**REPUBLIC OF AZERBAIJAN**

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# ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

# BIOTECHNOLOGICAL POTENTIAL OF EDIBLE SPECIES OF XYLOTROPHIC MACROMYCETES SPREAD IN SOUTH REGION OF AZERBAIJAN

Speciality: 2430.01 – Micology

Scıentıfic fıeld: Biology

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**Baku–2021**

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## INTRODUCTION

**Relevance of the subject.** One of the problems of our time is the increasing of anthropogenic impact on the environment, and as a result, changes in the ecosystem occur. Thus, "*the relationship between plants, animals, fungi and other biodiversity determines the state of the ecosystem, and any factor that causes them to change, at the same time, causes a change in the ecosystem as a whole*"[1]. These changes are often characterized by a negative aspect, it it explained by the observation of the unpleasant situations as an increase in the number of diseases and deaths among their manifestations etc. As a result, there is an increase in cardiovascular and oncological diseases, a weakening of the immune system, and they continue to grow both in number and in area. Their prevention is one of the topical areas of modern research.

The focus of these studies is "*aimed at obtaining functionally active drugs with antibacterial, antiviral, antifungal and antitumor effects, which generally strengthen the human immune system*."[2] Obtaining such medicines is important from natural sources, primarily from "*biological resources*"[3]. In this regard, one of the main directions of modern microbiology, mycology and biotechnology is to determine the possibility of using fungi for these purposes, including edible species of basidiomycetes, belonging to xylotrophs.

Nowadays, xylotrophic macromycetes are in the focus as manufacturers of compounds containing many biologically active substances, including proteins, enzymes, lipids, polysaccharides,

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[1] Flandroy, L., Poutahidis, Th., Berg, G., Clarke, G., Maria-Carlota D., Decaestecker, E.The impact of human activities and lifestyles on the interlinked microbiota and health of humans and of ecosystems//Science of The Total Environment, 2018, v.627, p.1018-1038

[2] Elkhateeb, W.A., Daba, G.M., Thomas, P.W., Wen, T.C. Medicinal mushrooms as a new source of natural therapeutic bioactive compounds. // Egypt Pharmaceut J, 2019, v.18, p.88-101

[3] Newman D.J. and Cragg G. M. Natural Products as Sources of New Drugs from 1981 to 2014.//J. Nat. Prod. 2016, 79, 3, p.629-661

polyacetylenes, organic acids, vitamins, etc., and even in some countries, production processes were followed suit. These substances are biologically active, including pharmacologically active, less toxic than substances obtained by chemical synthesis, and their use does not cause to side effects.

As of today, "*more than 3000 substances have been extracted from fungi, including antibiotics, antifungal, immunomodulatory, hypolipodemic, hepatoprotective, antihelminthic, lipotensive, insecticidal, thrombotic and antidiabetic compounds"*[4-5]. Antibiotics and other drugs obtained from mushrooms "*have effective antibacterial, antifungal and anti-AIDS effects, in addition to stimulating the immune system in the human body*"[6-7]. Considering that, these medicines do not have side effects on the body, and are cheaper and more economical raw materials than plant and animal sources, so the degree of relevance of research in this area becomes clear.

Although it is currently impossible to give a specific number of species of xylotrophic macromycetes, there is no doubt that this number exceeds 1000. The prevalence of xylotrophic macromycetes in Azerbaijan has been confirmed in the study. According to the results of these studies, "*the number of species in Azerbaijan is 2012*"[8]. A small number of xylotrophic macromycetes, registered

[4] You, Q., Yin, X., Ji, C. Pulsed counter-current ultrasound-assisted extraction and characterization of polysaccharides from *Boletus edulis*. // Carbohydr Polym, 2014, 101, p.379–385

[5] Zhang, L. X., Zhang, Y. J. & Zhang, L. P. Extraction and purification of polysaccharide from *Ganoderma lucidum* and its immunological activities. // *Journal of Northwest A & F University* 2014, 9, p. e86216–e86216.

[6] Adotey, G., Quarcoo, A, Holliday, J.C., Fofie, S., Saaka, B. Effect of an immunomodulating and antiviral agent of medicinal mushrooms (immune assist 24/7) on CD4+ T-lymphocyte counts of HIV-infected patients. // Int J Med Mushrooms, 2011, v.13(2), p.109-13.

[7] Sum, W.Ch., Indieka, S.A. and Matasyoh, J.C. Antimicrobial activity of Basidiomycetes fungi isolated from a Kenyan tropical forest. // African Journal of Biotechnology, 2019, Vol. 18(5), p.112-123

[8] Akhundova N.A., Orucova S.B., Bahshaliyeva K.F., Muradov P.Z. and Rahimov E.A. Evaluation by the Oxidase Activity of Xylotropic Macromycetes Causing White Decay. //Advances in Bioscience and Biotechnology, 2019,10, p.179-187

both in the world and in Azerbaijan, were involved in research as producers of biologically active substances. One of the results of these studies is the difference in the biosynthetic ability of xylotrophic macromycetes, as well as the amount of biomass formed in a particular biotope.

