REPUBLIC OF AZERBAIJAN

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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

ECOBİOLOGİCAL CHARACTERİSTİCS OF TOXİGENİC SPECİES OF FUNGİ BELONGİNG TO THE GENERA *PENİCİLLİUM* LİNK AND *ARTHROBOTRYS* CORDA DİSTRİBUTED İN AZERBAİJAN

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INTRODUCTION

Relevance and degree development of the topic. Fungi, which are stable components of biocenoses where organic matter is present, perform diverse and important ecological functions there. Thus, the fact that fungi play an active role in the realization of all processes occurring in any ecosystem, such as degradation, production, regulation of biodiversity, and so on, is a reality that is unequivocally accepted by the scientific community today. Therefore, fungi have always been the subject of research in various aspects and there is no doubt that they will continue to be so in the future. Because as a result of the increasing interference of humans with the environment, changes in the ecological situation in areas where fungi are spread are inevitable, and it is an undoubted reality that it is also important to re-research those places from time to time in order to restore the natural state of the changed conditions. At least for the reason that in order to restore any place to its previous state, the essence of the processes that occur there with the participation of living organisms, including fungi, must be clarified.

In studies conducted on fungi, their impact on individual biocenotic processes, structure, and ecobiology of living organisms has been clarified to one degree or another, but the scope and depth of these studies are weak compared to plants. Therefore, fungi are an open object for research today. On the other side, the level of study of individual ecotrophic groups of fungi is also different. For example, although the fungi distributed in this or that biotope are studied mainly according to the species composition and the heterogeneity of their populations "*phytopathogens have been studied more comperehensively in this regard*"[1]. From this aspect, it can be noted that insufficient attention has been paid to the study of saprotrophic or saprotrophically inclined species of micromycetes distributed in natural and anthropogenically affected cenoses.

Among the fungi that have not been studied are the genera

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[1] Peng Y., Li S.J., Yan J. et alş Research Progress on Phytopathogenic Fungi and Their Role as Biocontrol Agents// Front. Microbiol., 2021, 12:670135. doi: 10.3389/fmicb.2021.670135

Penicillium Link and *Arthrobotrys* Corda. However, a number of fungal species belonging to the genus *Penicillium*, which was the source of the first antibiotic, as well as to the nematophage-characterized *Arthrobotrys*, have long been the subject of research in certain aspects, and theirin this or that biotope "composition of the distributed species has been determined, the prospect of obtaining secondary metabolites frem them has been shown, and evenrelated to the creationof recombinant strains based on them"[2,3,4] has been conducted research. This does not mean that the potential of fungal species belonging to the *Penicillium* Link and Arthrobotrys Corda genera has been fully realized, as the number of species studied in this direction constitutes a small fraction of the species known to science belonging to the mentioned genera. For example, according to recent data, " 483 species belong to the genus *Penicillium*"[5], "*70 species belong to the genus Arthrobotrys*"[6], but the species that have been thoroughly studied do not even make up 10% of those listed. If we add to what has been said that "*the species currently known to science constitute only 7-8% of those actually existingin nature*"[7], then it can be noted that the number of species studied is less than 1%.

In the Republic of Azerbaijan, even in the XXI century "*were studied species belonging to the genera Penicillium Link and*

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[2] Wang, X.C., Chen, K., Zeng, Z/Q. *et al.* Phylogeny and morphological analyses of *Penicillium* section *Sclerotiora* (Fungi) lead to the discovery of five new species. //Sci Rep, -2017, **7**, 8233 (). https://doi.org/10.1038/s41598-017-08697-1

[3] El Hajj Assaf, C., Zetina-Serrano, C., Tahtah, N. et al. Regulation of Secondary Metabolism in the *Penicillium* Genus//International Journal of Molecular Sciences, -2020, 21(24), 9462. https://doi.org/10.3390/ijms21249462

[4] Hsueh Y.-P., Gronquist, M.R., Schwarz, E.M. et al.Nematophagous fungus *Arthrobotrys oligospora* mimics olfactory cues of sex and food to lure its nematode prey//*eLife*, *2017*, 6:e20023. https://doi.org/10.7554/eLife.20023

[5] Petersen, C., Sørensen, T., Nielsen, M.R. et al. Comparative genomic study of the Penicillium genus elucidates a diverse pangenome and 15 lateral gene transfer events// IMA Fungus, -2023, 14, 3. https://doi.org/10.1186/s43008-023-00108-

[6] https://www.mycobank.org/

[7] Hawksworth, D.L., Lücking R. Fungal Diversity Revisited: 2.2 to 3.8 Million Species. //Microbiol Spectr, -2017, 5:10.1128/microbiolspec. funk-0052-2016. https://doi.org/10.1128/microbiolspec.funk-0052-2016

Arthrobotrys Corda in one or another way"[8,9,10], but in almost all of the conducted studies fungi belonging to this genus have been studied mainly against the background of the general mycobiota of a specific biotope, and there are no studies dedicated to the comprehensive study of these fungi at the genus level. Thus, the number of species belonging to the genus *Penicillium* recorded in studies conducted in various biotopes of Azerbaijan varies between 4-17 species, of which there are no more than 25 species in total. The number of fungi belonging to the genus *Arthrobotrys* is between 5-6 species, according to research conducted in the XXI century. In other words, the study of fungi belonging to the mentioned genera, especially in terms of evaluating the secondary metabolites they synthesize, is weaker in Azerbaijani conditions.

The aims and tasks. The purpose of the presented work was dedicated to the study the toxigenicity and ecobiological characteristics of toxigenic species of fungi belonging to the genera *Penicillium* Link and *Arthrobotrys* Corda, which are distributed in various areas of the Republic of Azerbaijan.

