REPUBLIC OF AZERBAIJAN

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ABSTRACT

of the dissertation for the degree of Doctor of Science

MYCOBIOTAS OF ESSENTIAL PLANTS OF AZERBAIJANI FLORA, BACTERIAL AND FUNGICIDE CHARACTERISTICS OF THEIR ELEMENTS

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Field of science:	Biology
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Baku – 2021

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INTRODUCTION

Rationale and level of development of the research topic. The Republic of Azerbaijan has outstandingly rich and colourful vegetation. It was determined that "*the flora of Azerbaijan includes about 4700 plant species.*"[1] Therefore, the study of plant resources, the analysis of their distribution patterns, bioecological, phytocenological, and ontogenetic features, as well as the study of microbiological, mycological, ecological, and biotechnological bases of their use for practical purposes are very important issues. This is one of the priority areas of a number of sciences, including microbiology.

It should be noted that "*1547 species of plants of the flora of Azerbaijan are medicinal, about 800 of which are essential oils*"[1]. The presence of complex biologically active substances in essential oils has become the focus of researchers and has led to large-scale research in various aspects. Studies show that during their growing season, essential oil plants "*release bactericidal, fungicidal, and protistocidal substances of phytoncide nature"*[2,3] from metabolic products as a result of life activities in the environment.

These substances are a key factor in the formation of naturally occurring immune systems in plant organisms. In addition, phytoncide compounds are antagonistic to microorganisms, including micromycetes. In other words, "*essential oil plants that synthesize*

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[1]Mehdiyeva, N.P. Biodiversity of medicinal flora of Azerbaijan / NP Mehdiyeva, -Baku: Letterpress, -2011, -186 p.

[2]Getko, N. V. Shutova, A. G., Pobolovets, T. A. Titok V. V. Chemical composition of fugitive essential oils released into the atmosphere by the leaves of a specimen of the Lauraceae Juss family. in greenhouse culture, and their antimicrobial activity // Reports of the National Academy of Sciences of Belarus, -2016, vol. 60, no. 6, -p. 91-97

[3]Adorjan, D., Buchbauer, G.: Biological properties of essential oils: an updated review // Flavour Fragr. J., -2010, -vol. 25, - p. 407–426

phytoncide substances either have a fungicidal effect or create fungistatic conditions by preventing microorganisms that cause phytopathological diseases, including microscopic fungi, from settling on these plants and their further development”[4].

It is known that in recent years, the global bioecological balance has undergone major changes in the direction of imbalance. This, in turn, creates real difficulties in the vital activities of the living world, including plants, animals, and humans. As can be seen, the demand for natural products with a therapeutic and prophylactic effect by living things, including humans, is constantly growing in the unfavourable environment. Generally, plant products have such properties that “*these properties are also realized by the biologically active substances they contain*. *Such substances include alkaloids, essential oils, flavonoids, glucosides, coumarins, vaccine ingredients, resins, comedones, etc*."[5]. Among antifungal medicines of natural or synthetic origin, preparations derived from aromatic or medicinal plants are distinguished by low toxicity and high activity. Therefore, seeking antifungal agents in the wild plant flora is considered more relevant and opens the way for future research.

It should be noted that there are a number of research works on the antifungal activity of essential oils, saponins, flavonoids, and other biologically active substances isolated experimentally from Eupatorium cannabinum, Satureja thymbra, Salvia ponifera, Salvia desoleana, Monarda didyma, Thymus vulgaris, Pimenta racemosa, Cymbopogon citrates, Curcuma longa, Thymus capitellatus, Bergamot, Coriandrum sativum, Chaerophyllum byzantinum, Macrophomina phaseolina, Chenopodium botrys, Acroptilon repens,

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[4]Baxşəliyeva, K.F. Azərbaycanın müxtəlif biotoplarında yayılan toksigen göbələklərin say və növ tərkiblərinə görə xarakteristikası//-Bakı: Azərbaycan Aqrar Elmləri jurnalı, - 2016. № 5, -s. 92-95

[5]Natu, K.N., Tatke, P.A. Essential oils—Prospective candidates for antifungal treatment? //J. Essent. Oil Res., -2019, v.31, -p.347–360

Telekia spcioza, Myrica gale, Artemisia absinthium L., etc. in the scientific literature. These species are also found in the flora of Azerbaijan but the antifungal activity of essential oil plants growing in different ecological areas of Azerbaijan has not been adequately studied until our research, i.e. essential oil plants with antifungal properties are considered insufficiently studied in this aspect in the Azerbaijani flora.

On the other hand, conducted researches show that essential oils can be formed in almost all organs of plants, both vegetative and generative but comparative experiments show that essential oils accumulate more in the above-ground organs. At the same time, it should be noted that in practice, there is a fact that the flower part has the highest amount of essential oil among the vegetative and generative organs. This means that essential oil plants are more likely to synthesize essential oils during the flowering period of their growing season. Thus, in addition to proteins, carbohydrates, lipids, and vitamins, which are the main products of the metabolic process in the plant cell during the flowering phase, secondary substances (organic acids, aromatic compounds, glucosides, vaccine ingredients, rubbers, alkaloids, antibiotics, and essential oils), which are also important, are synthesized. Essential oils, which belong to the group of secondary metabolic products, play an extremely important role in plant life. Thus, essential oils are characterised by different aromas due to their different components. Therefore, plants that contain essential oils of different compositions differ sharply from each other with their specific aromas.

Essential oils in essential plants are a mixture of different substances that are chemically coordinated with one another, and their components are mainly oxidized terpenes, phenols, aldehydes, esters, acetates, lactones, and ketones. In general, it should be noted that essential oils are considered natural substances that consist of hundreds of components. Almost all of the components of essential oils derived from plants are substances of organic origin. This means that essential oil is a complex organic substance that forms different conformation in each plant in a peculiar combination. Clarification of its role in the formation of bactericidal and fungicidal properties is one of the issues of both practical and scientific interest but so far, this issue has been neglected in many researches.

Purpose and objectives. The purpose of this work is to assess the species composition and manifestations of ecotrophic specialisation of a number of essential oil plants of the flora of Azerbaijan, as well as the bactericidal and fungicidal properties of various plant materials.

In order to achieve the set goal, the following objectives are planned to be implemented:

- General characterisation of essential oil plants and their constituent components in the flora of Azerbaijan;
- Characterisation of mycobiota of essential oil plants in the flora of Azerbaijan by species composition, frequency of occurrence, and ecological-food web;
- Bactericidal properties of materials obtained from some essential oil plants in the flora of Azerbaijan and characterisation of this property according to the constituent components of essential oils;
- Fungicidal properties of materials obtained from some essential oil plants in the flora of Azerbaijan and characterisation of this property according to the constituent components of essential oils;
- Study of bactericidal and fungicidal properties of the composition of essential oils from plants in the flora of Azerbaijan with various substances.

Research methods. The accuracy of the results of the dissertation is confirmed by the fact that it allows statistical processing of the results obtained from the repetition of experiments performed and applied using microbiological, mycological, phytopathological, biochemical, as well as ecological methods and approaches that are widely used in similar work and meet modern requirements. The degree of purity of the reagents used in the analysis, the required level of accuracy of the devices and equipment, and the fact that they are intended for use in research in this area are also arguments that strengthen the accuracy of the results.

The main provisions of the dissertation submitted for defence:

- Both true fungi and species of fungal organisms are involved in the formation of mycobiota of essential oil plants in the flora of Azerbaijan, all of which are included in the pathogenic mycobiota of plants;
- Species involved in the formation of mycobiota of essential oil plants are characterised by a wide variety of both eco-food web and the degree of distribution in different economic regions;
- Materials derived from essential oils (aqueous extracts - AE and essential oils - EO) contain components with both bactericidal and fungicidal properties, the quantitative value of which can vary depending on the biological properties of both test organisms and the plants from which the source of the material is obtained, as well as the component composition of the essential oils;
- The composition of plant-derived EOs with each other, as well as with white Naftalan oil, is an approach that opens up additional opportunities for efficient use of resources**.**

The scientific novelty of the research. As a result of the research, mycobiotics of more than 100 essential oil plant species, grown and cultivated in the economic regions of the Republic of Azerbaijan, such as Absheron, Aran, Ganja-Gazakh, Lankaran-Astara, Guba-Khachmaz, Mountainous Shirvan, and Sheki-Zagatala, have been studied for species composition, eco-food web, distribution of fungi on individual plants, as well as bactericidal and fungicidal activity of various materials derived from essential oil plants.

It was found that 161 species of fungi and fungus-like organisms are involved in the formation of mycobiota of the studied plants, and 93.2% of them belong to real fungi and 6.8% to fungal organisms.

It was also determined that this is the first time that information has been obtained on the distribution of registered fungi such as *Mucor ramosissimus* Samouts, *Exserohilum longirostratum* (Subram.) Sivan., *Fusarium dimerum* Penz., *Nigrospora maydis* (Garov.) Jechová, *Penicillium simplicissimum*(Oudem.) Thom, *Phoma eupyrena* Sacc., *Ph.medicaginis* Malbr. & Roum, and *Sclerotinia graminearum* Elenev ex Solkina in Azerbaijan.

It was found that the distribution of fungi registered in different economic regions is different, and the mycobiota of essential oil plants in the Lankaran-Astara economic region is richer (66.5% of total fungi), while in Absheron – the poorest (40.4%). Comparison of Sorensen species compatibility coefficient of fungi registered in different economic regions also showed that these two economic regions are more distant from each other (32%), while the degree of compatibility of mycobiota specific to essential oil plants of Mountainous Shirvan, Guba-Khachmaz, and Sheki-Zagatala economic regions showed to be closer to each other (68-70%).

