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Mycobiota of Medicinal Plants of Azerbaijan and Mycological Safety of their Use

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In the presented work, the mycobiota of several medicinal plants that make up the flora of Azerbaijan was studied in order to create basic information for the development of principles of mycological safety of their use. The results showed that the mycobiota of the studied plants is rich in species composition, since 186 species are involved in the formation of the mycobiota of medicinal plants included in the flora of Azerbaijan, 94.1% of which are real fungi (Mycota), and 6.9% are mushroom-like organisms (Chromista). Among the detected fungi, a significant proportion of opportunistic (opportunistic), allergenic and toxigenic fungi, which are an indirect or direct source of danger to all living things, including humans. This circumstance justifies the need to prepare documents regulating the mycological safety of plant materials intended for medical purposes, and the first approach when using these plants should take into account the number of mycobiota, the quantitative indicator of which should not exceed 103 CFU/g (dry weight).

Keywords: Medicinal Plants; Mycobiota; Mycological Safety; Numerical Composition; Phytopathocomplex; Pathogenicity.

It is known that microscopic fungi called micromycete are in a close relation and interaction with other organisms in terrestrial ecosystems and first of all with plant[1,2]. Study of principles and relationships that exist in the artificial and natural ecosystems, particularly in a "fungus-plants" system is a significant problem of mycology. Within this aspect, the impact of micromycete on the other soil microorganisms is well studied[3] but there is quite poor information in the literature on the impact of the plants, especially valuable when we are talking in terms of practice, on the community of soil fungi.

Over 19,000 fungi are known to cause diseases in crop plants worldwide[4]. In addition, they can significantly affect its target (food, fodder, medicinal, decorative, etc.) quality.[5]. Among pathogenic micromycete there also exist toxigenic, allergenic and opportunistic species that pose a serious threat to people with reduced immunological status[6-7].

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In this regard, an interest in medicinal plants has grown up significantly in these latter days[8]. Among 500000 species (4750 in Azerbaijan) of higher vascular plants of the planet around 77.000 (1547 in Azerbaijan) have medicinal value[9-10].

They are widely used in the alternative medicine as these plants contain various substances having pharmacological as well as antibacterial and antifungal activity[11-12]. In addition, certain medicinal plants are used for other (food, feed, source of dyes, in gardening and others.) purposes. However, they are also a habitat area for various organisms, including fungi[13], are characterized by phytopathogenic many of which properties and cause various diseases always leading to yield losses and reduction of biological activity. In order to prevent the results of such phenomena it is necessary to study medicinal plants thoroughly, especially their relations with fungi. All this in its turn require accurate determination of the species composition of microorganisms inhabiting the medicinal plants.

The need for the study of medicinal plants is still connected with the fact that many medicinal plants are used without preliminary heat treatment in alternative medicine, i.e. they are not sterile in both the mycological and microbiological aspects[14].

The aim of the present work is to study the microbiota of a number of medicinal plants (grass, shrubs and woody), included in the flora of Azerbaijan, according to their species composition, ecological and trophic relations, and the establishment of the basic information for the development of mycological safety principles and their use.

MATERIALS AND METHODS

In the 2010-2018 timeframe mycological study of medicinal plants (tab. 1) of Azerbaijan in order to identify the species composition of fungi was carried out.

Samples collection was carried out in compliance with the common requirements. Samples of plants were selected in vivo and agrophytocenosis in the budding stage and initial blossom in mid June. About 3,500 plant samples with obvious signs of fungal diseases were collected and analyzed.

Isolation of microscopic fungi from the plant was carried out by the standard method[15]. Prepared samples were transferred to Petri dishes with Czapek's and Saburo medium. The experiment shall be repeated 4-5 times. The crops were incubated in an incubator at a temperature of 26-28^{0}Ñ.