In order to achieve the set goal, the following tasks was planned to be solved during the research:

1. Isolation of species belonging to the genera *Penicillium* and *Arthrobotrys* from different areas of Azerbaijan, creation of a collection of their various strains;
2. Evaluation of species belonging to the genera *Penicillium* and *Arthrobotrys* for toxic activity;
3. Endogenous and exogenous metabolites of fungi with high toxic activity and comparative evaluation of their biological activity;

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[8] Baxşəliyeva, K.F. Azərbaycanda yayılan toksigen göbələklərin ekobioloji xüsusiyyətləri/biologiya üzrə elmlər doktorluğu dissertasiyanın avtoreferatı/ - Bakı, 2017, -45s.

[9] Namazov, N.R. Azərbaycan florasına aid olan efiryağlı bitkilərin mikobiotası, onların tərkib elementlərinin bakterisid və fungisid xüsusiyyətləri:/ biologiya üzrə elmlər doktorluğu dissertasiyasının avtoreferatı/-Bakı, 2021, -62s

[10] Масмалиев Ф.А. Нематофаговые хищные грибы Пиркулинского Государственного Заповедника. /Материалы Международной конференции «Биологическое разнообразие Кавказа и Юга России. Махачкала, 2013, с.409-410

4. Study of the metabolic activity of toxigenic species belonging to the genera *Penicillium* and *Arthrobotrys* in ecophysiological aspects and identification of the mycotoxins they synthesize.

Research methods. To solve the tasks set in the dissertation, were used classical methods of mycology, as well as methods that are currently widely used in mycological, microbiological and physiological-biochemical studies in this direction. To fulfill the set tasks, the accuracy of the devices and equipment used in the experiments, the purity of the chemical reagents were at the required level, and all experiments in the studies were performed in at least 4 repetitions, which allowed for statistical processing of the obtained results. The degree of accuracy of the results obtained was determined based on the formula $P \geq 0.05$.

The main provisions of the dissertation submitted for defense.

- The characterization of the Absheron Peninsula as a geoecologically critical problematic area does not prevent the spread of fungi belonging to the genera *Penicillium* and *Arthrobotrys* in its territory;
- Although metabolites of fungi belonging to the genera *Penicillium* and *Arthrobotrys* have phytotoxic activity, among them, the number of species with strong phytotoxic activity is small;
- Although the metabolites of fungi belonging to the genera *Penicillium* and *Arthrobotrys* that cause phytotoxic activity are mainly exogenous in nature, they are not proteinaceous in nature;
- Although there is no clear correlation between total mycotoxin synthesis and biomass yield in optimized environments for the growth of *Penicillium* and *Arthrobotrys*, the lack of optimal environmental parameters intensifies mycotoxin synthesis.

Scientific novelty of the research. In the conducted studies, a total of 58 species, 17 of which belong to the genus *Arthrobotrys* and 41 to the genus *Penicillium*, were isolated from the soils of the Absheron Peninsula of the Republic of Azerbaijan. The isolated species were studied comprehensively for their phytotoxic activity, the nature of their endogenous and exogenous metabolites, and the mycotoxins they synthesized.

It became clear that among the recorded fungi, species such as *Penicilium atrovenetum, P.clavigerum, P.duclauxii, P.palitans*, and *P.viridicyclopium* are new to the mycobiota characteristic of Azerbaijani nature. Although species belonging to the genera *Penicillium* and *Arthrobotrys* have toxic activity to one degree or another, those with strong toxic activity are quite few, and this is confirmed by the example of species such as *Penicillium chryzogenum* (41.2%) and *P.cyclopium* (43.7%). Of the recorded species, 58.6% (11.2-35.4%) have moderate phytotoxic activity, while 38% have weak activity (1.1-8.2%).

A comparative study of endogenous and exogenous metabolites of the most phytotoxic species of the *Penicillium* and *Arthrobotrys* genera showed that the metabolites causing phytotoxic activity are mainly exogenous in nature, and the difference between the phytotoxic activity of endogenous and exogenous metabolites is 7.3-16.5 times in the *Arthrobotrys* genus and 4.8-10.0 times in the *Penicillium* genus. In the course of research, it was determined that both endogenous and exogenous metabolites of species belonging to the genera *Penicillium* and *Arthrobotrys*, which cause phytotoxic activity, are not of protein nature.

It was found that there was no clearly expressed linear relationship between biomass yield and total mycotoxin content, on the contrary, environmental optimization leads to a decrease in total mycotoxin yield, i.e. an inverse relationship is observed. Thus, the amount of mycotoxins per unit biomass is 1.07-1.29 times less in the *Arthrobotrys* genus than in the carbon-deficient variant, and 1.05-1.28 times less than in the nitrogen-deficient variant. In the genus *Penicillium*, this indicator is 1.12-1.25 and 1.08-1.21 times, respectively.

It has been shown that in conditions of environmental scarcity of basic nutrients such as carbon and nitrogen the formation of mycotoxins occurs more intensively in all species belonging to the genera *Penicillium* and *Arthrobotrys*, and this is manifested at a higher level in nitrogen deficiency. Mycotoxins produced by species belonging to the genera *Penicillium* and *Arthrobotrys* in optimized environments have been identified and it has been determined that species belonging to the genus *Penicillium* synthesize pharmacologically important mycotoxins such as aflatoxin, ochratoxin, citrinin, chrysogin, roquefortin, melagrine, penicillin, xanthocillin X, ergosterol penicillic acid, etc. while the metabolites synthesized and having toxic effects by fungi belonging to the *Arthrobotrys* genus consist of compounds such as oligosporan and oligosporol and their derivatives, and have weak pharmacological activity.