It was determined that the specific weight of toxins, allergens, and conventional pathogens also plays an important role among the fungal species involved in the formation of mycobiota of essential oil plant biota of the studied areas. Thus, 57.8% of the registered fungi has properties characteristic of allergens, 41.9% - of opportunistic ones and 65.2% - of toxicogenic ones. In addition, 85.1% of fungi involved in the formation of mycobiota of essential oils of Azerbaijan are more or less prone to pathogenicity.

It was also clear from the research that *Alternaria alternata*, *Botrytis cinerea*, *Fusarium moniliforme*, *Penicillium cyclopium* and *Verticillium dahilae* are the dominant species of mycobiota of essential oil plants in the studied areas, and their frequency varies between 42.7-53.6%.

It was found that aqueous extracts and essential oils from some essential oil plants in the flora of Azerbaijan have a negative effect on the growth of both bacteria and fungi, and their effect can be bacteriostatic and fungiostatic, as well as bactericidal and fungicidal, depending on the biological properties of both plants and test cultures.

The study of the component composition of the essential oils of some plants and the fact that essential oils with major components of thymol and tsineol have a higher bactericidal and fungicidal effect than menthol have been studied for the first time.

The composition of essential oils with each other, as well as with White Naftalan oil from Naftalan crude was prepared, which allows to increase their quantitative bactericidal and fungicidal activity up to 20%, as well as creates additional opportunities for more efficient use of natural resources.

Theoretical and practical significance of the research. The results of the research are a collection of information on the distribution of fungi and fungal-like organisms, manifestations of ecotrophic specialisation, and factual material that serves to expand understanding of the fungicidal and bactericidal effects of essential oil plants.

The composition of essential oils from different types of essential oil plants in the flora of Azerbaijan with each other and White Naftalan oil allows increasing their bactericidal and fungicidal properties, as well as more efficient use of natural resources.

Some of the results of the dissertation were included as an important result in the annual report of ANAS and tested with a positive result in field research.

Publication, approbation, and application of the dissertation. 37 scientific works on the topic of the dissertation have been published and the dissertation materials were presented at the II International Conference on “Actual problems of biochemical theories” (Ganja, 2011), VII International scientific conference on

"Ecology and protection of life activity" (Sumgayit, 2012), international scientific-practical conferences on "Actual problems of biological and chemical ecology" (Moscow, 2012; 2014), the 5th Congress of Russian Mycologists (Moscow, 2017), and the Republican Conference on "Actual Problems of Modern Biology" (Sumgayit, 2018).

The structure and scope of the dissertation. The dissertation consists of an introduction, a literature review (Chapter I), a description of the research materials and methods (Chapter II), a presentation of the results and their interpretation (Chapter III-V), a final analysis of the research, the main results, a list of references, and a list of abbreviations used in the dissertation. The dissertation consists of 245 computer pages (400350 symbols in total), including tables and figures.

LITERATURE REVIEW
CHAPTER I
GENERAL CHARACTERISTICS OF ESSENTIAL OIL PLANTS, THEIR MICOBIOTAS AND ANTIMICROB ACTIVITY

Section 1.1 of the dissertation analyses information about essential oil plants and their components, their effects and areas of use.

Section 1.2 of the dissertation describes the essential oil plants as one of the habitats of fungi and the research, conducted in the world and Azerbaijan, related to the formation of their mycobiota.

Section 1.3 of the dissertation analyses the work on the study of bactericidal and fungicidal activity of essential oils in essential oil plants, substantiates the prospects of their use for this purpose and justifies the fact that this issue, as well as the study of mycobiotics of essential oil plants, is an open research object in the world and Azerbaijan.

CHAPTER II
MATERIALS AND METHODS

2.1. General characteristics of the studied areas

Essential oil plants in the cultural and wild flora of Azerbaijan and their mycobiota were selected as the object of research, and for this purpose, samples were taken from the territories of seven ecologically different economic regions of Azerbaijan (Absheron, Aran, Guba-Khachmaz, Ganja-Gazakh, Sheki-Zagatala, Lankaran-Astara, and Mountainous Shirvan) (Fig. 2.1). The economic regions sampled for the study differ from each other in terms of the total area of their territory, natural and climatic conditions, as well as the natural resources of the area, vegetation, the nature of the economic activity, and other criteria, and these differences were also taken into account during sampling.

Figure 2.1. An overview of the sample areas (◯) for the study

More than 3,000 samples were taken from the vegetative and generative organs of cultivated and wild essential oil plants in the mentioned areas in the study and analysed in accordance with the purpose of the work.

2.2. Methods used for sampling and analysis of research

Samples for the study were taken from the territories of the mentioned economic regions in 2011-2020. Methods based on the selection of planned routes and permanent sites were used in sampling, sampling, on-site certification, preparation for laboratory analysis, growing fungi in pure culture, and determination of species composition were carried out according to "*known methods*"[6,7,8]. Sampling was carried out by seasons, as well as at different phases of vegetation and phenological development of plants, mostly in the flowering phase. In total, more than 3,000 samples were taken during the study and analysed for the purpose of the study.

Wheatgrass juice agar (WJA), rice (RA), starch (SA) and potato dextrose agar (PDA), and Czapek and Czapek-Dox agar were used as nutrient media for the growing of mushrooms in pure culture. The media were prepared, sterilised, and poured into a Petri dish in accordance with "*known methods*"[9] accepted in microbiology.

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

[7] Bensch K. Species and ecological diversity within the Cladosporium cladosporioides complex (Davidiellaceae, Capnodiales)/ K.Bensch, J.Z. Groenewald, J.Dijksterhuis [et al.]// Studies in Mycology. -2010. -v. 67, p.1-94.

[8] Seifert K.A. The genera of Hyphomycetes./K.A.Seiferi. -Utrecht:CBS-KNAW Fungal Biodiversity Centre, 2011. -997 p

[9] Нетрусов, А.И. Практикум по микробиологии./ А.И.Нетрусов, М.А.Егорова, Л.М. Захарчук [и др.]. М.:Издательский центр «Академия», -2005, -608с.

The naming and classification of fungi were carried out in accordance with the data on the official website of the International Mycological Association.

Mushrooms grown in pure culture were also characterised by growth rates in standard nutrient media, using a growth factor (GF). "*The formula GF = DiHS/T*"[10] was used to calculate the GF. Here, Di is the diameter of the colony (in mm), H is the height of the colony (mm), D is the density of the colony determined by visual image (from 1 to 5), T is the cultivation period (days).

The formula P = (n/N) x100 was used to characterise the frequency of fungal infections, as well as the prevalence of fungal diseases, where P is the frequency of fungi in the samples (or the prevalence of the disease caused by the pathogen – in %), n is the number of fungi detected (number of infected plant individuals in the study area, in numbers), and N is the total number of samples (total number of studied plant species in the area).

To compare the degree of conformity of individual plant cenoses, Sorensen's species compatibility coefficient (K) was used, and *"the formula K = 2C/A + B"*[11] was used to calculate it, where A and B are the number of species in each plant cenosis, C is the number of species that are the same in the comparable cenoses.

Czapek medium was used for growing mushrooms in a liquid nutrient medium, the composition of which was as follows (g/l): Glucose– 14,0; $CaCO_3$ – 0,7; KNO_3 – 0,7; $MgSO_4$ – 0,35; NaCl – 0,35; K_2HPO_4 – 0,35; $FeSO_4$ – traces; distilled water - 1 l.

Meat peptone broth (MPB) and meat peptone broth agar (MPBA) were used to grow the bacteria.

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

[11] Миркин Б.М., Розенберг Г.С. Толковый словарь современной фитоценологии. М.: Наука, 1983, с. 54–55.

Air-dried aboveground biomass of essential oil plants was used to obtain essential oils, and this process was carried out by hydrodisillation.

Agilent Technolgies Gas chromatography–mass spectrometry method was used to study the chemical composition of the essential oil.

Different proportions of aqueous extracts from air-dried aboveground parts collected during their flowering phase and different ratios of EO diluted in alcohol were used to determine the antifungal and antibacterial activity of the essential oil plants studied. The process was evaluated both for "*the diameter of the lysis zone"*[12] (DZ) and for *"the amount of biomass"*[13] (AB) formed. The methods and approaches used in the work of various authors were utilised to implement these tasks.

Both bacteria and toxigenic fungi were used as test-culture, some of which were presented by the Institute of Microbiology of ANAS, and some of them were grown in pure culture by us in the course of research.

During the research, all experiments were performed in 4-6 repetitions, and the results were *"statistically proceeded"*[14]. In all cases, the results corresponding to the formula $\sigma / M = P \leq 0.05$ (where M is the average value of repetitions, σ is the standard deviation, P is the Student's criterion) were considered valid and included in the dissertation.

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[12] Егорова Н. С. Руководство к практическим занятиям по микробиологии. Учеб. Пособие. 3-е изд., перераб. и доп.М.: Изд-во МГУ, 1995, 224 с:

[13] Пименова М. Н. Руководство к практическим занятиям по микробиологии./ Пименова М. Н., Гречушкина Н. Н., Азова Л. Г. [и др.]. – М.: Изд. МГУ. -1995. -224 с.