For this reason, a fungal strain isolated from a particular biotope is more likely to be more productive, and the relevance of research in this area remains high.

Finally, it should be noted that xylotrophic macromycetes include species belonging to the category of edible fungi, "*some of which (mainly Agaricus bisporus, Pleurotus ostreatus and Lentinula edodes) are intensively cultivated for many years for food and product (fruit body) is measured in millions of tons, and this volume is growing*"[9-10]. Intensive cultivation of xylotrophic macromycetes is also observed in Azerbaijan, but there are no exact data on the amount of products produced and the number of cultivated species, but, according to personal observations, the cultivated species are mainly *Agaricus bisporus* and *Pleurotus ostreatus*.

The number of xylotrophic macromycetes cultivated both in the world and in Azerbaijan is rather small, and this is because many fungi belonging to this category can form fruit bodies only in natural conditions, and their intensive cultivation has not yet found practical application. On the other hand, the cultivation of these fungi in pure culture is already in the focus of attention, but in this respect, the parameters necessary for the use of xylotrophic macromycetes as biological resources are not fully determined. If we add to the above mentioned that the research carried out in this direction in Azerbaijan is mainly of an episodic nature, then it is important to conduct research to clarify this issue in the conditions of Azerbaijan.

**The purpose and objectives.** The purpose of this work is a comprehensive study of edible species of basidiomycetes belonging

[9] Zhang Y., Geng W., Shen Y., Wang Y. and Dai Y-Ch. Edible Mushroom Cultivation for Food Security and Rural Development in China: Bio-Innovation, Technological Dissemination and Marketing Sustainability, 2014, 6, p.2961-2973

[10] Grimm, D. and Wösten H.A.B. Mushroom cultivation in the circular economy// Appl Microbiol Biotechnol., 2018, v.102(18), p.7795–7803.

to the xylotrophs, widespread in the southern region of Azerbaijan, as well as in other ecologically different regions, depending on their species composition, frequency of occurrence, as well as the amount of fruit body (FB) in natural conditions, and is devoted to a comprehensive study of biotechnological parameters of vegetative mycelium.

To achieve this goal, it is considered appropriate to solve the following tasks:

- Determine the species composition, frequency occurrence and ecological-trophic relations of xylotrophic macromycetes common in the southern region of Azerbaijan, as well as in other regions;
- Evaluation of edible species of xylotrophic macromycetes by the number of FB formed in natural conditions, the number of formation waves and stocks;
- Release of edible species of xylotrophic macromycetes into pure culture, screening of isolated strains for biomass release and selection of an active producer;
- Comparative assessment of biochemical composition and biological activity of edible species of xylotrophic macromycetes in natural conditions and in pure culture.

**Research methods**. In order to conduct the research in the dissertation, mycological (registration of the distribution of macromycetes, their release into a pure culture and their classification by species composition, frequency of occurrence, etc.), physiological and biochemical (determination of the biomass yield in the phase of vegetative growth and optimization of the environment for its appearance,) determining the biochemical composition of FB and VM, determining the biological activity of metabolites, etc.) and biotechnological methods and approaches (methods for determining the amount of MC in vivo, obtaining feed additives, etc.) have been used.

The accuracy of the data obtained in researches lies in the fact that currently various methods of mycological research are of widely used. The accuracy of the equipment and instruments used for analysis, the required level of purity of chemical reagents and

reagents used for analysis, the repetition of experiments allows statistical processing results and the fact that the deviations do not exceed the accepted level, i.e. correspond to the formula m / M = P≤0.05, the results are repeatedly discussed at international and local conferences and published in prestigious scientific journals.

**The main points of the dissertation defense:**

- The wide variety of forests in the southern part of Azerbaijan also makes it richer in xylomycobiota than in other zones;
- Edible fungi are also involved in the formation of xylomycobiota of forests in the southern region of Azerbaijan, which also differ from each other in the amount of FB that they naturally produce and the amount of biomass they produce during the vegetative growth phase;
- Although both natural fruiting bodies and pure cultures (vegetative mites) of edible fungi found in the southern part of Azerbaijan are suitable for use as a source of biologically active substances, the use of vegetative mycelium is more efficient from economic and technological point of view;
- Edible fungi are good producers of not only food, but also feed additives.

**Scientific innovations:** As a result of the research, the species composition of xylomycobit in the forests of the southern region of Azerbaijan and belonging to the category of edible fungi included in the physiological-biochemical and potential both FB and VM in pure culture was determined as the biotechnology of the source of biologically active substances was evaluated from the point of view of oji.

It was found that 53 species of xylotrophic macromycetes are spread in the studied territories, 9 of which belong to the category of edible fungi. They also differ in the frequency of occurrence in natural conditions, ecological-trophic relations, distribution in different substrates, and in the case of vegetative mycelium - in the amount of biomass formed and the quantitative indicator of the biological activity of their metabolites.