Theoretical and practical significance of research. The results obtained in the studies are factual material that serves to expand the understanding of fungi belonging to the genera *Penicillium* and *Arthrobotrys*, as well as the mycobiota of Azerbaijan.

The results obtained in the studies allow for the regulation of mycotoxin synthesis, which in the future will allow for more effective results in the extraction of specific mycotoxins from fungi.

The cultural morphological signs and illustrative materials related to this or that fungus in the dissertation can be used as a textbook during the teaching of mycology.

Publication, approval and application of dissertation. 14 scientific works related to the topic of the dissertation have been published, of which 6 are scientific articles and 8 are theses and conference materials. The materials of the dissertation were presented at the Republican conference on "Diversity of Karabakh, soil and water resources: past, present and future" (Baku, 2021), at the International scientific conference on "Actual problems of modern natural and economic sciences" (Ganja, 2022), at the Republican scientific practical conference "New trends and innovations: prospects for the development of microbiology in Azerbaijan" (Baku, 2022), at the III international conference (Baku, 2023), at the International Scientific Conference on "Actual Problems of Medical Prevention" (Baku, 2023), at the V International scientific and technical conference on "Actual issues of modern research directions" (Russia F., Penza, 2023), at the I international scientific practical conference on "Innovative biotechnologies for environmental protection: from theory to practice” (Belarus R., Minsk, 2024).

The organization where the dissertation was carried out. The dissertation work was carried out in the microbiological biotechnology and experimental mycology laboratories of the Institute of Microbiology of the Ministry of Science and Education of the Republic of Azerbaijan.

The structure and volume of the dissertation. The dissertation consists of an introduction, a literature review (Chapter I), materials and methods of work (Chapter II), an experimental part (Chapters III-IV), a final analysis of the research materials, main results, lists of used literature and abbreviations. The total volume of the dissertation is 215500 characters.

CHAPTER I

THE GENERA *PENİCİLLİUM* LİNK ADN *ARTHROBOTRYS* CORDA: SPECİES COMPOSİTİON, DİSTRİBUTİON, ECOPHYSİOLOGİCAL CHARACTERİSTİCS AND TOXİGENİC METABOLİTES

In section 1.1 of the dissertation are analyzed the place and species composition of fungi belonging to the genera *Penicillium* and *Arthrobotrys* in the modern system, in section 1.2, their distribution regularities and ecotrophic relationships, and in section 1.3, information about toxigenic species and their ecophysiological properties, and the points that should be paid attention to in order to achieve the planned goal of the study are clarified.

CHAPTER II
MATERIAL AND METHOD OF RESEARCHS

2.1. General characteristics of the research areas

The research was conducted in large geomorphological units of the Republic of Azerbaijan, in the territories of the Republic of

Azerbaijan located in the Greater Caucasus, primarily on the Absheron Peninsula, as well as in the Kur-Araz Lowland. Samples for the study were taken from both relatively clean and anthropogenic pollution soils, as well as on the plants there, and analyzed in accordance with the purpose of the study.

2.2. General characteristics of the methods used during sampling and analysis in research

In the studies, sampling, on-site passporting, preparation for laboratory studies, preparation of suspensions and nutrient media from samples, inoculation and incubation of nutrient media with suspensions, and obtaining pure cultures were carried out based on the "classical"[11] methods accepted in mycology, as well as the eləcə də bu sahədə son illərdə aparılan işlərə "*methodos and approaches used by various authors*"[6, 12,13] in recent years in this field.

For the isolation of fungi were used "*standard nutrient media*"[12], more precisely, agarized malt juice, Saburo agar, Potato agar, etc.

The identification of cultures, the purity of which was controlled by microscopy, was carried out based on the macroscopic and microscopic characteristics of the cultures, based on "known determinants"[14,15,16,17] and their taxonomic affiliation and current name were determined based on the data on the "official website of the

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[11] Maheshwari, R. 2016. Fungi: Experimental Methods In Biology, 2th Edition. CRC Pres, 358p.

[12] Visagie C.M. . Houbraken J., Frisvad C. Et al. Identification and nomenclature of the genus Penicillium//Studıes ın mycology, 2014, v.78. -p.343–371

[13] Ефимочкина Н.Р., Седова И.Б., Шевелева С.А., Тутельян В.А. Токсигенные свойства микроскопических грибов // Вестн. Том. гос. ун-та. Биология., 2019. №45., -с.6-33

[14] Гарибова, Л.В. Популярный Атлас Определитель Грибы. /Л.В.Гарибова. - Москва: Просвещение /Дрофа, 2009, 352с.

[15] Kirk P.M., Cannon P.F., Minter D.W., Stalpers J.A. Dictionary of the fungi, 10th edn. CABI publishing – Wallingford (UK), -2008, 784p

[16] Li D.W., Magyar D., Kendrick B. Color Atlas of Fungal Spores. A laboratory identification Guide. ACGIH, 2023, 862p.

[17] Саттон, Д. Определитель патогенных и условно-патогенных грибов -М.: Мир, -2001, -486с.

International Association of Mycology”[6].

For separate endogenous and exogenous metabolites of fungi and determine their phytotoxic activity were used the biomass and culture solution formed during cultivation of fungi in liquid Chapek medium for 5 days. In this case, the determination of phytotoxic activity was carried out based on the effect on the germination capacity of plant seeds, using “*methods and approaches”*[8] used in similar studies.