Quantitative and qualitative analysis of mycobiota was carried out by visual inspection using Petri dishes (on the 3-14th day of incubation) with the following microscopic examination and identification using an optical microscope *OMAX 40X-2500X*.

Identification of taxonomy according to the set of cultural and morphological peculiarities was carried out using identifiers and mycological atlases[16-17].

Toxigenic, opportunistic and allergenic to the human kinds were considered species of micromycete according to various identifiers[18] .

Determination of the antimicrobial activity of plant materials was carried out by the method of holes[19].

The repetition of all experiments is fourfold. The data obtained are statically processed[20].

RESULTS AND DISCUSSION

It is known that the structure of the complex of soil micromycete can serve as an informative parameter of soil monitoring[21] including those being in agricultural use. In order to study the structure of the complex of micromycete of medicinal plants there were determined their species composition and taxonomy, found typical and casual species, their representativity in the complex. As a result of the study, it was found that mycobiota of wild and cultivated medicinal plants included in the flora of Azerbaijan includes 186 species, 94.1% of which are related to the true fungi (*Mycota*), and 6.9% - to funguslike organisms (*Chromista*) (tab. 2).

The data received also indicate that the selected mycobiota of medicinal plants is characterized by the predominance number of members of the genus called *Colletotrichum*, which is represented by 17 species and genus called Septoria represented by 14 species. The genus

called *Ascochyta, Fuzarium, Penicillium, Phoma, Alternaria* and *Aspergillus* are represented by 8-13 species. Other genera (*Botrytis* (1), *Cephalosporium* (2), *Cercospora* (1), *Bjerkandera* (1), *Cerrena* (1), *Daedalea* (1), *Daedaleopsis* (1), *Diplodina* (2), *Eryshiphe* (3), *Fomes* (1), *Fomitopsis* (2), *Ganoderma*(1), *Ýnonotus*(1), *Laetiporus* (1), *Lentinus*(1), *Lenzites*(1), (2), Monilia(1), Peronospora(4), Pestolotia(2), Phellinus(3), Phomopsis(1), Plasmopara(1), *Macrosporium Plectosphaerella*(1), *Pleurotus*(1), *Polyporus*(1), *Puccinia*(2), *Rhisopus*(1), *Schizophyllum*(1), *Sclerotina*(1), *Sporotrichum*(1), *Stemphylium*(4), *Stereum(*1), *Trichothecium*(1), *Trametes*(4), *Trichoderma*(4), *Urocystis*(1), *Uromyces*(2), *Ustilaqo*(3) inckuded 1-6 species.

It should also be noted that fungi *Mucor corticola* Hagem, *Ascochyta anethicola* Sacc., *Asc. pinodes* (Berk.et. Blox) Jones., *Asc. pseudopinodella* Bond- Mont et. Xassi, *Diplodina lactucae* (Oudem) Sacc., *Dicoccum asperum* (Corda) Saccardo, *Penicillium stoloniferum* Thorn., *P.puberulum* Bainier, *Verticellium pulverulentum* Couwenteg., *V.lateritium* Berk., *Phoma roumii* Fron., *Ph. minulella* Sacc et. Penz., *Septoria petroselini* Desm., *S.sojina* Thuern and *Phellinus chrysoloma* (Fr.) Donk) are new not only for mycobiota of medicinal plants, but discovered within the territory of Azerbaijan for the first time.

It was found that 44.8% of fungi, marked on medicinal plants according to ecological-trophic relations are referred to saprotrophs, 55.2% to the biotrophic but biotropism and saprotrophic characteristics of 80.5% of fungi has polythrophic (or optional) character (fig. 1), which shows a high pathogenic potential of microbiota of medicinal plants. In addition, among these fungi there are a lot of species that are either opportunistic (*Aspergillus nidulans* (Eidam) Wint., *F.semitectum* Berk et Rav., *P.purpurogenum* Stoll. Et al) or allergenic (*Botrytys cinerea* Pers.: Fr., *Monila sitophila* Montagne) Saccardo, *T.viride* Pers. And others.) or possessed both (*Alternaria alternata* (Fr) Keysel., *A.flavus* Lk., *A.fumigatus* Fres., *A.niger* v.Tiegh, *A.ocraceus* Wilhelm, *A.versicolor* Vuil Tirab, *Cladosporium cladosporioides* (Fresen .) GA de Vsries., *C.herbarium* (Pers.:Fr) Lk., *Mucor racemosus* Fres., *Penicillium chrysogenum*