The dominant *Laetiporus sulphureus* and *Pleurotus ostreatus*, *Armillaria mellea* and *Polyporus squamosus* were found to be the

most common, while the rest are random and rare, with only *A. mellea* being biotrophic and the rest being polytrophic.

For the first time in the study, a mathematical approach was used to calculate the number of fruit bodies formed by 9 edible fungi in Azerbaijan in natural conditions, and it was determined that 216.72 tons of fruit bodies can be formed in the southern region of Azerbaijan per year.

It has been established that both the MS, formed by fungi in natural conditions, and the biomass formed in the phase of vegetative growth, can be used as a source for obtaining biologically active substances. Nevertheless, the use of VMs is beneficial both from an economic and a technological point of view.

It has been established that the use of fungi *G.lucidum* B-09, *L.sulfureus* B-18 and *P.ostreatus* B-25 as producers of biologically active substances and their carbon source for this purpose is glucose (0.97-1.0%). It is more expedient to grow such products for 5-7 days under conditions of liquid-phase fermentation (deep cultivation) at 28°C in an environment with a nitrogen source NH4NO3 (0.036-0.038% nitrogen) and peptone (0.28-0.30%).

**Theoretical and practical importance of work.** The obtained results are factual material for the bank of information on xylotrophic macromycetes, which unambiguously confirms the prospects of their use in the near future.

The methodological approach presented in the study makes it possible to assess more accurately the bioresource potential of xylotrophic macromycetes, especially their edible species, both in natural and in laboratory conditions.

Because of the research, conditions that allow the use of edible species of xylotrophic macromycetes in Azerbaijan as producers of various biologically active substances, which creates a real basis for organizing the future production of various products (food, feed and medicinal) have been identified.

*Pleurotus ostreatus* B-25, which is reported to be widespread in Azerbaijan, has been tested in the poultry industry as a feed additive, creating new opportunities for both more efficient resource use and increased livestock production.

**Approval and application of publication and dissertation.** 12 scientific works on the topic of the dissertation were published, nine of which are scientific articles. The materials of the dissertation were presented at the International Scientific Conference "Actual Problems of Biological and Chemical Ecology" (Russia, Moscow, 2014), Scientific-Practical Conference on "Actual Problems of Modern Biology and Chemistry" (Ganja, 2014), 5th. reported at an international conference (Ukraine, Kiev, 2019).

The results of the dissertation were tested with a positive result in poultry industry in production conditions jointly with the the Veterinary Research Institute of the Center for Agrarian Science and Innovations of the Ministry of Agriculture of the Republic of Azerbaijan.

**The organization where the dissertation work was implemented.** The dissertation work was carried out in the laboratories of biologically active substances and microbiological biotechnology of the Institute of Microbiology of ANAS.

**Structure and volume of dissertation:** The dissertation consists of an introduction, a summary of the literature (Chapter 1), a description of research materials and methods (Chapter 2), a presentation of the results and their interpretation (Chapters 3 and 4), a final analysis of the research, results, bibliography and abbreviations list used in the work. The dissertation consists of a total of 137 pages and contains 222565 characters.

## CHAPTER I.

## XYLOTROPIC MACROMISCETS: SPECIES COMPOSITION, MECHANISMS, PERSPECTIVES AS PRODUCERS OF BIOLOGICAL ACTIVE SUBSTANCES

This part of the dissertation provides with general data on the species composition of xylotrophic macromycetes and their ecological functions in nature. Then, treats that indicate the perspectives of their use as producers of biologically active substances were revealed, including pharmacologically active ones,

and according to literature data, their use for economic, environmental and technological reasons is justified. Finally, the literature on edible species of xylotrophic macromycetes and their use in modern food products, feed, medicine, etc. is analyzed. .In this regard, it is justified that the study of edible species of xylotrophic macromycetes, widespread in Azerbaijan, is not studied completely.

## CHAPTER II

## MATERIALS AND METHODS

The research was conducted mainly in the southern forests of the Republic of Azerbaijan. At the same time, in order to compare a number of indicators the samples were taken from plain and mountain forests located in large geomorphological units such as the Greater and Lesser Caucasus too.

The traditional route (width 5-6 m, length 500 m to 5000 m) and permanent area (size 50mx50m) were used to take samples of xylothorphic macromycetes, more precisely the MC formed in natural conditions, and more than 500 samples were collected totally. The species composition of the fungus was determined based on the morphological description of the FB and the data obtained from the microscopic examination.

The frequency of occurrence of fungi in natural conditions (P) was determined according to the following formula:

$$P(pc/ha)= N/S \quad (1)$$

Here, N is the number of fruit body (pc) recorded along the selected route and belonging to a particular species, S is the area of the selected route (ha). In order to find the area of the route, the dimensions en (a) and length (b) expressed by it were used, i.e S = ab.