The total amount of mycotoxins in fungal materials was determined using an “*exspress method*”[18], based on the use of a special Veratox immunoenzyme kit, and individual mycotoxins were determined using an “*immunoenzyme analysis(İFA, ELİSA)*”[19]. In the studies, all experiments were performed in at least 4 replicates and the results were “*statistically*”[20] analyzed. In this case, the results that meet the formula $\sigma/X_0 \leq 0.05$ were included in the dissertation, where σ is the mean square deviation, X_0 is the average value of the repetitions.

CHAPTER III
PENICILLIUM LINK AND *ARTHROBOTRYS* CORDA GENUS AND GENERAL CHARACTERISTICS OF THEIR SPECIES DISTRIBUTED IN AZERBAIJAN

3.1. Isolation of fungi belonging to the genera *Penicillium* and *Arthrobotrys* from natural sources, determination of species composition, and creation of a collection of pure cultures

As a result of the analysis of about 300 samples taken from the study areas, a total of 265 strains were isolated into pure culture, and as a result of their identification using classical mycological

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[18] https://soctrade.ua/equipment/shop/test-mikotoksiny-Veratox/

[19] ГОСТ 31653-2012 Корма. Метод иммуноферментного определения микотоксинов. М., 2012, 15с.

[20] Кобзарь, А. И. Прикладная математическая статистика/ А. И. Кобзарь, -М.: ФИЗМАТЛИТ, - 2006, -816 с.

methods, revealed that among of isolated strains included both *Penicillium* and *Arthrobotrys* genera too(tab. 3.1). As seen, 35,1% of the total recorded strains, 93 strains, belong to the studied genera. Since the purpose of our research was to study fungi belonging to these two genera, these strains were used in the further course of research. Initially, were conducted studies to determine the species composition of strains isolated from various biotopes, which classical mycological methods were used at this time.

Table 3.1.

Distribution of strains registered in studies by genus

Genus	Number of strains, un.	Share in total, %
Arthrobotrys	25	9,4
Penicillium	68	25,7
Others (*Alternaria, Aspergillus, Cladosporium, Fusarium, Trichoderma* and oth.)	172	64,9
Total	265	100

It was found that the 93 isolated strains are characterized by their species composition as follows(tab. 3.2). As seen, the isolated strains belong to a total of 58 species, of which 17 belong to the genus *Arthrobotrys* and 41 to the genus *Penicillium*. The recorded fungi are species that have been recorded in studies conducted by different researchers at different times, that is, known to science, and there are no new ones among them. However, among them, there are species that are new to the mycobiota specific to Azerbaijani nature, more precisely, their distribution in the territory of Azerbaijan was determined precisely as a result of these studies, such as *P. atrovenetum, P. clavigerum, P. duclauxii*, *P. palitans* and *P. viridicyclopium*(fig. 1).

3.2. Annotated list of species belonging to the genera *Penicillium* and *Arthrobotrys* recorded in Azerbaijan

As mentioned, the number of species belonging to the genera

Table 3.2

Species of fungi belonging to genera *Arthrobotrys* and *Penicillium*, whose distribution has been determined in studies

Genus	Species
Arthrobotrys	*A.aggregatus, A.apsheronica, A.azerbaijanicus, A.bakunika, A.chazaricus, A.conoides, A.doliiformis, A.fruticulosus, A.longiphorus, A.microscahoides, A.longus, A.musiformis, A.nematopagus, A.oligosporus, A.shahriari, A.soprunovii, A.tabrizica*
Penicillium	*P.atrovenetum, P.brevicompactum, P.canescens, P.chrysogenum, P.citrinum, P.citreoviride, P.clavigerum, P.corylophiloides, P.corymbiferum, P.cyaneum, P.cyclopium, P.decumbens, P.duclauxii, P.expansum, P.funiculosum, P.galucolanosum, P.granulatum, P.hordei, P.janthinellum, P.jensenii, P.kojigenum, P.lanosogriseum, P.lanosoviride, P.lanosum, P.lavendulum, P.martensii, P.notatum, P.ochraceum, P.olivirinoviride, P.oxalicum, P.palitans, P.puberulum, P.purpurogenum, P.purpurescens, P.resticulosum, P.spinulosum, P.stoloniferum, P.tardum, P.variabile, P.viridicyclopium* and *P.waksmanii*

Penicillium and *Arthrobotrys* distributed in the sampled area during the research was 58.

In mycological studies, an annotated list of recorded fungi species is usually compiled, which mainly includes information about the taxonomic affiliation of the fungi, their place of origin, some characteristic cultural-morphological features, etc. Taking this into account, it was considered appropriate to compile an annotated list of fungi recorded in the course of our research. This list is compiled based on the correct name of the fungi currently on the website of the International Association of Mycology, the area of their distribution, some cultural-morphological characteristics of the fungi whose distribution was first recorded in Azerbaijan, relevant