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

EXPERIMENTAL SECTION
CHAPTER III
SPECIES COMPOSITION OF ESSENTIAL OIL PLANTS AND THEIR MYCOBIOTA DISTRIBUTED IN ECOLOGICALLY DIFFERENT AREAS OF AZERBAIJAN

3.1. Species characteristics of essential oil plants in the flora of Azerbaijan, mycobiota of which is planned to be studied and general characteristics of the chemical composition of essential oils obtained from some of them

The research sampled 103 species of essential oil plants that grow wild in different parts of Azerbaijan and are grown in agrocenoses. The sampled plants include mainly monocotyledonous and dicotyledonous species of angiosperms, as well as several species of gymnosperms and pteridophytes. In short, the plants used for sampling are characterised by a wide variety of aspects.

The sampled plants also differed in their life forms. Thus, the sampled plants include *Albizzia julibrissin, Betula pendula, Crataegus pentagyna, Fraxinus excelsior, Pinus sylvestris, Tilia caucasica* and other trees, *Juniperus communis, Rosmarinus officinalis, Thea sinensis* and other shrubs, *Artemisia lerchiana, Achillea millefolium, Apium graveolens, Thymus collinus* and others herbs, as well as ephemerals and ephemeroids. Among these plants, there are many valuable nutrients, fodder, as well as species widely used in folk medicine.

3.2. Characteristics of the mycobiota of the essential oil plants to be studied according to the species composition

As a result of the analysis of samples taken from the mentioned essential oil plant species in 2011-2020, it was determined that a total of 161 species are involved in the formation of their mycobiota.

Information on the taxonomic structure of these fungal species is summarised in Table 3.2. As can be seen, the distribution of fungi by

Table 3.2

General characteristics of the taxonomic structure of fungi and fungal-like organisms in some essential oil plants in the flora of Azerbaijan

Kingdom	Phylum	Class	Order	Family	Genus
Chromista	*Oomycota*	1	2	2	4(11)
Mycota	*Zygomycota*	1	1	2	4(14)
	Ascomycota	5	9	12	30(109)
	Bazidiomycota	2	5	8	15(27)
Total	4	**9**	**17**	**24**	**53(161)**

individual taxa is characterised by different quantitative indicators, and in almost all cases, taxa belonging to the sac fungi division are characterised by relatively high rates. Thus, 67.7% of the total registered mushrooms fall to their share. Of the remaining fungi, 6.8% belong to Chromista, 8.7% to Zygomycota, and 16.8% to Bazidiomycota. At the same time, their anamorphic species make 93.6% of sac fungi and 63.4% of total fungi.

As can be seen from Table 3.2, along with true fungi, fungal-like organisms are also involved in the formation of mycobiota of essential oil plants, which account for 6.8% of the total mycobiota. All of these species of fungal-like organisms (Oomycota) are the cause of one or another pathology.

It should be noted that most of the fungi recorded in the studies were found at different times in different biotopes by other researchers, i.e. most of the recorded species are involved in the formation of mycobiota, which are inherent in the nature of Azerbaijan. However, among the registered species, there are those whose distribution in the territory of Azerbaijan was recorded as a result of these studies, and their number was equal to 8.

Among the first recorded fungi in Azerbaijan, anamorphs of sac fungi have a relative abundance and account for 75% of those that meet this characteristic (*Exserohilum longirostratum (Subram.) Sivan.,Fusarium dimerum Penz., Nigrospora maydis (Garov.) Jechová, Penicillium simplicissimum (Oudem.) Thom, Phoma eupyrena Sacc., Phoma medicaginis Malbr. & Roum.*). Of the registered fungi, 12.5% belong to the telemorphs of sac fungi (*Sclerotinia graminearum Elenev ex Solkina*) and 12.5% to zygomycetes (*Mucor ramosissimus Samouts*).

3.3. General characteristics of the distribution of fungi registered in the research by economic regions

Samples for the study were taken from the territory of seven economic regions of Azerbaijan, which differ in certain indicators, according to which the distribution of registered fungi (Table 3.3) and the degree of compatibility with each other were also different

Table 3.3

Distribution of fungi by economic regions

№	Economic regions	Number of species		Share in total mycobiota (in %)
		Fungi	Plant	
1	Absheron	65	38	40,4
2	Aran	87	47	54,0
3	Mountainous Shirvan	79	40	49,1
4	Ganja-Gazakh	94	52	58,4
5	Guba-Khachmaz	98	60	60,9
6	Lankaran	107	62	66,5
7	Sheki-Zagatala	95	53	59,4

(Table 3.4). As can be seen, the richest mycobiota is characterised by essential oil plants spread Lankaran-Astara, and the poorest - in the Absheron economic region. Interestingly, fungi are considered the

Table 3.4

Characteristics of mycobiota of different economic regions according to the coefficient of concordance (in %)

	1	2	3	4	5	6	7
Absheron(1)	**100**	47	45	49	51	54	50
Aran(2)	47	**100**	52	56	58	60	57
Mountainous Shirvan(3)	45	52	**100**	54	55	59	54
Ganja-Gazakh(4)	49	56	54	**100**	60	63	59
Guba-Khachmaz(5)	51	58	55	60	**100**	64	60
Lankaran(6)	54	60	59	63	64	**100**	63
Sheki-Zagatala(7)	50	57	54	59	60	63	**100**

most drought-resistant organisms but in our opinion, the humidity factor plays the main decisive role in this issue, as the climate is humid in the first region and dry subtropical in the second one. As for the similarity of the fungi registered in different economic regions, more precisely, the Sorensen coefficient of species compatibility, apparently, the two economic regions are more distant from each other due to mycobiota (Table 3.4). As can be seen, the degree of conformity of mycobiota, which is characteristic of essential oils plants of the three economic regions - the Mountainous Shirvan, Guba-Khachmaz, and Sheki-Zagatala economic regions, is higher. In our opinion, this closeness is due to the fact that these economic regions are located close to each other, in adjacent areas, and their soil-climatic conditions and vegetation are relatively close to one another.

However, in other areas that meet this characteristic, especially in the Aran, the closeness of others is not so great. There is a point that if we take into account that the Aran economic region is the most irrigated area of the country's population and the cultivation of crops occupies a large part of this area, then, we can justify this issue.

3.4. Characterisation of ecotrophic relationships of fungi recorded in studies

It is known that as fungi are heterotrophic organisms, they require preformed organic compounds as energy sources, and nutrients from dead or decomposing organic matter serve as the main source for this. Naturally, essential oil plants in the presented studies perform this function. Therefore, the next stage of research is devoted to the clarification of trophic, i.e. nutrient relations between fungi and essential oil plants. Research in this area uses two approaches, the first of which is an approach that reflects the trophic relationships between fungi and plants, or rather the lifestyle of fungi, and according to this approach, fungi are divided into Saprotrophs, Biotrophs, and Polytrophs. According to the division in the research conducted in this direction, it is common to characterise the fungi registered in one or another plant or plant group, and it can be said that most of the fungi known to science have been characterised in this aspect. This issue is not so relevant today, except for some controversial points. However, in recent years, new approaches to the characterisation of fungi in this aspect – characterising fungi based on the criteria defined as a manifestation of ecotrophic specialisation, have become more relevant. Among these manifestations, special attention is paid to being Allergenic, Opportunistic and Toxicogenic.

For this reason, the fungi first recorded in the course of our research were characterised in this respect. The results showed that among the fungi recorded in the study, many meet the specified characteristics, and the number of toxins is higher than both allergens and opportunistic ones (Table 3.5). As can be seen, the quantitative

Table 3.5

Characteristics of fungi registered in different economic regions according to the forms of ecological-trophic specialisation

№	Economic regions	Allergenic		Opportunistic		Toxicogenic	
		1	2	1	2	1	2
1	Absheron	36	55,4	28	43,1	46	70,7
2	Aran	49	56,3	34	39,1	57	65,5
3	Mountainous Shirvan	41	51,9	32	40,5	52	65,8
4	Ganja-Gazakh	54	57,4	40	43,0	64	68,1
5	Guba-Khachmaz	57	58,2	41	41,8	70	71,4
6	Lankaran	65	60,7	50	46,7	77	72,0
7	Sheki-Zagatala	52	54,7	39	41,1	62	65,3
Total		93	57,8	67	41,9	105	65,2

Note. 1 - Number of species and 2 - Share in total mycobiota (%)

indicator of common fungi of all forms of ecotrophic specialisation differs to some extent in different economic regions, and it is interesting that the relative number of fungi corresponding to all three characteristics is higher in Lankaran-Astara economic region. The second approach used to characterise fungi in this direction is to determine the participation of the recorded fungi in the formation of pathogenic or epiphytic mycobiota, which also includes characterisation of the fungi recorded in essential oil plants in this study.The results showed that most of the recorded fungi have the ability to cause pathology to one degree or another (Fig. 3.1).

As can be seen, only 14.9% of the recorded fungi are involved in the formation of epiphytic mycobiota, at least because they belong to the true saprotrophs due to their ecotrophic relationships, and the formation of epiphytic mycobiota occurs at the expense of this group. It is worth mentioning that it is not correct to consider all the remaining fungi as pathogens. It is true that all other fungi belong to either biotrophs or polytrophs, and it is possible for the fungi of this characteristic to meet their food needs at the expense

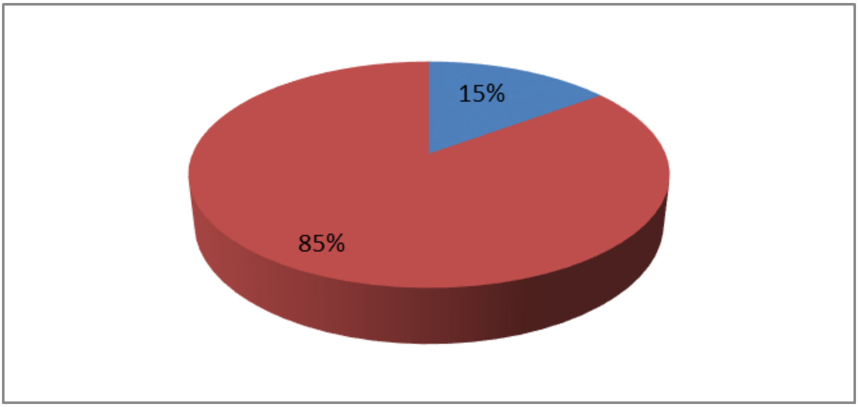

Figure 3.1. **Characterisation of epiphytic and pathogenic species of fungi in essential oil plants.**

of living things. Although this is a point of concern in pathogenicity, neither the name nor the symptoms of the pathology caused by many fungi are known today. Therefore, based on the results obtained, it can be noted that although 85% of the fungi recorded in essential oil plants meet their food needs to some extent due to living materials, it is incorrect to attribute all of them to real pathogens. It seems logical to divide them into three conditional groups, namely pathogens, prone to pathogenicity, and those that lead to the development of pathogens, which may be more appropriate to make it the subject of a separate study.