The following mathematical calculations were used to determine the number of fruit bodies formed by fungi in natural conditions.

$$M = [(X_1+X_2+.....+Xn)/n]x(SP) \quad (2)$$

Here, M – the weight of the fruit body formed by the fungus in natural conditions (t), $X_1$, $X_2$, ..... Xn - the mass of FB formed by the fungus in natural conditions 24 hours after plucking (kq), n – FB number (n), S – the total area of the studied forest (ha), P – the frequency of occurrence of FB determined according to formula 1

Obtaining a pure culture of the fungus in agar malt juice (AMC) was carried out according to a known method. Cultivation of pure cultures in liquid glucose-peptone medium (DGPM) was carried out under deep cultivation at 26-280 ° C for 5 days in accordance with similar methods. At the end of the period, the resulting biomass is crushed in a magnetic stirrer and used as planting material. During the evaluation of edible fungi for biomass formation in the vegetative phase, cultivation was carried out for 3-10 days under deep cultivation conditions (DP, 160-180 cycles / min, 26-280 ° C). The obtained biomass is separated from the nutrient medium by centrifugation (10 min, 5000 rpm) and evaluated in accordance with the method of reducing dry weight at 1050 ° C, and the biomass output is expressed in g / l or mg / ml.

Evaluation of the growth of fungi in a solid nutrient medium was carried out and the calculation of the growth factor (GF) was conducted according to the following formula:

$$GF = dhs/t \quad (3)$$

Here, d – diameter of the fungus colony (mm), h – height of the fungus colony (mm), s – The density of a colony on a 5-point scale is determined by visual image və t – cultivation date (day).

The determination of the toxicity of fungal cultures in relation to the infuser (*Tetrahymena pyriformis*), as well as the ability of seeds to germinate, was carried out according to "*the method used in the work of K.F. Bakhshaliyeva*"[11].

When determining the biochemical composition of FB and VM specific for fungi, the amount of proteins was determined by the Keldahl method (N × 6.25; N × 4.38), the total amount of carbohydrates - by the Bertrand method, and the amount of water-

[11] Baxşəliyeva, K.F. Azərbaycanda yayılan toksigen göbələklərin ekobioloji xüsusiyyətləri: /B.e.d....dissertasiyanın avtoreferatı. / -Bakı: 2017, -43 s.

soluble carbohydrates according to the Shomody-Nelson method, the amount of nucleic acids (the sum of RNA and DNA) was determined spectrophotometrically on an SF-2000 instrument, and the amount of fat was determined on the basis of a Soxhlet instrument.

The cultivation during the production of the feed additive from *P. ostreatus* was carried out in accordance with "*the methods used in the work of P.Z. Muradova*"[12] in solid-phase fermentation.

The experiments were carried out in at least 4 repetitions, and the results were "*statistically processed*"[13].

## III CHAPTER
## CHARACTERISTICS OF CYSLOTROF MACROMISETS SPREAD IN THE SOUTHERN REGION AND OTHER TERRITORIES OF AZERBAIJAN ON SPECIES COMPOSITION, FREQUENCY OF OCCURRENCE AND SPREADING ON SUBSTRATES

### 3.1. Characteristics of xylotrophic macromycetes, widespread in the southern region of Azerbaijan, in terms of species composition, ecological-trophic relations and distribution in substrates

During 2014-2018, analysis of samples collected in forests located in the study areas revealed the distribution of 93 species of xylotrophic basidiomycetes, 52 of which were found in the forests of the southern region of Azerbaijan. They are characterized due to their ecological and trophic relationships, as well as the rot color that they form in natural conditions, their hyphal systems, their distribution in substrates, etc. For example, 7.7% of registered fungi are biotrophs, 7.7% are saprotrophs, and the remaining 84.8% are

[12] Muradov P.Z. Bitki tullantılarının biodeqradasiyası prosesində hidrolaza və oksidazaların aktivliyinin dəyişilməsi. /B.e.d....dissertasiyanın avtoreferatı. / -Bakı: 2004, -45 s.

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

fungi whose saprotrophy and biotrophy are not real, i.e. optional or polytrophic. Under natural conditions, 84.6% of fungi cause white rot and 15.4% brown rot.

A similar difference was observed in the fungal hypha system and in the frequency of occurrence (FO). Thus, among the registered fungi, both monomycetes (for example, *F. nigrescens*, etc.), Dimitics (*Trametes zonatus*, etc.), and trimitics (*Pseudotrametes gibbosa*, *Pycnoporus cinnabarinus*, etc.) are found due to their hyphal systems. 5 species of the registered fungi are recorded as dominate *(Fomes fomentarius, Fomitopsis pinicola, Ganoderma applanatum, Phellinus igniarus* and *Trametes versicolor*) (1.1–2.7 units / ha), 25 species as widespread (0.12–0.92) units / ha) and the remaining 27 species are characterized by random and rare (0.00043-0.09 units / ha). Regarding the distribution of the registered fungi by substrates, it found that most of the fungi belong to eutrophils. More precisely, 86.5% of registered fungi are eurytropics, 7.7% are conditional stenotrophic, and 5.8% are stenotrophs.