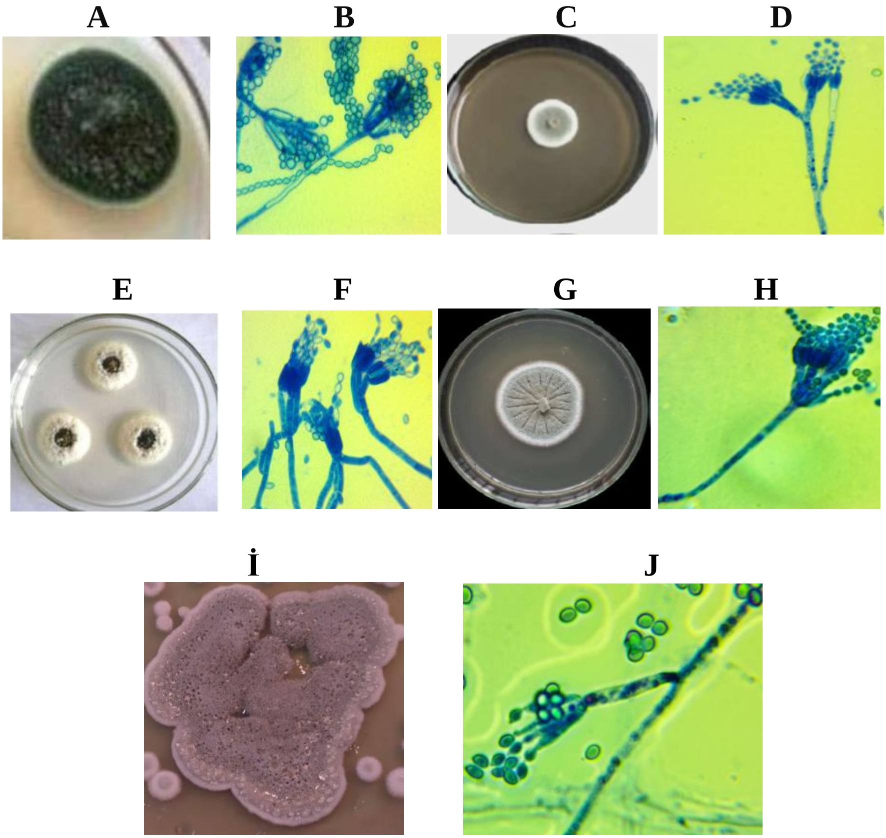

Figure 1. General view of a colony and microscopic view of its conidia formed by the fungus *Penicilium atrovenetum*(A,B), *P.clavigerum* (C, D), *P. duclauxii* (E,F), *P.palitans* (G,H) and *P.viridicyclopium* (I, J)

illustrativematerials, as well as information about their metabolites of practical interest.

It should be noted that fungi are constantly exchanging energy and nutrients with their environment to continue their life activities, and they enrich the environment they live in with the metabolites they produce as a result of their life activities. In general, it should be noted that the metabolites produced by fungi are generally divided into two groups: endogenous and exogenous, each of which has similar and specific functions to one degree or another. From a biotechnological perspective, exogenous metabolites are considered more valuable, so it was considered appropriate to first characterize the fungi recorded in the studies according to the phytotoxic activity of their exogenous metabolites. It was clear from the results that although there are cultures with toxic activity among species belonging to the genera *Penicillium* and *Arthrobotrys*, among them there are quite a few with strong toxic activity, which is confirmed by the example of species such as *P.chryzogenum* and *P.cyclopium* (tab. 4.1). As seen, more than half of the recorded fungi have moderate, and about 1/3 have weak phytotoxic activity. The total toxigenic content of fungi belonging to the genus *Penicillium* is relatively higher than that of those belonging to the genus *Arthrobotrys*, which allows us to note that they have a different role in the ecological functions occurring in the soil.

Therefore, in order to more comprehensively evaluate the phytotoxic activity of the genera selected as the object of research, culture selection for further studies was carried out on both genera and it was considered appropriate to select 3 species from each genus(tab. 4.2).

4.2. Comparative study of the phytotoxic activity of endogenous and exogenous metabolites of selected fungal species as active producers

As is known, the wide diversity of fungi creates resources that are useful in terms of the diversification of biosynthetic gene clusters and the enormous potential for metabolic variation, which is of practical use. It is no coincidence that the colonization of our planet by organisms with a mycelial structure is associated with the strong development of secondary metabolism in them. In addition, secondary metabolites have biological activity, which has provided the basis for the production of life-saving drugs and agrochemicals. Toxic metabolites, i.e. mycotoxins, are also products of secondary metabolism that contaminate human food, animal feed, and the environment. At the same time, mycotoxins are considered

Table 4.1

Evaluation of recorded fungal species for phytotoxic activity (PTA)

PTAdegree(%)	**PTA**(%)	Compatible species	Share in total, %
Strong	41,2-43,7	*P.chryzogenum* və *.cyclopium*	3,4
Average	11,2-35,4	*A.nematopagus, A.compactus, A.doliiformis, A.fruticulosus, P.citrinum, P.citreoviride, P.clavigerum, P.duclauxii, P.expansum, P.funiculosum, P.galucolanosum, P.granulatum, P.hordei, P.janthinellum, P.jensenii, P.kojigenum, P.lanosogriseum, P.lanosoviride, P.lanosum, P.lavendulum, P.martensii, P.notatum, P.ochraceum, P.olivirinoviride, .oxalicum, P.palitans, P.puberulum, P.purpurogenum, P.purpurescens, P.resticulosum, P.spinulosum, P.stoloniferum, P.variabile, P.viridicyclopium, P.waksmanii*	58,6
Weak	1,1-8,2	*A.gregatus, A.apsheronica, A.azerbaijanicus, A.bakunika, A.chazaricus , A.longiphotrus, A.microsporus, A.longus, A.musiformis, A.oligosporus, A.shahriari, A.soprunovii, A.tabrizica, P.atrovenetum, P.brevicompactum, P.canescens, P.corylophiloides, P.corymbiferum, P.cyaneum P.decumbens, P.tardum*	38,0

Table 4.2

Species considered active producers after initial screening of fungi recorded in studies

Genus	Compatible species	Phytotoxic activity (%)
Arthrobotrys	*A.nematopagus*	17,6
	A.compactus,	15,4
	A.doliiformis	16,3
Penicillium	*P.chryzogenum*	41,2
	P.cyclopium	43,7
	P.janthinellum	35,4

determinants of fungal diseases of plants, animals, and humans, characterized by a wide variety of their chemical composition and biological activity. However, they also have similar characteristics, and the characterization of mycotoxins from this aspect is currently one of the issues being widely studied. Considering this, it was considered appropriate to evaluate the metabolism, or more precisely, the metabolic activity, of the active fungi selected in the studies for both endogenous and exogenous metabolites. Before proceeding to the presentation of the obtained results, it would be appropriate to clarify one point, which is related to endogenous and exogenous metabolites. More precisely, what is meant by these "terms"?

