distribution of which is the presence of organic matter in the environment, are active participants in almost all processes occurring in nature. The fact that fungi perform such a diverse and wide range of ecological functions is the basis of their practical interest. Fungi, which are characterized as producers in an ecological sense, are the only organisms that are capable of natural production (for example, enriching the soil with vitamins and other biologically active substances), destruction (in the mineralization of organic matter, especially the degradation of lignocellulose-containing substrates), regulation of biodiversity (the presence of phytopathogens among 8-25 thousand species) and perform the indication functions.

The natural soil-climatic, flora and fauna of the environment in which fungi are distributed, i.e., abiotic and biotic factors, also affect the presence and formation of these features. Thus, some studies have repeatedly confirmed that certain characteristics of fungi that spread in any biotope are at least quantitatively different. For this reason, there is no doubt that in fungi distributed in a certain area, at least quantitatively different traits can be found. If we recall the idea that "fungi are the offspring of the devil and were created to disturb the harmony in nature and confuse researchers," then we can once again state that fungi are an open and interesting object for research of any kind.

Considering this, following the tasks set for the research, soil, plant, and water samples were taken from the territories of several economic regions located in the territory of the Greater Caucasus, which is one of the largest geomorphological units of Azerbaijan. They were transferred into pure culture and the species composition was determined. The endogenous and exogenous metabolites synthesized by these fungi were evaluated according to their phytotoxic and zootoxic activities. The chemical nature of those metabolites was determined. The results obtained during the performance of these tasks are presented in the following conclusions.

CONCLUSIONS

1. A collection consisting of 50 species and 76 cultures of true fungi (Mycota or Fungi) belonging to the phyla *Ascomycota* (65.2%), *Bazidiomycota* (26.1%), and *Mucormycota* (8.7%) has been created as a result of the taking samples from soil, water, and plants in various parts of Azerbaijan. 16 cultures included in the collection belong to macromycetes, and 60 to micromycetes[1-6, 8-10, 12-13].

2. During the evaluation of micro- and macromycete fungi recorded in the studies based on the degradation of polymer-containing plant waste, it became clear that only 10 of the macromycetes belonging to the *Basidiomycota* phylum have the qualitatively determined activity of enzymes such as laccase, peroxidase, and tyrosinase, which are considered to be involved in lignin biodegradation. All these enzymes are causative agents of white rot under natural conditions. The remaining 10 fungi have no activity of these enzymes, that is, they are brown rot agents. Cultures belonging to the remaining 26 species have the activity of one or sometimes two of the mentioned enzymes, which suggests them being agents of soft rot[5, 7].
3. When the phytotoxic activity is characterized according to the taxonomic affiliation of the recorded fungi, it is clear that 26.7% of the fungi belonging to the *Ascomycota* phylum have strong (reducing the seed germination capacity by more than 40%), 56.6% - moderate (between 20-40%), 10% - weak (more than 20%), and 6.7% have no phytotoxic activity. None of the fungi belonging to the phylum *Basidiomycota* have either strong or moderate phytotoxic activity, and only 50% of the remaining fungi have weak phytotoxic activity, and 50% do not have such a characteristic, and some (*Cerrena unicolor*, *P.ostreatus* and *L.sulphureus*) even cause stimulation (4-7%). All fungi belonging to the *Zygomycota* phylum have weak phytotoxic activity[6, 9, 11, 15].
4. During the comparative study of the phytotoxic activity of fungi of the same species isolated from different biotopes, it was clear that the 5,difference between the strains of the same species isolated from soil and plants is greater than in water. This trend is related to the fact that the fungal colonization of soil and plants differs from that in water

because the aquatic environment is transient for the fungi recorded in the studies i.e., they are migrants of the aquatic environment. Sometimes, the phytotoxic activity is close to those isolated from plants, and sometimes to those isolated from the soil, which allows us to assume from which environment they fall into the water[9, 11, 14].

5. It was found that the endogenous and exogenous metabolites of the recorded fungi also have zootoxic activity. 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 and by 1.05-1.65 times due to the effect of endogenous metabolites. Although both endogenous and exogenous metabolites of fungi with weak phytotoxic activity do not significantly decrease the viability of infusors, no stimulation occurs either. Exogenous as well as endogenous metabolites obtained from xylotrophic macromycetes alone lead mainly to the increase in the growth ability of infusors. Thus, a different effect is observed between fungi with a fixed place of residence and those capable of inhabiting soil, plants, and water[11].
6. When clarifying whether the toxic metabolites synthesized by fungi have a protein or polysaccharide nature, neither phytotoxic nor zootoxic activity was revealed in both endogenous and exogenous metabolites precipitated by organic solvents and this situation is justified both in the vegetative mycelia and in relation to the culture solution. Both toxic and stimulatory phenomena are mainly observed only in the fraction that does not precipitate with organic solvents, which indicates a non-protein nature of the metabolites[15].

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