It should be noted that the fungi found in essential oil plants are characterised not only by their taxonomy and ecotrophic relationships, but also by their ecophysiological characteristics. Thus, among them, there are hydrophils, thermotolerants, alcohol tolerant, and facultative aerophiles.

3.5. General characteristics of mycobiota specific to individual essential oil plants and their vegetative and generative organs in the flora of Azerbaijan

As a result of research conducted at this stage, it was considered relevant to characterise the fungi involved in the formation of mycobiota of individual plants by the number of species. As noted, samples of 103 essential oil plant species were selected for the study, and the average number of samples taken from one plant ranged from 10 to 30. From the analysed samples, it was determined that the share of fungi, grown in pure culture and identified as species in the formation of mycobiota of individual plants, is characterised by different quantitative indicators. Taking this difference into account, it was considered relevant to divide the plants into three groups. The first group is characterised by rich mycobiota, which is represented by more than 30 species that are involved in the formation of mycobiota of plants of this group. The second group includes plants characterised by relatively low mycobiota, of which 10-30 species of fungi are involved in the formation of mycobiota. The last group includes plants characterised by weak mycobiota, in which less than 10 species of fungi are involved in the formation of mycobiota. According to this division, the studied plants – Common wormwood, Mugwort, Tanacetum, and Hemlock belong to the last group, i.e. these plants are characterised by the weakest mycobiota. Crataegus, Tilia, Shamrock, Lilac, and other plants belong to the first group, which is characterised by a richer mycobiota. In general, when characterising all the sampled plants according to this division, it becomes clear that 27 plant species (26% of the total species) belong to the first group, and 71 species (68%) - to the second group. In general, Tilia (43 species) is characterised for the richest mycobiota, while Mugwort and Salvia (with seven species) – for the weakest mycobiota.

One of the points of attention in the study of the mycobiota of essential oil plants is related to the distribution of fungi on different vegetative and generative organs of plants. When analysing fungi from this point of view, it became clear that some fungi are found in all organs, that is, they are universal, while others are found in only one organ, that is, they are specific (Table 3.6). As can be seen, the

Table 3.6

Distribution of fungi in essential oil plants by plant organs

The name of the body found	Number of registered fungi species	Share in total mycobiota (%)
Stem	83	51,6
Leaf	87	54,0
Root	29	18,0
Flowers and fruits	46	28,6
Specific ones	24	14,9
Universal ones	68	42,2

stems and leaves of essential oil plants are the most common sources of fungi, while the roots are the least common. Universal ones make up 42% of the total mycobiota, which is not a favourable indicator from a phytosanitary point of view.

Thus, universality can be characterised as an indicator that serves to expand the adaptive properties of these fungi.

3.6. Characteristics of the frequency of occurrence of fungi forming the mycobiota of essential oil plants

The last issue identified in the study of mycobiota of essential oil plants is the frequency of occurrence of recorded fungi in the total studied areas. Determining this is an important indicator for understanding the share of fungi in the functions they perform in these areas as an ecosystem. The following indicators are taken as the basis for the frequency of occurrence (Table 3.7). When characterising the fungi recorded in the study based on the above-mentioned indicators, the number of species dominating in all study areas is equal to five (Table 3.8). As can be seen, the number of dominant species in individual economic regions is slightly higher

Table 3.7

Indicators for grouping the fungi according to the frequency of occurrence

Group name according to the frequency of occurrence	Quantitative indicator of the frequency (%)
Dominant species	40 and above
Common species	10-40
Rare and random species	Less than 10

Table 3.8

Characteristics of registered fungi species according to the frequency of occurrence in different economic regions

№	Economic regions	Number of dominant species, pcs (%)	Common species, (pcs.)	Rare and random species (pcs.)
1	Absheron	5(47,8-59,5)	28	32
2	Aran	7(46,4-61,2)	38	42
3	Mountainous Shirvan	6(50,2-68,7)	29	44
4	Ganja-Gazakh	7(43,5-61,6)	48	39
5	Guba-Khachmaz	8(45,5-57,7)	46	43
6	Lankaran	9(42,8-63,4)	48	50
7	Sheki-Zagatala	8(48,1-60,1)	43	44
On all studied areas		5(42,7-53,6)	75	81

than the total and varies from five to nine. It should be noted that in other studies, the number of fungi registered as dominant in studies conducted in one or another biotope of the Republic corresponded to this indicator. However, it is interesting to note that although the number is always similar, the participation combination of the dominant species varies depending on where the sample is taken. For example, species from soil samples such as *Aspergillus niger, Chaetomium globosum, Mucor globosus, Penicillium chryzogenium* and *Trichoderma liqnorum* are characterised by a frequency of dominance, while in our case, the dominant species are *Alternaria alternata, Botrytis cinerea, Fusarium moniliforme, Penicillium cyclopium* and *Verticillium dahilae*.

Interestingly, there is no doubt about the phytopathogenicity of 4 of the 5 species registered as dominant in the mycobiota of essential oil plants in the studied areas of Azerbaijan, and they cause alternariosis, gray rot, fusarium wilt, and wilting. Although there is no literature on *P.cyclopium* causing any serious pathology, it is also known to be toxigenic. When describing the most common, as well as random and rare species from this aspect, it is clear that there are many species among them characterised by their activity due to both dangerous pathogens and other forms of ecotrophic specialisation. All this is characterised as one of the sources of nourishment and habitat of essential oil plant, fungi, including plants themselves, as well as species that pose a serious threat to human health.

3.7. Annotated list of fungi forming the mycobiota of essential oil plants

An annotated list of 161 species of fungi in the flora of Azerbaijan, which are involved in the formation of mycobiota of 103 species of essential oil plants, was also compiled. The list was compiled using the information on the taxonomic relevance of fungi, some cultural and morphological features to be considered during identification, the substrate and location where they were first recorded, as well as the enzymatic activity of some fungi.

CHAPTER IV
EVALUATION OF MATERIALS FROM ESSENTIAL OIL PLANTS BSED ON THE BACTERIAL AND FUNGISIDAL PROPERTIES

4.1. Total amount and components of essential oil of some essential oil plants of the flora of Azerbaijan

A number of studies have shown that essential oil plants also contain biologically active substances with different compositions, which also have different levels of bactericides and fungicides, as well as antivirals, and other features.

Since this seemingly contradictory point is of scientific and practical interest, in the second stage of the research, essential oils were used in this aspect, i.e. various materials derived from them, primarily aqueous extracts and essential oils.

Research in this area first studied the chemical composition of essential oils in a number of essential oil plants. It became clear from the results that the plants studied differed from one another both in the amount of essential oils and in the major components found in it (Table 4.1). Admittedly, the same components are characterised by a major indicator in some plants. For example, menthol is a major component for catnip and peppermint, while thymol is a major component for yarrow and wormwood but these are very rare.

Since this difference affects the bactericidal and fungicidal properties of these plants both from a scientific and practical point of view, it is considered relevant to clarify these issues in the course of research. For this purpose, aqueous extracts of essential oil plants, more precisely infusions and essential oils were used. Both classical and new cultures were used as test cultures. More precisely, along with bacterial cultures such as *Bac.subtilus, Stafilococcus aureus, Pseudomonas aureginoza*, *Echericha coli*, and *Candida alpicans*, which are currently used in similar studies, fungi like *Aspergillus niger, A.ochraseus, Fuzarium oxysporium, Penicillium citrinum,* and *P.cyclopium* have been also used. The choice of the latter is due to the fact that all of these species belong to toxins and synthesise mycotoxins, which cause serious complications for human health and are comparable in effect to weapons of mass destruction. The fact

Table 4.1

General characteristics of essential oils in plants used in research

Plants	The total amount of EO (in %)	The amount of the main components in the EO (in %)
Catnips (*Nepeta cataria* L.)	2,5-3,0	Menthol- 98,5 Limenton- 0,92
Peppermint (*Mentha piperita* L.)	2.5-6,0	Menthol – 89,87 Menthon – 6,02
Yarrows (*Achillea millefolium* L.)	0,75-1,0	Thymol- 57,24 Menthol -17,89
Perforate St John's-wort (*Hypericum perforatum* L)	0,4-0,6	γ – amorfen -30,6 δ-kadinen – 7,1 (E)-β-farnezen -5,4
Common wormwood (*Artemisia absinthium* L.)	0,2—0,5 %	Thymol- 30,97 Evcaliptol -24,13
Mugwort (A.vulgaris L)	0,1-0,3	Iso-thymol – 71,81 Evkaliptol – 17,15
Apium (*Apium graveolens* L.)	0,1-0,2	Carbocrol -63,83 o-cumene – 21,0
Rosmarinus (*Rosmarinus officinalis* L.)	1,0-2,4	α-pinen -30,21 β-pinen -30,14 kamfen -20,23
Lemon (*Citrus limon* (L.) Osbeck)	0,18-0,28	α-limonen -89,32 sitral – 3,25
Salvia (*Salvia officinalis* L).	0,6-1,5	Tsineol – 15 α-tuyon -10 β-tuyon -8 D-α-pinen- 7

that these fungi, as well as the toxins they synthesize, are natural contaminants of plants grown for food, makes it necessary to limit their activities in modern sciences such as microbiology, mycology, etc., which is why we chose them as test cultures.