As is known, after the synthesis of this or that metabolite occurs inside the cell, it either participates in intracellular processes or is secreted into the extracellular or intercellular spaces. They also characterize the corresponding metabolites and divide them into two groups: endogenous and exogenous. Endogenous metabolites are generally considered to be those that are synthesized intracellularly and released from the cell only as a result of autolysis or mechanical damage, while exogenous metabolites are those that are secreted into the extracellular and intercellular spaces after the synthesis process. Unlike endogenous metabolites, after synthesis of exogenous metabolites, a certain part is secreted outside the cell and a certain part remains inside. For this reason, sometimes this division is conditional and cannot clearly define the boundary between endogenous and exogenous metabolites. Therefore, in our studies, the exogenous metabolites were taken as the basis for the culture solution obtained after separating the biomass formed by fungi during cultivation in a liquid nutrient medium, and the endogenous metabolites in the biocultural solution, which are those that enter the solution after disrupting the cellular structure of the biomass formed by the fungus, were taken as the source of the metabolites. In the work of some authors, endogenous metabolites are also called cell-associated metabolites, because the part of the biomass that is in the intercellular spaces during the disruption of the cell structure is also mixed with endogenous metabolites.

As a source of endogenous and exogenous metabolites in the selected fungi, their biomass formed under deep cultivation conditions in liquid Chapek medium for 5 days and the culture solution separated from it by filtration were taken, respectively and initially their phytotoxic activity was studied comparatively. From the obtained results, became clear that the metabolites causing phytotoxic activity in all selected fungi were exogenous in nature(tab. 4.3). As seen, the difference between the phytotoxic activity of endogenous and exogenous metabolites is 7.3-16.5 times in the genus *Arthrobotrys* and 4.8-10.0 times in the genus *Penicillium*. This fact also allows us to note that secondary metabolites in both fungal genera are exogenous in nature.

Table 4.3

Comparative study of the phytotoxic activity of endogenous and exogenous metabolites of selected fungal species

Compatible species	Phytotoxic activity (%)	
	endogenous	Exogenous
A.nematopagus	2,1	18,1
A.compactus	1,1	16,1
A.doliiformis	1,7	15,4
P.chryzogenum	5,8	40,6
P.cyclopium	7,3	44,1
P.janthinellum	4,4	34,9

At the same time, this can say about the metabolites that cause nematophagous and enzymatic activities of fungal species belonging to the genus *Arthrobotrys*. The determination of what other different or similar properties the toxic metabolites of the studied species have was clarified by clarifying whether they are polysaccharide or protein in nature. From the obtained result became clear that the metabolites synthesized by the fungal species belonging to the genera studied in this issue, which have phytotoxic activity to one degree or another, do not have protein nature. Thus, phytotoxic activity was shown by both endogenous and exogenous metabolites, and not the part that was precipitated by acetone in the ratio of 1:2, but the part that was not precipitated(tab. 4.4). So that At 5000 rpm, the dissolved proteins precipitate from the solution, while the polysaccharides remain in solution. This also suggests that the toxic substances of the studied species are similar in nature.

Table 4.4

Determination of the biochemical nature of the part of endogenous and exogenous metabolites that cause phytotoxic activity

Species	Phytotoxic activity (%)			
	Endogenous		Exogenous	
	Preci-pitated	Remai-ning in solution	Preci-pitated	Remaining in solution
A.nematopagus	0	3,2	0	20,5
A.compactus	0	1,8	0	18,6
A.doliiformis	0	2,5	0	17,8
P.chryzogenum	0	8,8	0	43,4
P.cyclopium	0	9,3	0	46,7
P.janthinellum	0	7,2	0	38,2

4.3. Ecophysiological characteristics of toxigenic species belonging to the genera *Penicillium* and *Arthrobotrys*

The study of the metabolism of fungi, or metabolome according to modern views, is related to both their widespread distribution in nature and their synthesis of biologically active substances with various functions, primarily antitumor, antimicrobial, antioxidant, and other activities. For this reason, it was considered appropriate to evaluate the active fungi selected in the final stage of the research for their metabolic activity and to clarify the nature of the impact of environmental factors on them.

First, it was clarified whether there was a relationship between biomass yield and the total amount of mycotoxins in the sample of exogenous metabolites. To clarify this issue, each fungus was simultaneously cultivated in 3 variants of the same nutrient medium (Čapek medium) that differed in composition (optimal, nitrogen and carbon deficient), one of which was optimized for maximum fungal biomass yield, while the other two were low in basic nutrients such as carbon and nitrogen. The results showed that there was no clear linear relationship between biomass yield and total mycotoxin content, contrary, the optimization of the environment leads to a decrease in the total yield of mycotoxins, that is, an inverse dependence is observed (tab. 4.5). As can be seen, the amount of mycotoxins per unit

Table 4.5

Effect of changing the composition of the nutrient medium on the total yield of mycotoxins

Species of fungi	Optimal variant		Nitrogen - deficient		Carbon-deficient	
	BM*	MT	BM	MT	BM	MT
A.nematopagus	2,87	0,0120	1,65	0,0073	1,78	0,0081
A.compactus	4,12	0,0178	2,87	0,0130	3,12	0,0143
A.doliiformis	3,12	0,0214	2,04	0,0179	2,25	0,0202
P.chryzogenum	3,74	0,1050	2,15	0,0740	2,54	0,0877
P.cyclopium	4,37	0,1086	3,14	0,0853	3,46	0,0975
P.janthinellum	3,25	0,1001	2,08	0,0697	2,23	0,0811