Table 1. Taxonomy of medicinal plants being studied that are part of the flora of Azerbaijan

Division	Class	Family	Genus	Species
Magno-liophyta	*Magno-liopsida*	*Asteraceae,Fabaceae Lamiaceae Rosaceae, Apiaceae, Brassicaceae Ranunculaceae, Malvaceae Caryophyllaceae, Rubiaceae, Chenopodiaceae Solanaceae, Scrophulariaceae Polygonaceae Boraginaceae Euphorbiacea*	44	146
	Liliop-Sida	*Poaceae, Orchidaceae Cyperaceae, Liliaceae Alliaceae , Hyacinthaceae Juncaceae, Ýridaceae Amaryllidaceae*	23	60
Totally	2	26	67	206

Table 2. General characteristics of fungi and funguslike microorganisms common for medicinal plants of Azerbaijan

Kingdom	Division	Class	Order	Family	Genus	Species
Mycota	Zygomycota	1	1	2	2	7
	Ascomycota	5	9	13	26	137
	Bazidiomycota	2	6	11	21	31
Chromista	Oomycota	1	2	2	3	11
Totally	9	18	27	52	186	

Thom., *P.citrinum* Thom, *P.cuclopium* Westling, *P.expansum* Lk., *Rhisobus stolonifer* (Ehr.:Fr) Vuill etc.) peculiarities. Furthermore, among these fungi were the species that are toxigenic (*A.fumigatus, A.ocraceus, C.herbarium, F.oxysporum* (Schlecht) Snyd. Et Hans, *F. moniliforme* Sheldon, *F.sporotrichiella* Bilai, *P.cuclopium*, *Verticillium album* (Preuss) Pidopl et al.). Many of these fungi synthesize mycotoxins[22], which are dangerous for many organisms that overlay the Earth. In addition, they cause various allergies in humans[6-7].

The use of medicinal plants without heat treatment in alternative medicine, makes it possible to characterize them as one of the sources of transmission of fungal diseases, according to the data we managed to receive[21]. Thus, the microbiota of medicinal plants includes many fungi which both cause disease in a variety of plants intended for food, feed and medical purposes and pollute them by their own different metabolites. Mycotoxins which adversely affect the health of all living beings, including humans are among these metabolites[23]. Therefore, addressing the issue related to the regulation of the amount of mycotoxins in various products is very important[24]. In this regard, during the studies we have examined the antimicrobial activity of some medicinal plants, which differ in the numerical composition of microbiota. The results showed that the numerical index of antimicrobial activity changes depending on the numerical strength of the fungi inhabiting on one and the same species of plants (table. 3). It can be seen that once the numerical index of fungi on plants being studies is greater than 10^3 CFU/g,

Table 3. Antimicrobial activity (lysis zone diameter in mm) of extracts of medicinal plants, which differ in the numerical composition of mycobiota