First, studies were conducted to clarify the bactericidal and fungicidal properties of aqueous extracts from essential oil plants. It became clear from the results that AE obtained from all the plants used can affect the growth of both bacteria and fungi, and in all cases, it manifests itself by weakening the growth. In this case, the quantitative indicator of the effect varies depending on the source of AE. This can be clearly seen from the data given in the examples of some of the plants studied (Table 4.2). As can be seen, in no case

Table 4.2

The effect of AE obtained from essential oil plants on the growth of fungi and bacteria

Composition	Test cultures	Activity	
		By biomass output (by control %)	By the diameter of the lysis zone (mm)
1	2	3	4
Salvia (1/10)	*Bac.subtilus*	27,6	14
	St.aureus	34,3	11
	Ps.aureginoza	25,4	17
	Ech. coli	23,2	19
	Candida alpicans	28,4	17
	Fuzarium oxysporium	29,8	16
	Aspergillus niger	21,3	19
	A.ochraseus	20,3	21
	Penicillium citrinum	18,9	22
	P.cyclopium	17,2	23

Continuation of Tab. 4.2.

1	2	3	4
Rosemary (1/10)	*Bac.subtilus*	43,3	8
	St.aureus	39,5	12
	Ps.aureginoza	38,4	16
	Ech. coli	35,7	17
	Candida alpicans	32,1	12
	Fuzarium oxysporium	35,6	11
	Aspergillus niger	32,1	10
	A.ochraseus	34,8	16
	Penicillium citrinum	40,2	14
	P.cyclopium	37,6	17
Yarrows (1/10)	*Bac.subtilus*	34,5	12
	St.aureus	32,2	13
	Ps.aureginoza	25,4	11
	Ech. coli	29,2	14
	Candida alpicans	21,4	15
	Fuzarium oxysporium	17,8	19
	Aspergillus niger	21,2	16
	A.ochraseus	22,3	17
	Penicillium citrinum	18,1	18
	P.cyclopium	21,4	20
Control		100	100

does the growth of bacteria or fungi stop completely, i.e. the effect ends mainly with growth retardation. More precisely, it would be more accurate to evaluate the effect of the AE used as bacteriostatic and fungiostatic. However, the effect of aqueous extracts from some plants is higher than others, and their use in the future for the production of medicines that restrict the growth of bacteria and fungi has certain prospects. It is worth mentioning one of the points given in Table 4.2, which is related to the nature of the bactericidal and fungicidal properties of AE of the plants used. As noted, the effect of AE on the growth of test cultures is different, and the fact that this difference is directed at bacteria and fungi does not allow a general trend to be observed. More precisely, aqueous extracts from plants do not affect both Gram (+) and Gram (-) bacteria and fungi equal. For example, although aqueous extract from the plants shown in the table – Yarrows has the highest effect on the bacterium *St.aureus* (Gram (+)), this feature associated with *Ps.aeroginoza* (Gram (-)) is characteristic of the Salvia. It is observed no plant has a universal effect on fungi. For example, while Yarrows have the highest effect for *F.oxysporium* fungus, a similar effect for *P.cuclopium* is observed when using AE derived from Salvia. Similar examples can be seen in the example of other plants, such as Mugwort, Common wormwood, Peppermint, etc., which are not shown in the table but have been tested in this regard in research.

In the next stage of the study, the bactericidal and fungicidal properties of plant-derived EO were studied, and it became clear from the results that, unlike AE, the effect of EO is characterised by a higher quantitative value (Table 4.3). This manifests itself in both bactericidal and fungicidal properties, and the situation observed with AE is almost repeated with some quantitative differences. Only in this case, a complete cessation of growth is observed, i.e. the effect of EO can be characterised more as a bactericidal or fungicidal property.

It should be noted that about 20 plants were used to obtain EO; the data on the bactericidal and fungicidal properties of some of them are given in Table 4.3. In general, depending on the source of the tested plants, both bactericidal and fungicidal properties are characterised by different quantitative indicators, and according to the results of research conducted at this stage, it was considered relevant to select the tested plants in three acceptable aspects:

1. Those with relatively high bactericidal properties;
2. Those with relatively high fungicidal properties; and

3. Universal, i.e. those with relatively high bactericidal and fungicidal properties.

Table 4.3

Bactericidal and fungicidal properties of essential oils from essential oil plants

Composition	Test cultures	Activity	
		By AB (by control %)	By DZ (mm)
1	**2**	**3**	**4**
Salvia	*Bac.subtilus*	1,2	31
	St.aureus	2,3	26
	Ps.aureginoza	2,4	25
	Ech. coli	1,1	30
	Candida alpicans	0	27
	Fuzarium oxysporium	0	26
	Aspergillus niger	0	29
	A.ochraseus	0	31
	Penicillium citrinum	0	32
	P. cyclopium	0	33
Rosemary	*Bac.subtilus*	33,2	13
	St.aureus	29,3	17
	Ps.aureginoza	28,3	21
	Ech. coli	25,6	22
	Candida alpicans	12,2	18
	Fuzarium oxysporium	15,2	17
	Aspergillus niger	12,2	16
	A.ochraseus	14,4	22
	Penicillium citrinum	20,0	20
	P. cyclopium	17,3	23

Continuation of Tab. 4.3

Yarrows	*Bac.subtilus*	3,4	22
	St.aureus	3,2	23
	Ps.aureginoza	3,5	21
	Ech. coli	2,9	24
	Candida alpicans	0	32
	Fuzarium oxysporium	1,8	23
	Aspergillus niger	1,0	25
	A.ochraseus	0	29
	Penicillium citrinum	0	30
	Penicillium cyclopium	0	32
Control		100	0

According to the above-mentioned conventional aspects, i.e. wormwood was selected for its bactericidal properties; pepper mint and celery for their fungicidal properties; and sage for universal properties. After various studies, the selection of these plants was evaluated from three aspects due to their use in humans as a source of medicines to regulate and reduce the number of the normal bacterial and fungal biota.

As noted, the component composition of EO is different and is generally divided into major and minor in amount. According to the literature, as well as the results of our research, the component of EO includes about 500 items, some of which are listed in Table 4.4. Studies to determine whether there is any relationship between the major components of EO and their bactericidal and fungicidal properties have shown that EO has the same and different major components. The difference in both bactericidal and fungicidal properties of EO with the same major component is among the results obtained as well (Table 4.4). As can be seen, the major component of the essential oils of the Catnips (Nepeta cataria) consists of menthol, while that of plants such as Yarrows and Common wormwood contains thymol. Although there is no clear dependence of their bactericidal or fungicidal properties when compared with each other, both bactericidal and fungicidal properties of thymol-containing EO are higher than those of menthol. In sage, which has bactericidal and fungicidal properties, tsineol has the status of a major component. In short, the unequivocal opinion about the bactericidal and fungicidal nature of one or another plant according to the major components can be expressed only after the individual components of the EO have been obtained in pure form, noting that the thymol-containing EO has relatively higher bactericidal and fungicidal properties.

Various methods are also used to enhance the bactericidal and fungicidal properties of EO alone obtained from individual plants, one of which is the use of their composition with different substances. Considering this, in the next and final stage of the research, the composition of EY, which was distinguished by its activity, as well as the composition of White Naphthalene Oil (WNO) obtained on the basis of high purification technology from Naftalan oil was used. It became clear from the results that although the activity of the components of some compositions did not change sometimes, in some variants, there is an increased effect, i.e. the bactericidal and fungicidal properties of the composition increase. Moreover, the growth effect can be up to 20%, depending on the source of the components of the composition and the biological

Table 4.4

Bactericidal and fungicidal activity of plants in which the major elements of the component composition of essential oils are similar and different

Plants that are the source of the EO used	Test cultures	Activity	
		By AB (by control %)	By DZ(mm)
1	2	3	4
Catnips (Nepeta cataria L. Menthol- 98,5 Limenton- 0,92	*Bac.subtilus*	6,4	20
	St.aureus	6,2	21
	Ps.aureginoza	6,5	19
	Ech. coli	5,9	22
	C. alpicans	2,1	28
	F.oxysporium	0	32
	A.niger	2,0	22
	A.ochraseus	2,3	21
	P.citrinum	0	32
	P.cyclopium	0	34
Yarrows (Achillea millefolium L.) Thymol- 57,24 Menthol -17,89	*Bac.subtilus*	3,4	22
	St.aureus	3,2	23
	Ps.aureginoza	3,5	21
	Ech. coli	2,9	24
	C. alpicans	0	32
	F.oxysporium	1,8	23
	A.niger	1,0	25
	A.ochraseus	0	29
	P.citrinum	0	30
	P.cyclopium	0	32

Continuation of Tab. 4.4

1	2	3	4
Common wormwood (Artemisia absinthium L.) Thymol- 30,97 Evcaliptol -24,13	*Bac.subtilus*	2,4	25
	St.aureus	2,2	26
	Ps.aureginoza	2,5	24
	Ech. coli	1,9	27
	C. alpicans	0	33
	F.oxysporium	0	28
	A.niger	0	30
	A.ochraseus	0	32
	P.citrinum	0	34
	P.cyclopium	0	37

characteristics of the test cultures. For example, there are certain changes in the bactericidal and fungicidal activity of EO compositions prepared with WNO from different plants, which leads to a growth effect (Table 4.5). A similar situation, i.e. an increase in activity, also occurs in the EO/ EO composition (Table 4.6).