Note: *BM- biomass (g/l), MT- mycotoxins (mkg/l)

biomass is 1.07-1.29 times less in the *Arthrobotrys* genus than in the carbon-deficient variant, and 1.05-1.28 times less than in the nitrogen-deficient variant. In the genus *Penicillium*, this indicator is 1.12-1.25 and 1.08-1.21 times, respectively. Interestingly, mycotoxin production is more intense in all species of both fungal genera under nutrient deficiency, and all fungi are more sensitive to nitrogen deficiency, which is more pronounced in biomass yield. However, the amount of mycotoxins per unit biomass is higher in carbon deficits. Since this situation is manifested in all species, it can be noted that the formation of secondary metabolites occurs by a similar mechanism in both genera of fungi.

During the study of the metabolic activity of the toxigenic species of the genera Penicillium and Arthrobotrys recorded in the studies in ecophysiological aspects, it became clear that they have both similar and different characteristics from each other. Differences are more noticeable between genera, and similar sign are more noticeable between species belonging to the same genus (tab. 4.6). As can be seen, fungi belonging to the genus *Arthrobotrys* are almost identical in terms of cultivation temperature, minimum, maximum and optimal initial pH of the medium.

Table 4.6

The influence of cultivation temperature and initial acidity of the medium on the biomass yield of selected fungi

Species	Cultivation temperature (^{0}C)			Initial pH of the medium		
	MN*	OP	MX	MN	OP	MX
A.nematopagus	5	30	45	3,3	6,2	8,5
A.compactus	5	29	45	3,3	6,1	8,4
A.doliiformis	5	29	45	3,3	6,2	8,4
P.chryzogenum	2	28	41	3,0	5,5	8,0
P.cyclopium	3	28	40	3,0	5,6	8,0
P.janthinellum	3	28	40	2,9	5,5	8,0

Note: * - MN – minimal, OP – optimal and MX - maximum

A similar situation is repeated in species belonging to the genus *Penicillium*. When analyzing the mentioned indicators by genus, it becomes clear that in species belonging to the genus *Arthrobotrys*, both indicators, both minimum and maximum, as well as optimal temperature, are 2-5^{0}C higher than in the genus *Penicillium*, and by 0.4-0.7 higher in pH. This shows that the sensitivity of these fungi to the mentioned parameters is regulated at the genus level. Therefore, at the end of the research, it was determined which environment is optimal for both fungi to produce maximum mycotoxins. From the results obtained when using a medium prepared on the basis of Chapek's medium, it became clear that reducing the indicators considered optimal for biomass yield to one degree or another allows obtaining the highest indicator of total mycotoxin yield(tab. 4.7). An increase in the amount of mycotoxins per unit mycelial mass was observed in the absence of basic nutrients(tab. 4.5), now factors affecting this process are also includes temperature and the initial pH of the environment.

Table 4.7

Optimal environmental parameters for maximum mycotoxin production in the studied fungi

Species	Carbon source (g/l)	Nitrogen source (g/l)	T^{0}C	pH
A.nematopagus	Sucrose (22)	$NaNO_3$(2,1)	28	6,0
A.compactus	Sucrose (20)	$NaNO_3$(2,0)	26	5,7
A.doliiformis	Sucrose (20)	$NaNO_3$(2,1)	27	5,9
P.chryzogenum	Sucrose (21)	$NaNO_3$(2,0)	26	5,2
P.cyclopium	Sucrose (22)	$NaNO_3$(2,0)	25	5,2
P.janthinellum	Sucrose (21)	$NaNO_3$(2,1)	24	5,1

4.4. Characterization of fungi belonging to the genera *Penicillium* and *Arthrobotrys* according to the mycotoxins they synthesize

As a result of the research, it was determined which mycotoxins the selected fungi produced in the optimized environment, and it became clear that the mycotoxins of fungi belonging to the genus Penicillium are characterized by a wider variety(tab. 4.8). Nevertheless, it should be noted that the mycotoxins of both genera differ in their biological activity, and the mycotoxins belonging to the genus Penicillium are more biologically active and medically important.

Table 4.8

Mycotoxins produced by the studied fungi

Species	Carbon source (g/l)
A.nematopagus	Oligosporan, oligosporol A and B , hydroxyoligosporone
A.compactus	Oligosporan, 4′,5′- dihydrooligosporone, oligosporol A,
A.doliiformis	Oligosporan, oligosporol A and B, 10′,11′-epoxyoligosporone
P.chryzogenum	Aflatoxin, ochratoxin, citrinin, chrysogin, roquefortin, melagrin, penicillin, xanthocillin X and ergosterol
P.cyclopium	Penicillin and cyclopiazonic acids, xanthomegnin, viomellein, patulin, ochratoxin
P.janthinellum	Penicillin acid, fumitrmorqin A, B and C, penitrem A, B, C and E

For example, penicillin acid once led to the beginning of the antibiotic era and is still used for medical purposes today. The toxic metabolites synthesized by fungi belonging to the genus *Arthrobotrys* consist of compounds such as oligosporan and oligosporol and their derivatives, and have weak pharmacological activity.