It was found that the registered species, such as *Polyporus umbellatus* and *Pluteus aurantiorugosus*, are new to the xylomycobiota, which is unique to the nature of Azerbaijan.

### 3.2. Edible species of xylotrophic macromycetes distributed in the southern region of Azerbaijan and their general characteristics

The edible species of fungi found in the course of investigation have been evaluated in accordance with the species composition and resource potential. It was found that 10 fungi transferred into pure culture and isolated from other territories, including the southern region of Azerbaijan, belong to the category of edible fungi, and 9 of them (*A.mellea* – RT=0,25 units/ha, *F.hepatica* - RT=0,000021 units/ha, *F.velutipes*- RT=0,11 units/ha, *G.lucidum*- RT= 0,12 units/ha, *L.sulphureus*- RT=0,78 units/ha, *P.tigrinus*- RT=0,12 units/ha, *P.ostreatus*- RT=0,76 units/ha, *P.squamosus* - RT=0,12 units/ha, və *P.umbellátus* - RT=0,0017 units/ha) is found in the

southern regions of Azerbaijan. During the study period, *K.mutábilis* (FO = 0.0012 units/ha) was found only in the Greater Caucasus.

With regard to the distribution of fungi on individual trees (substrates), it was found that 9 fungi belong to eutrophics, and 1 to stenotrophics (*P.umbellátus*). The FO of registered fungi is also characterized by different indicators, since *L.sulfureus* and *P.ostreatus* are the highest among fungi, species such as *A.mellea*, *P.tigrinus, P.squamosus, G.lucidum* and *F.velutipes* are moderate, *P. .umbellátus, K..mutábilis* and *F.hepatica* were registered with the lowest rates.

It was found that 4 species of fungi are monomitic (*A.mellea, F.hepatica, F.velutipes* and *P.ostreatus*), 5 species are dimitic (*K.mutábilis, L.sulphureus, P.tigrinus, P.squamosus* and *P.umbellátus)* and one species has a trimitic *(G.lucidum)* hyphal system.

## CHAPTER IV
## ECOLOGICAL, PHYSIO-BIOCHEMICAL AND BIOTECHNOLOGICAL BASIS FOR ESTIMATION OF BIORESOURCE POTENTIAL OF XYLOTROPIC MACROMISETS DISTRIBUTED IN AZERBAIJAN

### 4.1. Obtaining pure cultures of edible species of xylotrophic macromycetes and their evaluation by biomass output

In the course of the investigation, it was considered to conduct a comparative study of FB and VM as a source of products for various purposes. Therefore, in the first study, 32 strains of edible xylotrophic fungi isolated from pure culture (20 from the southern regions, 8 from the Greater Caucasus, and 4 from the Lesser Caucasus) were evaluated from biomass output from a Glucose Peptone Agar (GPA). It became clear that all strains differ from each other for biomass they produce, and this difference applies even to individual strains of the same species (Table 4.1.1).

As you can see, the highest biomass is observed in the *P.ostreatus* strain, the lowest in the P.umbellátus strain, and the difference between them is a maximum of 1.96 times.

Table 4.1.1

Evaluation of edible species of xylotrophic macromycetes recorded in studies for biomass output in the vegetative phase

| Fungi species | Number of strains(pcs) | Cultivation date (day) | Biomass output (g/l) |
|---|---|---|---|
| *Armillaria mellea* | 5 | 5 | 3,2-4,1 |
| *Fistulina hepatica* | 1 | | 2,9 |
| *Flammulina velutipes* | 3 | | 3,1-4,0 |
| *Ganoderma lusidum* | 5 | | 4,0-4,7 |
| *Kuehnerómyces mutábilis* | 3 | | 2,7-3,9 |
| *Laetiporus sulphureus* | 5 | | 3,7-4,6 |
| *Panus tigrinus* | 4 | | 3,0-4,0 |
| *Pleurotus ostreatus* | 5 | | 4,3-4,9 |
| *Polyporus squamosus* | 3 | | 2,7-4,0 |
| *P.umbellátus* | 1 | | 2,5 |

It should be noted that durıng the evaluatıon of the growth factors of pure cultivated strains of fungi, including xylotrophic macromycetes, has been conducted for fungi with high GF-the fungi with GF over 50, for moderate GF over 30-50, for weak GF- less than 30. It was found that the biotic potential of 4 of the tested strains (GF = 50.2-60.5) was fast, 16 (GF= 35.4-46.7) and 12 (GF= 20.1-32.1) had the low biotic potential. As a result of this stage, the strains *G.lucidum* B-09, *L.sulfureus* B-18 and *P.ostreatus* B-25 were recognized as the most active in terms of biomass output.