All this promises broad prospects for the acquisition of better quality new medicines of therapeutic and prophylactic value, and on the other hand, allows the efficient use of natural resources. The latter data given in Tables 4.5 and 4.6 should also be mentioned, which is related to the components of EO used in the preparation of the composition. As noted, the compositions were performed in different proportions of EO/EO and EO/WNO combinations. When describing the results obtained in this direction, it is clear that the effectiveness of the compositions prepared with the EO/WNO ratio is relatively high. The presence of one of the constituent elements, thymol-containing EO in compositions prepared in the EO/EO ratio, increases the effect, which can be considered as an additional argument to support the above-mentioned idea that thymol compounds are characterised by higher activity.

Table 4.5

Bactericidal and fungicidal properties of the composition of essential oils with White Naftalan oil

Composition	Test cultures	Activity (DZ, mm)
Salvia officinalis + White Naftalan oil (1:1 ratio)	Bac.subtilus	35
	Ech. coli	34
	Klebsellia sp.	30
	Ps.aureginoza	30
	St.aureus	31
	Aspergillus flavus	38
	A.niger	35
	A.ochraseus	38
	C.alpicans	30
	C.cladosporides	35
	F.moniliforme	34
	F.oxysporium	31
	P.citrinum	35
	P.cuclopium	34
Artemisia absinthium + White Naftalan oil (1:1 ratio)	Bac.subtilus	36
	Ech. coli	39
	Klebsellia sp.	35
	Ps.aureginoza	32
	St.aureus	31
	Aspergillus flavus	29
	A.niger	30
	A.ochraseus	32
	C.alpicans	31
	C.cladosporides	34
	F.moniliforme	32
	F.oxysporium	31
	P.citrinum	29
	P.cuclopium	32

Table 4.6

Bactericidal and fungicidal properties of compositions made of EO with different major components

Composition	Test cultures	Activity	
		By AB (by control %)	By DZ (mm)
1	**2**	**3**	**4**
Catnips/Sage 1:1	*Bac.subtilus*	5,4	21
	St.aureus	5,2	22
	Ps.aureginoza	5,5	20
	Ech. coli	4,9	23
	C.alpicans	1,7	29
	F. oxysporium	0	31
	A.niger	1,5	23
	A.ochraseus	2,0	23
	P.citrinum	0	29
	P. cyclopium	0	30
Yarrows/Sage 1:1	*Bac.subtilus*	2,4	24
	St.aureus	2,2	25
	Ps.aureginoza	2,5	23
	Ech. coli	1,9	26
	C.alpicans	0	34
	F. oxysporium	1,1	25
	A.niger	0,5	27
	A.ochraseus	0	30
	P.citrinum	0	31
	P. cyclopium	0	33

Continuation of Tab. 4.6

1	2	3	4
Common wormwood/Sage 1:1	*Bac.subtilus*	1,1	31
	St.aureus	1,2	32
	Ps.aureginoza	1,3	29
	Ech. coli	1,0	31
	C.alpicans	0	34
	F. oxysporium	0	31
	A.niger	0	33
	A.ochraseus	0	34
	P.citrinum	0	36
	P. cyclopium	0	39

CHAPTER V
POSSIBILITIES OF USING ESSENTIAL OIL PLANTS TO ENSURE MICOLOGICAL SAFETY OF PRODUCING VEGETABLE PRODUCTS

5.1. Assessment of the possibility of using products from essential oil plants to improve the phytosanitary situation of agrocenosis used for the cultivation of vegetable crops

The research studied the possibility of using the products obtained from essential oil plants studied for improving the phytosanitary condition of the melon agrocenosis for this purpose, and Catnips, Yarrows, and Mugwort were used in this connection. Dry biomass and AE were used together with above-mentioned plants to improve the phytosanitary condition of agrocenosis, and their method of use was as follows.

1. The mass, collected from the aboveground part of any plant, is completely dried under normal conditions (under the sunlight) and processed into flour. The dry mass of flour was used for the agrocenosis at the rate of 2 kg (dry weight) per hectare. The assessment of the improvement of the phytosanitary condition of agrophytocenosis was estimated based on germination capacity of plant seeds and background indicator of phytococcal activity determined on the basis of specific weight of toxigenic fungi.

2. A 10% extract of the dried plant mass is prepared by infusion and infused for 2 hours (at 40^0C) and at the end of the period, the insoluble (more precisely not extracted) part of the plant is separated from the solution by filtration. The solution is used as a means to improve the phytosanitary condition of the agrocenosis. The obtained solutions were sprayed at the rate of 5 l/ha.

Research on the use of both means was carried out in the fields of Research Institute of Vegetable Growing under the Ministry of Agriculture of the Republic of Azerbaijan with an area of 0.25 ha each, where cabbage and tomato plants are grown. As a control, adjacent plots were planted without the addition of tools but the same plant was planted. It became clear from the results of the research conducted in 2019-2020 that both dry biomass and AE lead to a decrease in the phytotoxic background of the tested crop area, and in this case, the results differ from each other in terms of quantity(tabl.5.1). As can be seen, the phytotoxic activity of soils taken from the area used as a control (under cabbage cultivation) is higher, compared to the area where AE was used. A similar indicator is observed when using dry biomass. However, in these cases, the quantitative indicators of the effect are different and the use of dry biomass is weaker than that of AE. Thus, the background of phytotoxic activity of soils is 12%, 16%, and 24% less in cabbage and 15%, 20%, and 25% in tomatoes, respectively, compared to the control when using AE obtained from catnip, yarrows, and mugwort. A similar indicator is used for dry biomass from these plants, which

Table 5.1.

Effect of dry biomass and AE derived from some essential oil plants on the background of phytotoxic activity of agrocenosis

№	Plant used	By the background indicator of toxic activity of soils			By the number of toxins fungi		Gross output (t/ha)
		Number of seeds used (pcs)	Number of non-germinated seeds (pcs)	Activity (%)	Total number of recorded fungi species	Number of phytotoxic fungi	
Cabbage							
1	Catnips	150	24/22*	4/12	30/32	20/21	16,0/16,3
2	Yarrows		23/21	8/16	34/33	22/22	16,2/16,7
3	Mugwort		21 / 19	16/24	32/33	21/22	16,6/17,2
4	Control		25	100	34/33	21/19	15,8
Tomato							
1	Catnips	150	18/17	10/15	26/27	17/17	10,4/10,7
2	Yarrows		19/16	5/20	23/23	16/15	10,7/11,0
3	Mugwort		17/15	15/25	25/26	16/17	11,0/11,5
4	Control		20	100	24	15	10,2

Note: * - Data obtained from the effect of dry biomass is given in numerator; data obtained from the effect of AE – in denominator.

is 4%, 8%, and 16% less in cabbage and 10%, 5%, and 15% in tomatoes, respectively. This clearly shows that the recorded effect is higher in the second case than in the first.

A similar situation is observed when the toxigenic fungi are evaluated for their specific weight in the total mycobiota. However, in this case, the effect is not systemic, and in general, it is difficult to determine in which case the effect is good. Thus, first, according to the background indicator of toxic activity of soils, in all cases, i.e. in areas where both cabbage and tomato plants are grown, the means obtained from ordinary mugwort are more effective but there is no such dependence on the number of species of toxigenic fungi. Second, the quantitative value of the effect determined by the toxic background of soils is higher than that determined by the specific weight of the toxigenic species. Third, the final product indicators are in line with the toxic background of the soil. Therefore, in our opinion, it is more relevant to determine the experiments on such issues on the background of toxic activity of soils of agrocenoses. This is also favourable for both economic and technological reasons.

Thus, it takes less energy and material to determine the level of activity of the background indicator of toxic activity of soils, and it is easier in terms of time. Thus, in the second case, a sample should be taken from the soil, all fungi should be removed from the sample to a pure culture, and then the species composition of toxins between them should be determined. All this takes much time, as well as more energy.

It would also be appropriate to address the issue of the effectiveness of the results obtained from field research (Table 5.1), which is related to which of the tested materials is more appropriate to use. As noted, it was clear from the results that the use of both materials has a positive effect on improving the phytosanitary condition of agrocenosis but the quantitative indicator of the effectiveness of AE obtained from the same plant is higher. This is due to the fact that compounds with fungicidal or bactericidal effect in the dry biomass of any given plant are in the dry state and are less likely to come into contact with phytopathogenic species, which worsens the phytosanitary situation, and when AE is added, there is no doubt that this contact is a distinct possibility. This is confirmed by the specificity of the heterotrophic nutrition of fungi. Thus, small amounts of water and water-soluble substances in the soil can penetrate from any part of the fungal cell. Therefore, it is more convenient to use liquid means to improve the phytosanitary condition of agrocenoses. The convenience of this can be explained by another reason, which is related to the efficient use of resources, primarily bioresources. Thus, when using dry biomass, it is important to collect more material related to any given plant, which is not conducive to the use of resources in accordance with the principles of sustainable development. To get AE, one saves on those resources at least 10 times. Thus, the amount of plant material added to the solution to obtain AE makes 10%. At the same time, the effect of this dry biomass per kg is high when using AE. In short, the use of AE is more favourable for environmental, economic, and technological considerations.