FINAL ANALYSIS OF THE RESULTS OBTAINED IN THE RESEARCH

One of the current research directions of the modern era is the study of living organisms, primarily fungi, that synthesize metabolites with toxic effects as a result of their life activities. Thus, as a result of the activity of these fungi, food and feed products, and raw materials are contaminated with mycotoxins synthesized by them. As a result of research conducted in this direction since the 60s of the last century, it became clear that the most dangerous source of this problem is fungal species belonging to the genera *Fusarium, Aspergillus, Penicillium*, and *Alternaria*. Although the distribution of these fungi is predominant in products and raw materials for one or another purpose, the diversity of the chemical composition of products and raw materials and the different storage conditions make it necessary to conduct regional studies to determine the ability of these fungi to produce individual toxins. Therefore, there is not enough literature on this topic. On the other side, about 20 years ago, the number of species of toxigenic fungi was 250, and the number of mycotoxins synthesized by them was 300, currently, these numbers vary between 300 and 500, respectively, that is, both the species of these fungi and the number of mycotoxins synthesized by them continue to increase. Interestingly, one fungi can synthesize several toxins, and several fungi species can synthesize one toxin. If we add to the above that a number of fungal species have not become the subject of research in this direction, the synthesis of toxic metabolites of this or that fungus is affected by the abiotic and biotic factors of the environment in which the fungus is distributed, and the fungi currently known to science constitute a small part (4-7%) of those actually found in nature, then it can be said with confidence that even the study of species known to science in this aspect is a very relevant issue. Although the distribution of toxigenic fungi in the territory of the Republic of Azerbaijan has become the subject of some studies conducted in recent years, neither the toxic metabolites of many species nor the issues related to their identification have been addressed in those studies.

Therefore, taking into account the above, the aim of the presented study was to determine the phytotoxic activity of fungi belonging to the genera *Penicillium* and *Arthrobotrys*, which are widespread in Azerbaijani conditions, the factors affecting the synthesis of mycotoxins, that is, their ecophysiological properties, as well as the mycotoxins they synthesize.

RESULTS

1. A total of 265 strains were isolated from the samples taken from the soils of the Absheron Peninsula of the Republic of Azerbaijan, and as a result of their identification by classical mycological methods, it was found that 35.1% (93 strains) of the isolated strains belong to the genera *Arthrobotrys* and *Penicillium*, and as a result of determining their species composition, it was found that there are 17 species belonging to the genus *Arthrobotrys* and 41 species belonging to the genus *Penicillium*. This is the first time that 5 species of the genus *Penicillium*(*P.atrovenetum, P.clavigerum, P.duclauxii, P.palitans* and *P.viridicyclopium*) have been discovered in the nature of Azerbaijan[1, 5, 7-9, 12, 14].

2. It has been determined that although all species belonging to the genera *Penicillium* and *Arthrobotrys* have toxic activity to one degree or another, those with strong toxic activity are quite few, and this is confirmed in the example of species such as *Penicillium chryzogenum* (41.2%) and *P.cyclopium* (43.7%). Of the recorded species, 58.6% (11.2-35.4%) have moderate phytotoxic activity, while 38% have weak activity (1.1-8.2%) [5, 7, 13].

3. A comparative study of the endogenous and exogenous metabolites of the species with the highest phytotoxic activity of the studied *Penicillium* and *Arthrobotrys* genera revealed, that, metabolites that cause phytotoxic, nematophagous and lipolytic activities are mainly exogenous in nature. Thus, the difference between the phytotoxic activity of endogenous and exogenous metabolites is 7.3-16.5 times in the genus *Arthrobotrys* and 4.8-10.0 times in the genus *Penicillium*. It has been determined that both endogenous and exogenous metabolites that cause phytotoxic activity in species belonging to the genera *Penicillium* and *Arthrobotrys* are not proteinaceous in nature[6-7, 10-11, 13].

4. It was clear from the conducted research that there was no clearly expressed linear relationship between the yield of

biomass and the total amount of mycotoxins, on the contrary, the optimization of the environment leads to a decrease in the yield of total mycotoxins, that is, an inverse relationship is observed. Thus, the amount of mycotoxins per unit biomass is 1.07-1.29 times less in the *Arthrobotrys* genus than in the carbon-deficient variant, and 1.05-1.28 times less than in the nitrogen-deficient variant. In the genus *Penicillium,* this indicator is 1.12-1.25 and 1.08-1.21 times respectively [4].

5. In the absence of basic nutrients such as carbon and nitrogen in the environment, the formation of mycotoxins occurs more intensively in all species belonging to the genera *Penicillium* and *Arthrobotrys*, and all fungi are more sensitive to nitrogen deficiency, which is more pronounced in biomass yield. This also occurs when the cultivation temperature and initial pH of the medium decrease relative to the optimum. Since this situation is observed in all species, it can be noted that the formation of secondary metabolites occurs by a similar mechanism in both genera [4, 13].

6. Mycotoxins produced by species belonging to the *Penicillium* and *Arthrobotrys* genera in optimized environments have been identified and, it has been determined that while species belonging to the genus *Penicillium* synthesize aflatoxin, ochratoxin, citrinin, chrysogin, roquefortin, melagrine, penicillin, xanthocillin X, ergosterol, penicillic acid, and other pharmacologically important mycotoxins, the toxic metabolites synthesized by fungi belonging to the genus *Arthrobotrys* consist of compounds such as oligosporan and oligosporol and their derivatives, which have weak pharmacological activity [1, 4].

PRACTICAL RECOMMENDATIONS

1. It is advisable to use new information about species belonging to the genera *Penicillium* and *Arthrobotrys* isolated from various ecosystems of the Republic of Azerbaijan in the research as baseline data for the future assessment of the

ecological situation from a mycological aspect and the preparation of new regulatory documents in this regard.

2. The results obtained during the research are useful in terms of more efficient use of the studied fungal genera, especially species of the genus Penicillium, as active producers of secondary metabolites of practical importance, as well as in terms of clarifying the points that should be paid attention to for the future use of other fungi that match this characteristic.

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