Interestingly, all three strains were separated from the forests in the southern part of Azerbaijan during the study and differed from each other by the substrate in which they inhabited. Thus, strain *G.lucidum* B-09 formed in pure culture taken from FB of hornbeam, strain *L.sulfureus* B-18 - of linden, and strain *P.ostreatus* B-25 – of oriental peanuts.

## 4.2. Optimization of the environment for biomass output from fungi selected as active producer

It should be noted that environmental factors also play a role in the emergence of living things, and sometimes this is expressed in significant quantities. Therefore, the medium for the selected strains is optimized for the main parameters. The optimal indicators identified during the study are summarized in Table 4.2.1.

Table 4.2.1

Main parameters of an optimized environment

| Fungi | C source (content, %) | Mineral N (due to nitrogen %) | Organic N (%) | T, $^0$C | pH | Cultivation condition | Method and duration of preparation of planting material |
|---|---|---|---|---|---|---|---|
| *G. lusidum* B-09 | Glucose (0,97) | $NH_4NO_3$ (0,038) | Peptone (0,28) | 28 | 5,5 | DC | GPA 6 days |
| *L.sulphureus* B-18 | Glucose (0,95) | $NH_4NO_3$ (0,036) | Peptone (0,30) | 28 | 5,3 | DC | GPA, 7 days |
| *P.ostreatus* B-25 | Glucose (1,00) | $NH_4NO_3$ (0,040) | Peptone (0,28) | 28 | 5,8 | DC | GPA, 5 days |

As can be seen, the strains selected as an active producer differ in some respects from each other in the quantitative value of one or another parameter. However, the known parameters of the culture medium taken for cultivation, as the result of optimization, biomass output increased by 10% in the *G. lusidum* B-09 strain, by 8% in the *L. sulfureus* B-25 strain and by 5% in the *P.ostreatus* B-25 strain and the amount of produced biomass is 5.0, 4.9 and 5.4 g / l, respectively.

It should be noted that it was considered appropriate to compare strains with known producers selected as active producers in studies in various scientific centers and it was determined that the fungi recorded in the studies in one way or another, they are not inferior to known mushrooms or even surpass them. However, the development of more reliable criteria for cultures that will move from laboratory to production should not be overlooked.

## 4.3. Comparative study of the FB and VM biochemical composition of active producers.

In the next step of study the biochemical composition of the biomass of fungal cultures obtained from GPA and FB formed in natural conditions was compared (Table 4.3.1). As can be seen, the highest protein content is observed in the MV of *Pleurotus ostreatus,* and the lowest in the FB of *Armillaria mellea*.

Table 4.3.1

FB and VM biochemical compositions of xylotrophic macromycetes (dry weight, %)

| Fungi | Protein | | Carbohydrates (including soluted sugar) | | Fats | | Nucleic acid | |
|---|---|---|---|---|---|---|---|---|
| | FB | VM | FB | VM | FB | VM | FB | VM |
| *A.mellea* | 20,3 | 22,4 | 29,3(1,8) | 32,2(2,2) | 1,4 | 1,6 | 0,67 | 0,89 |
| *F.hepatica* | 27,1 | 30,2 | 26,7(1,5) | 27,5(1,4) | 2.2 | 2,5 | 0,64 | 0,81 |
| *F.velutipes* | 23,7 | 25,1 | 28,7(2,1) | 30,3(2,0) | 1,9 | 2,1 | 0,54 | 0,62 |
| *G.lusidum* | 26,5 | 29,3 | 31,2(2.4) | 33,4(2,7) | 2,1 | 2,2 | 0,47 | 0,76 |
| *K.mutábilis* | 27,5 | 22,5 | 27,3(1,5) | 28,5(1,7) | 1,7 | 1,9 | 0,59 | 0,87 |
| *L.sulphureus* | 28,5 | 29,0 | 30,5(2,3) | 32,1(2,5) | 1,1 | 2,7 | 0,54 | 0,78 |
| *P.tigrinus* | 23,2 | 24,1 | 29,4(1,9) | 30,3(2,3) | 1,8 | 2,0 | 0.59 | 0,90 |
| *P.ostreatus* | 29,4 | 32,2 | 29,3(2,4) | 30,7(3,2) | 2,3 | 2,7 | 0,52 | 0,64 |
| *P.squamosus* | 24,2 | 25,2 | 26,2(2,1) | 28,9(2,2) | 1,5 | 1,7 | 0,62 | 0,77 |
| *P.umbellátus* | 22,4 | 23,2 | 23,2(1,6) | 27,6(2,0) | 1,4 | 1,7 | 0,61 | 0,75 |

According to the total quantity of carbohydrates the highest indicator is observed in VM of the fungus Ganoderma lusidum, the lowest in the naturally produced FB of the fungus P.umbellátus. Although all component elements are found in both substances, the quantity of components in VM is rather higher than those in the FB.

It is observed in strains of fungi such as *G. lusidum, L.sulphureus* and *P.ostreatus* in high degree.