Medicinalplants	Numerical composition (CFU/g)	Culture study				
		St.aureus	*Bac. subtilis*	*Ps. aeruginosa*	*Esc.coli*	*Candidaalbicans*
Artemisia absinthium L.	$\leq 10^3$	*16*	*21*	*20*	*24*	*17*
	10^3-10^4	*14*	*18*	*17*	*21*	*15*
	$\geq 10^5$	*12*	*15*	*14*	*16*	*12*
Artemisia vulgaris L.	$\leq 10^3$	*17*	*18*	*19*	*18*	*18*
	10^3-10^4	*15*	*16*	*16*	*15*	*15*
	$\geq 10^5$	*14*	*14*	*15*	*13*	*13*
Cuminumcuminum L.	$\leq 10^3$	14	19	17	26	13
	10^3-10^4	12	17	16	22	11
	$\geq 10^5$	11	14	14	18	9
Foeniculumvulgare Mill.	$\leq 10^3$	17	19	15	21	12
	10^3-10^4	16	17	14	19	10
	$\geq 10^5$	14	15	13	16	8
Laurus nobilis L.	$\leq 10^3$	16	24	18	19	11
	10^3-10^4	15	22	16	17	9
	$\geq 10^5$	13	20	14	12	7
Mentha piperita L.	$\leq 10^3$	17	19	17	23	14
	10^3-10^4	13	15	14	20	11
	$\geq 10^5$	10	12	12	17	9
Salvia officinialis L.	$\leq 10^3$	15	19	20	18	14
	10^3-10^4	13	17	17	15	12
	$\geq 10^5$	11	14	15	12	11
Olea europaea L.	$\leq 10^3$	12	17	15	20	12
	10^3-10^4	10	14	12	16	10
	$\geq 10^5$	9	12	10	11	8
Thymus collinus Bieb.	$\leq 10^3$	16	20	16	24	12
	10^3-10^4	14	18	15	22	10
	$\geq 10^5$	12	15	13	17	8

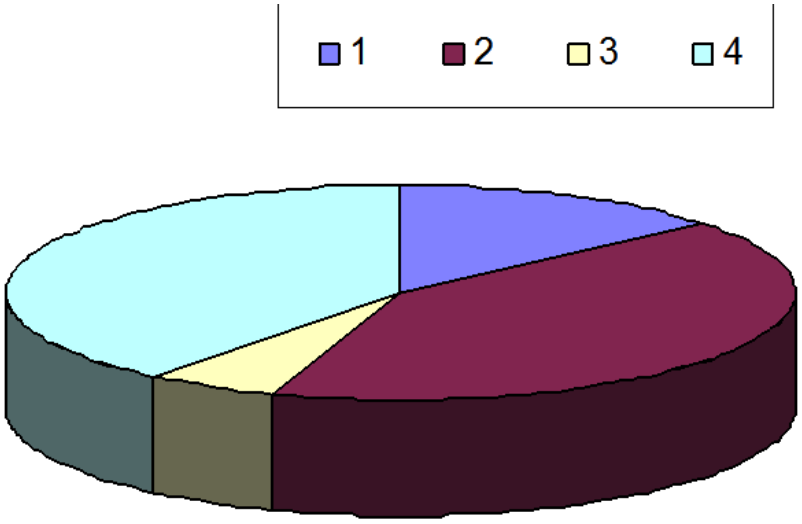

Fig. 1. Ecological and trophic characteristic of fungi found on the medicinal plants of Azerbaijan.
1 – real biotrophs; 2 –polybiotrophs 3- real saprotrophs 4 – polysaprotrophs

the antimicrobial activity of plants decreases. Given that some studies have confirmed that, the numerical index of plant mycobiota change their target quality. In this regard[25], then 10^3 CFU / g can be considered an acceptable limit for the number of fungi for medicinal plants.

CONCLUSION

Thus, the results showed that the medicinal plants being studied, which are part of the flora of Azerbaijan are characterized by a habitat for a great variety of fungi, including many opportunistic, allergenic and toxigenic, which could be a direct or indirect source of danger for all living beings, including human. This fact justifies the need for preparation of regulations governing the mycological safety èof plant materials for medical purposes, and the first approach to the use of these plants should be the numerical composition of the microbiota, the numerical index of which should not exceed 10^3 CFU/g (dry weight).

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Conflict of interest

The authors declare that there is no conflict of interest regarding this paper

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