5.2. Assessment of the possibility of using products from essential oil plants to ensure the mycological safety of some fruit plants

As noted, mycotoxins are metabolites that are formed as a result of the life activity of toxigenic fungi and cause deterioration of the quality of many plant products. The fact that its amount is within acceptable limits in the products produced, including dried fruits (walnuts, hazelnuts, almonds, pistachios, etc.) is one of the most important issues today for environmental and economic reasons. It is worth noting that the high toxicity of hazelnuts produced in Azerbaijan is well known to both scientists and practitioners. The amount of mycotoxins in it is higher than required by regulations. To prevent this, the most important action is to limit the number and activity of toxigenic fungi in the environment in which any given product is grown.

In general, it should be noted that the enrichment of plant products with mycotoxins occurs in several stages, the first of which occurs during the cultivation of the product in the field, and the second during its storage and processing. More precisely, in the enrichment of plant products with mycotoxins, both conventionally called “field” and “storage” fungi are involved. Preventive measures to limit the activity of fungi belonging to these conditional groups are different, and the applied methodological approach may vary depending on the product produced and storage conditions. Taking this into account, the research clarified the effectiveness of the use of products from essential oil plants in limiting the activity of toxigenic fungi, and this work was carried out jointly with the Institute of Fruit Farming and Tea Growing of the Ministry of Agriculture of the Republic of Azerbaijan. Experiments were mainly carried out on hazelnuts in the mentioned area. AE and EO obtained from plants such as Mugwort, Yarrows, and Catnips were used in the experiments. The use of products was carried out by spraying, the dose of which was 1l/10 m^2 and was done twice a year (in the 3rd decade of April and July). Here we are talking about the area related to the plant, which was also calculated as follows: the area was found based on the length of all 200 hazelnut plants (100 experiments and 100 controls), selected for the experimental work, and the size of the width of the branches. The width was measured at the widest and shortest points of the plant branches, and the average value was used. The process was evaluated based on the number of fungi inhabiting the leaves and fruits of the plant and the total amount of mycotoxins. The results of the study showed that both products derived from these plants reduce both the number of toxigenic fungi and the total amount of mycotoxins (tabl. 5.2). As can be seen, EO is more effective than AE, which is reflected in both the number of fungi and a decrease in the total amount of mycotoxins. Thus, the average value of the number of fungi in hazelnut trees selected as control is 5950 CFU/g, and the amount of mycotoxins is 1.88 mcg/g. A similar indicator is observed with a decrease of 1.16-1.27 and 1.18-1.45 times when using AE from all plants, and 1.24-1.38 and 1.28-1.69 times when using EO, respectively.

FINAL ANALYSIS OF THE STUDIES

The steady growth of the world's population within a stable area is intensifying human intervention in nature, and as a result, the bio-ecological balance has recently shifted to fundamental changes in the direction of global imbalances. This, in turn, creates certain changes in the vital activities of all taxonomic groups, primarily difficulties. These difficulties are manifested in the occurrence of various pathologies among living things and the emergence of new, more precisely sustainable forms of their perpetrators, etc. Prevention of these is one of the current research areas of modern biological and medical sciences. In the unfavourable environmental conditions associated with the solution of these problems, the demand for natural products with a therapeutic and prophylactic effect on living things and humans is constantly increasing.

One of these compounds, which is characterised as BAS (biologically active substances), is essential oils (EO). Medicinal essential oil plants are mainly used to obtain EO. However, a number of studies have shown that this type of plant is also one of the habitats of fungi, and there are allergens, opportunistic and toxicogenic ones among these fungi, which are characterised as manifestations of ecotrophic specialisation. In short, medicinal plants contain components with bactericidal and fungicidal activity, on the one hand, and fungi, which cause various pathologies in living things, as well as nutrients necessary for the survival of bacteria. The discovery of the mechanism of all this, the clarification of plant-

Table 5.2

Effects of some products from essential oil plants on the number of fungi (NF=x10^3 CFU/g) and the total amount of mycotoxins (MK) (mcg/g)

№	Names of plants used	I spraying				II spraying				Average price for two sprays.			
		NF		MK		NF		MK		NF		MK	
		SE	EY	SE	EY	SE	SE	EY	SE	SE	EY	SE	EY
1	Mugwort	4,8	4,52	1,36	1,2	4,56	4,12	1,24	1,02	4,68	4,32	1,30	1,11
2	Yarrows	4,85	4,5	1,62	1,5	4,62	4,35	1,46	1,40	4,74	4,43	1,54	1,44
3	Catnips	5,21	4,98	1,68	1,56	5,02	4,65	1,52	1,38	5,12	4,82	1,60	1,47
4	Control	6,23		1,92		5,67		1,84		5,95		1,88	

fungal relations is one of the issues of both theoretical and practical importance. For this reason, the purpose of this paper is to clarify the bactericidal and fungicidal properties of mycobiota and their components, which are characterised as medicinal essential oil plants in the flora of Azerbaijan.

The territories of 7 out of 10 economic regions of Azerbaijan were used for sampling in order to achieve the set goal and the tasks set for its implementation. No samples were taken from the Nagorno Karabakh and Lachin-Kalbajar economic regions for research purposes due to being under the occupation of the Republic of Armenia during the research, and from the Nakhchivan economic region due to technical reasons (distance, other researches conducted in those areas, etc.).

First, the species composition of the sampled plants was determined in the research. It was found that the number of plant species used for sampling was 103, including both monocotyledonous and dicotyledonous plants, as well as gymnosperms and polypodiopsidas. In addition, many of the sampled plants grow wild in the nature of Azerbaijan, and some are grown for one purpose or another. On the other hand, the sampled plants also differed in their life forms, as they include trees, shrubs, and grasses.

As for the fungi involved in the formation of the mycobiota of these plants, it became clear from the research that there is a wide variety in this area. Thus, studies conducted in 2011-2020 showed that 161 species of fungi (Mycota) and fungal-like organisms (Chromista) are involved in the formation of mycobiota in 103 species of plants. 93.2% of the fungi registered in the study belong to real fungi and 6.8% to fungal-like organisms.

When determining the taxonomic structure of true fungi, it became clear that the vast majority of recorded fungi, more precisely 72.7% (67.7% of the total recorded) were sac fungi (Ascomycota phylum), 18.0% were basidiomycetes (Basidiomycota), and 9.3% were zygomycetes.

It should be noted that today the systematics of fungi is a dynamically developing field, and therefore we have clarified our views in this regard, based on the information on the official website of the International Mycological Association, which is constantly updated and contains up-to-date information. According to the website, sac fungi now include indefinite fungi - deutromycetes, which were once considered a formal group at certain times, and in this regard, sac fungi are grouped into two groups. This grouping is also based on their sac stage. Accordingly, sac fungi are divided into anamorphic and telemorphic, the former being non-sac fungi, multiply only asexually, and the latter being sac fungi, reproducing both asexual and sexual states.

The fact that the anamorphs of fungi are more active in the formation of mycobiotics of any biotope than fungi of other groups is reflected in almost all studies conducted in Azerbaijan. When characterising the fungi recorded in the study from this aspect, it became clear that the vast majority of the 109 species of sac fungi recorded, i.e. 86, belong to the anamorphs of sac fungi. This is 72.7% of the actual fungi recorded in the study (53.4% of fungi and fungus-like organisms).

Most of the fungi and fungus-like organisms recorded in the studies, more precisely 153 species, are among the fungi found to be prevalent in various studies conducted in Azerbaijan. The same statement can not be said about species such as *Mucor ramosissimus Samouts, Exserohilum longirostratum (Subram.) Sivan., Fusarium dimerum Penz., Nigrospora maydis (Garov.) Jechová, Penicillium simplicissimum(Oudem.) Thom, Phoma eupyrena Sacc., Phoma medicaginis Malbr. & Roum,* and *Sclerotinia graminearum Elenev ex Solkina* as this is the first time to obtain experimental data on the distribution of these fungi in Azerbaijan. Based on this fact, it should be noted that the mycobiota, which is unique to the nature of Azerbaijan, has not been sufficiently studied, and today it is an object that is open and relevant for such research.

Taking into account that the research was conducted in seven economic regions of Azerbaijan, which differ from one another on a number of indicators, the distribution of fungi in those economic regions was also studied. It was determined that the mycobiota of essential oil plants in the territory of the Lankaran-Astara economic region is characterised by richer, and Absheron's is characterised by the poorest mycobiota. The main difference between the Lankaran-Astara economic region and the Absheron economic region is related to climate and soil, as the first has a humid subtropical climate, and the second has a dry subtropical climate.

Due to the fact that fungi are heterotrophic organisms, their food relationships with other living things, especially plants, are also one of the points of attention in microbiological and mycological research. Taking this into account, it was considered relevant to characterise the fungi registered in the researches from this aspect as well. For this purpose, first, the division of fungi into saprotrophs, biotrophs and polytrophs was characterised accordingly. It was found that 14.9% of the fungi recorded in the study belonged to true saprotrophs, and it is due to them that the epiphytic mycobiota of essential oil plants are formed. 20.5% of the remaining fungi are true biotrophs. Polytrophics account for 64.6% of fungi and fungus-like organisms. In short, 85.1% of the fungi involved in the formation of mycobiota of essential oil plants in Azerbaijan are prone to some degree of pathogenicity, and according to their attitude to plants, they can be divided into three conditional groups: true pathogens, prone to pathogenicity, and those that lead to the development of pathogens. If we characterise the fungi recorded in the study in this aspect, it is clear that 24 species are excluded from this division, i.e. they are actually engaged in “cleaning out” the remains of plants, as well as other living organisms, which lost their vitality due to pathogens and other biotic and abiotic factors. There are 33 species of true pathogens; 50 - prone to pathogenicity, and 43 - that lead to the development of pathogens. There is not enough research material to characterise the 11 species according to this division. Admittedly, they belong to polytrophics in terms of ecotrophic relationships, and at least they can be characterised as leading to other parasites.