From the studies carried out for the determination of the presence or not of biologically active components in the composition of extracts obtained from fungi it became apparent that both substances belonging to the selected fungi have biologically active components and this is evidenced by an increase in the number of infusoria in a medium where extracts belonging to fungi were added. At this time the effect of increase becomes different depending on the source of extract and the highest effect (2,75 times) is observed in the extract obtained from VM of the fungus *G.lucidum.*

A similiar situation shows itself in the germination ability of seeds as well and the germination ability of seeds depending on fungi increased by 3–7%. All of these, although not directly, allow us to note the presence of biologically active metabolites in the extracts obtained from the noted fungi.

The study also clarified the identification of the natural resources of individual fungi as bioresources. Based on the results of studies carried out in this direction, first of all, the approximate amount of MC formed by fungi in natural conditions was calculated, and it was determined that fungi have a different share in this process
(Table 4.3.2). As you can see, the largest share falls on *P. ostreatus* and *L.sulfureus,* and the amount of FB produced by them is 87.1% of the total.

Table 4.3.2.

Approximate amount of FB formed by xylotrophic macromycetes under natural conditions

| Fungi | The number of waves in FB formation (times) | Annual weight of FB (t) | Share in total (%) |
|---|---|---|---|
| *A.mellea* | *2* | 14,250 | 6,58 |
| *F.hepatica* | *1* | 0,0025 | 0,001 |
| *F.velutipes* | *2* | 0,093 | 0,043 |
| *G.lusidum* | *1* | 0,090 | 0,042 |
| *K.mutábilis* | *1* | 0,190 | 0,088 |
| *L.sulphureus* | *2* | 90,00 | 41,53 |
| *P.tigrinus* | *2* | 5,00 | 2,31 |
| *P.ostreatus* | *2* | 98,80 | 45,59 |
| *P.squamosus* | *2* | 8,20 | 3,78 |
| *P.umbellátus* | *1* | 0,090 | 0,042 |
| Total | | 216, 72 | 100 |

It should be noted that this figure may be more or less, since in all these fungi FB is annual, and its formation is also influenced by the climatic conditions of the environment. The method of intensive cultivation of most of these fungi has not yet been developed; therefore, it can be said with confidence that the natural resources of FB are limited, which can be noted as a stimulating effect to the growing interest in FB.

## 4.4. Obtaining of fodder products from edible species of xylotrophic macromycetes.

In order to the produce new feed products from xylotrophic macromycetes, the possibility of using the feed additive obtained by cultivating of *P.ostreatus* B-25 in wheat bran under solid phase fermentation (SPF) conditions was also studied. As a result of cultivation of the fungus on a substrate for 4 days, the amount of proteins, fats and soluble sugars in it changes both quantitatively and

qualitatively. The recorded changes are reflected in the change in the quantitative indicators of the components, as well as in the change in the enzymatic quality. There is a decrease in the amount of basic polymers in the initially obtained wheat bran, an increase in the amount of protein, lipids and minerals, as well as significant enzymatic activity.

The use of the product as a feed additive has been tested on the farm. Clarification of this issue was carried out on chickens specializing in the ovarian direction. Inspection of the received product was carried out jointly with the Veterinary Research Institute of the Ministry of Agriculture of the Republic of Azerbaijan. Adding 0.035 kg to chickens per 1 ton of feed leads to an increase in the ovulation capacity of the chickens, which is 5% more compared to the control. An increase in the number of eggs obtained was observed as their weight remained constant.

## FINAL ANALYSIS OF RESEARCH

Based on the study of edible species of xylotrophic macromycetes, while we assessed the situation in the Republic of Azerbaijan, which is characterized by the richness and diversity of natural conditions, it was found that the situation here is not unambiguously positive. Therefore, during 2014-2018 the studies conducted to assess the resources of both common and edible mushrooms in the xylotrophic mycobiota of forests located in the southern region of Azerbaijan, as well as in the Greater and Lesser Caucasus. It was found that during the period under study, 93 species of basidiomycetes were involved in the formation of the mycobiota of these forests, of which 52 species were found in the forests of southern Azerbaijan.

10 species of the common fungi, are classified as edible mushrooms, and 9 of them have been identified in the forests of the south, which are considered main research areas. This is 17.4% of the total number of mushrooms recorded in the southern zone. However, studies have shown that edible mushrooms are promising species in terms of biomass output both in vivo and in the vegetative

growth phase, that some are even dominant in terms of FO, and that most are biotrophic or saprotrophic. This information on the fungi used for food purposes actually. The fact that FB in many edible mushroom species registered in the southern zone, especially *L. sulphureus* and *P. ostreatus*, occurs twice a year, clearly demonstrates the abundance of natural resources and the prospects of the identified species of the registered fungi.

It should be noted that the recent expansion of both basic and practical research aimed at obtaining medicines, various foods and nutritional supplements from natural sources is of particular importance. In this regard, the presence of fungi among the objects of interest is of particular importance, since the environmental degradation, the reduction of the forest area, and the increased anthropogenic impact on the environment also lead to a decrease in traditional biological resources, including the natural resources of fungi. From this point of view, it is important to correctly determine, at least predict the amount of biological resources formed in natural conditions in order to use natural resources more efficiently and develop methods and approaches to their use in nature.