It should be noted that recent mycological studies have used other indicators, more precisely allergenicity, toxigenicity and opportunic ones, which are characterised as manifestations of ecotrophic relationships between fungi and plants. When characterising the fungi recorded in our studies from this aspect, it became clear that the specific weight of toxicogenics, allergens, and conventional pathogens also plays an important role. Thus, 57.8% of the registered fungi are allergenic, 41.9% are opportunistic, and 65.2% are toxicogenic. Characterising this fact from the literature, it is clear that the ecotrophic structure of fungi, involved in the formation of mycobiotics of essential oil plants, is generally not characterised by favourable indicators. This suggests the need to be more careful when using them, as well as the development of normative documents containing the principles of microbiological and mycological safety of their use.

Determining the distribution of fungi on individual organs of plants is important information from a practical point of view. Thus, in folk medicine, as well as for food and feed purposes, various parts of plants are used, and in most cases, this is done without heat treatment. For this reason, which part of the plants is most affected by fungi may be important when using them. Taking this into account, it was considered relevant to determine the distribution of fungi on different organs of plants. It was found that 51.6% of the registered fungi and fungus-like organisms are found in the trunk, 54.0% in the leaves, and 18.0% in the roots. 42.2% of fungi involved in the formation of mycobiota are universal, 14.9% are specific and can live only in a plant-specific organ.

Although there are differences in the distribution of fungi by economic regions, plants, and plant organs, there are some commonalities in the study. This is the frequency of occurrence of fungi on essential oil plants in general. Studies have shown that species such as *Alternaria alternata*, *Botrytis cinerea*, *Fusarium* *moniliforme, Penicillium cuslopium,* and *Verticillium dahilae* are the dominant species of mycobiota of essential oil plants, with a frequency of 42.7-53.6%. Of the five species of mycobiota dominant in essential oil plants, four are phytopathogens and cause alternariosis, gray rot, fusarium wilt, and wilting. *P.cuslopium* is a potent toxin, although no known disease has been reported related to it. This fact can be considered as a dangerous situation, as well as an indication of greater caution when using essential oil plants.

Aqueous extracts and essential oils from some essential oil plants in the flora of Azerbaijan have been found to contain components that adversely affect the growth of both bacteria and fungi, which effect can be bacteriostatic and fungiostatic, bactericidal and fungicidal, depending on the biological properties of both plants and test cultures, and have bactericidal and fungicidal properties.

EO derived from essential oil plants have a complex component and are generally divided into major and minor. Today, it is not clear which of these components is the basis of bactericidal or fungicidal activity. For this reason, it was determined that the bactericidal and fungicidal effects of both AE and EO obtained from essential oil plants containing major components thymol and tsineol are higher than those of menthol, and therefore, the use of EO from mugwort and common wormwood is more promising.

During the research, the bactericidal and fungicidal activity of EOs obtained from some essential oil plants in the flora of Azerbaijan, as well as compositions prepared with White Naftalan oil from Naftalan oil on the basis of high purification technology were studied. The obtained results allowed determining the optimal composition of the components used in the preparation of compositions, which allows increasing the quantitative indicator of bactericidal and fungicidal activity by up to 20%. This also creates additional opportunities for more efficient use of natural resources. On the other hand, higher bactericidal and fungicidal activity of individual compounds in the EO component, primarily the major components thymol and tsineol, was observed. In this regard, it should be noted that the study of which component of EO plant, which contains a large number of components, is the basis of its bactericidal or fungicidal activity, and the results can be considered as the first step in this direction. However, an unequivocal answer to this question is possible only after clarifying the bactericidal and fungicidal properties of each of the components in the EO.

The results of the study showed that the products obtained from plants in the flora of Azerbaijan are useful both for improving the phytosanitary condition of agrocenosis, where vegetables are planted, as well as for ensuring the mycological safety of hazelnut production. Thus, in the course of the research, the possibility of determining the synthesis of essential oil plants metabolites capable of restricting the growth of fungi, including toxins and the possibility of using plants to ensure the safety of plant products against mycotoxins were investigated. It has been found that both aqueous extracts and essential oils from plants, especially Mugwort, can be useful as a source of medicines suitable for this purpose, and the number of fungi and the amount of mycotoxins decrease 1.16-1.27 and 1.18-1.45 times when using AE from essential oil plants, and 1.24-1.38 and 1.28-1.69 times when using EO, respectively.

RESULTS

1. The research conducted in 2011-2020 in the economic regions of the Republic of Azerbaijan such as Absheron, Aran, Ganja-Gazakh, Lankaran-Astara, Guba-Khachmaz, Mountainous Shirvan, and Sheki-Zagatala revealed that 161 species of fungi and fungal organisms are involved in the formation of mycobiota of 103 species of essential oil plants growing and cultivating in these areas, of which 93.2% were true fungi and 6.8% were fungal-like organisms, all of which were pathogens in host plants[3, 5-6, 13-15, 18, 23, 25, 27, 30, 35].
2. It was determined that 153 species of registered fungi and fungal-like organisms are known species of mycobiota specific

to the nature of Azerbaijan, while *Mucor ramosissimus Samouts, Exserohilum longirostratum (Subram.) Sivan., Fusarium dimerum Penz., Nigrospora maydis (Garov.) Jechová, Penicillium simplicissimum(Oudem.) Thom, Phoma eupyrena Sacc., Phoma medicaginis Malbr. & Roum, Sclerotinia graminearum Elenev ex Solkina* are among the species identified in these studies in Azerbaijan[25].

3. It was found that the distribution of fungi registered in various economic regions is different, and the mycobiota of essential oil plants in the Lankaran-Astara economic region is richer (66.5% of the total fungi), while in Absheron, they are characterised by the poorest (40.4%) by mycobiota. Comparison of Serenson species compatibility coefficient of fungi registered in different economic regions also showed that these two economic regions are more distant from each other (32%), while the degree of compatibility of mycobiota, which is characteristic of essential oilseeds of the mountainous Shirvan, Guba-Khachmaz, and Sheki-Zagatala economic regions is closer to each other (68-70%)[25, 27, 37].
4. Species involved in the formation of mycobiota of essential oil plant biota of the studied areas also have a significant share of toxins, allergens, and conventional pathogens, which are manifestations of ecotrophic interactions, as 57.8% of the total recorded fungi are allergens, 41.9% are opportunistic and 65.2% are toxigenic. In addition, among the fungi involved in the formation of mycobiota of essential oil plants, there are promising species as producers of biologically active substances[28, 34].
5. It was determined that the fungi involved in the formation of mycobiota of essential oil plants of Azerbaijan are also characterised by diversity due to their ecophysiological features, as they include species that react differently to moisture, temperature, pH, and oxygen. At the same time, 85.1% of the recorded fungi are more or less susceptible to pathogens, and

according to their attitude to plants, they can be divided into three conditional groups: pathogens, prone to pathogenicity, and those that lead to the development of pathogens[10, 18-19, 28, 33].

6. It was found that the distribution of fungi on different vegetative and generative organs of plants is also characterised by different quantitative indicators. Thus, 51.6% of the registered fungi are found in the trunk, 54.0% in the leaves, and 18.0 % in the root. 42.2% of fungi involved in the formation of mycobiota are universal, 14.9% are specific and can live in only one organ[28].
7. In the studied areas, it was determined that species such as *Alternaria alternata*, *Botrytis cinerea*, *Fusarium moniliforme*, *Penicillium cyclopium*, and *Verticillium dahilae* are the dominant species of mycobiota of essential oil plants, the occurrence frequency of which varies from 42.7 to 53.6%. Four of the five dominant species of mycobiota in essential oil plants are phytopathogens, causing alternariosis, gray rot, fusarium wilt, and wilting, while *Penicillium cuslopium* is a potent toxin, although no known disease has been reported[27].
8. It was found that aqueous extracts and essential oils from some essential oil plants in the flora of Azerbaijan contain components that adversely affect the growth of both bacteria and fungi, and their effect depends on the biological characteristics of both plants and test cultures and can have bacteriostatic, fungiostatic, bactericidal and fungicidal properties. This allows them to be used both to improve the phytosanitary condition of agrocenosis, where the vegetables are planted, as well as to ensure the mycological safety of hazelnut production[-2, 4, 7-9, 11-14, 17, 20, 24, 26, 31-32].
9. The bactericidal and fungicidal effect of both aqueous extracts and essential oils of essential oil plants containing major components thymol and tsineol is higher than that of menthol, and in this regard, the use of them derived from common wormwood and mugwort is more promising[29].

10. The research determined the optimal composition of the composition made from essential oils of essential oil plants of Azerbaijani flora and White Naftalan oil from Naftalan oil, which allows increasing the quantitative indicator of bactericidal and fungicidal activity of the individual components up to 20%. It also creates additional opportunities for more efficient use of natural resources[21-22].

THE LIST

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The defence will be held on 29 june 2021 at 11.00 at the meeting of the Dissertation Council BED 1.07 operating under the Institute of Microbiology of ANAS.

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Abstract was sent to the required addresses on 27 may 2021

Signed for print: 24.05.2021

Paper format: 60x84 1/16

Volume: 70804

Number of hard copies: 20