Therefore, a formula was proposed to calculate the annual amount of MC that can be produced in Azerbaijan under natural conditions of the species of edible mushrooms in Azerbaijan, and it was determined that the mushrooms mentioned in Azerbaijan produce just over 216 tons of FB in year.

Biomass formed by fungi both under natural conditions and during the vegetative growth phase has been found to contain biologically active metabolites and has been shown to be non-toxic to both plants and *Tetrahymena pyriformis*. This allows FB to address the scarcity of natural resources at the expense of VM. This can be done by obtaining pure cultures of edible fungi, selecting active strains for biomass production, and finding the optimal environment for the selected strains. For this purpose, the use of fungi such as *G.lucidum* B-09, *L.sulphureus* B-18 and *P.ostreatus* B-25 is more convenient.

As a result of the growing world population, interest in edible mushrooms has taken a new direction, one of which is the

processing of waste into fodder products. Taking this into account, the effectiveness of the use of mushrooms, recorded in studies in the production of feed additives, which play a direct role in food production, was also evaluated. Studies for this purpose have shown that P. ostreatus B-25 is a promising indicator for the production of feed additives and differs from well-known manufacturers in the biomass obtained when grown in wheat bran for 4 days under BFF conditions, and their use in poultry farming for this purpose. allows you to efficiently use resources, as well as buy additional products.

## RESULTS

1. Analysis of samples collected from forests in the south of Azerbaijan revealed the distribution of 52 species of xylotrophic basidiomycetes, of which only 10 (*Armillaria mellea* (Vahl) P. Kumm, *Fistulina hepatica* (Schaeff.) With, *Flammulina velutipes* (Curtis) Singer, *Ganoderma lusidum* (Curtis) P. Karst, *Kuehnerómyces mutábilis* (Schaeff.) Singer & AH Sm, *Laetiporus sulphureus* (Bull.) Murrill, *Panus tigrinus* (Bull.) Singer, *Pleurotus ostreatus* (Jacq.) P. Kumm, *Polyporus squamus*(Huds.) Fr. and *P.umbellátus* (Pers.) Fr.) belong to the category of edible fungi, *Pluteus aurantiorugosus* (Trog) Sacc. and *P.umbellátus* have been identified as new to mycobiota, which is unique to Azerbaijani nature[1-3, 6-7, 11].
2. The registered edible mushrooms are characterized by a variety in vivo due to their frequency, ecological and trophic relationships and distribution in individual substrates, and in the case of vegetative mycelium, the amount of biomass formed and the quantitative indicator of the biological activity of metabolites. Thus, *Laetiporus sulphureus* and *Pleurotus ostreatus dominant, Armillaria mellea* and *Polyporus squamosus* are the most common, and the rest are random and rare species, of which only *Armillaria mellea* is biotroph, and the rest are polytroph [5-6, 9-11].

3. Although the resources of fruit bodies naturally formed by fungi during the year are limited, it was determined that at least 216.72 tons of fruit bodies, typical of 10 mushrooms, could be formed in Azerbaijan, and 87.1% of them are *Laetiporus sulphureus* and *Pleurotus ostreatus* [4, 10].
4. Biologically active metabolites were found both in the natural fruit body of fungi and in the biomass formed at the vegetative stage, which has a stimulating effect on both plants and infusorian [9].
5. It was found that in order to obtain substances with one or another biological activity, it is advisable to use pure cultures of fungi. Thus, in this case, it is always possible to obtain from them enough biomass during the year. For this purpose is advisable to keep *G.lucidum* B-09, *L.sulphureus* B-18 and *P.ostreatus* B-25, which are selected as active producers, contain glucose (0.97-1.0%) as a carbon source and $NH_4NO_3$ (0,036-0,038% for nitrogen) and peptone(0,28-0,30%) as nitrogen source, at a temperature of $28^0$C under conditions of liquid phase fermentation (deep cultivation) for 5-7 days[5, 9, 12].
6. *P.ostreatus* B-25 is also a suitable edible fungi in Azerbaijan as its extraction from wheat bran cultivated in the SPF for 4 days is a method that allows both efficient use of resources and additional production in animal husbandry[8, 12].

## THE LIST

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The defense will be held on "31" may **2021** at **11-00** at the meeting of the Dissertation council FD 1.07 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at the Institute of Microbiology of ANAS.

Address: Az1004, Baku city, M.Mushfig street 103

The dissertation is available in the library of the Institute of Microbiology of ANAS.

Electronic versions of the dissertation and abstract are posted on the official website of the Institute of Microbiology of ANAS (https://www.azmbi.az/index.php/az/).

Abstract was sent to the required addresses on " 30" april 2021.

Signed: 27.04.2021

Paper format: A5

Volume: 33541

Circulation: 20