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ANTIFUNGAL ACTIVITY OF MYCELIA OF *TRAMETES GIBBOSA* REGARDING POTENTIALLY PATHOGENIC FOR HUMANS AND ANIMALS FILAMENTOUS FUNGI

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The antifungal activity (AFA) of 4 strains of *Trametes gibbosa* was investigated regarding potentially pathogenic for humans and animals filamentous fungi. AFA was evaluated by using 3 approaches: in mutual growing, using samples of culture liquid (CL) and mycelial extract (ME). In dual culture partial and complete overgrowth reactions on test fungi by *T. gibbosa* were observed. The CL and ME samples inhibited the growth rate of mycelia of test fungi up to 55.6% and 29.7% respectively. Thus, the *T. gibbosa* collections, especially CL samples, possess AFA against studied filamentous fungi and are perspective for further research as a source of antimycotic compounds.

***Keywords*:** *Trametes gibbosa*, antifungal activity, cultural liquid, mycelial extract.

Introduction. Nowadays, due to the deterioration of ecology, exposure of stresses and different infections, fungal diseases are enlarged. Use of different antibiotics, hormones and other drugs weaken human immune-system and it promotes the development of mycosis. Due to toxicity and side effects of existing antifungal drugs today, as well as the development of resistances against them by filamentous fungi, searching new, nontoxic for organism antifungal preparations with natural origin becomes necessary [1].

At present attention of scientists are aimed on higher fungi, particularly on tinder mushrooms (*Basidiomycota*, *Polyporales*), which are easily cultivated, undemanding to the medium, fast growing and forming fruiting body on culture. It is known that many species of polypore mushrooms produce bioactive compounds (polysaccharides, terpenoids, phenols, etc.), which have immune-modulating, anticancer, antibacterial, antifungal etc. effects [2, 3]. Among bioactive compounds produced by *polypores*, the antifungal effect was mainly reported for terpenoids (tri- and sesquiterpenes) and phenolics [4].

Polypore species from genus *Trametes* such as *T. versicolor (L.) Lloyd, T. zonata (Nees) Pilát, T. hirsuta (Wulfen) Lloyd and T. gibbosa (Pers.) Fr.,* possess antibacterial, antioxidant, immune-modulating, antivirus and hypoglycemic properties [5–7].

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There is some information about the antifungal activity (AFA) of different species of genus *Trametes* against pathogenic fungi. Thus, *T. versicolor* inhibits the growing of *Botrytis cinerea, Fusarium oxysporum* and *Mucor miehei* phytopathogenic fungi [8], as well as the methanol extracts of fruiting bodies of *T. versicolor* and *T. hirsute* show AFA against *Aspergillus fumigatous*, *Aspergillus niger*, *Aspergillus flavus* and *Penicillium sp.* pathogenic for humans and animals filamentous fungi [9, 10].

In our previous experiments *T. gibbosa, T. hirsuta, T. trogii, T. ochracea, T. versicolor* and *T. villosa* species shown AFA against *Chrysosporium keratinophilum, Microsporum gypseum, Trichophyton terrestre, Penicillium griseofulvum* potentially pathogenic for humans and animals filamentous fungi in dual culture conditions [11].

Thus, screened *Trametes* collection was considered to be a potential source of antifungal compounds and it was decided to enlarge the experiments by studying the antifungal activity of cultural liquid and mycelial extract samples of the collection.

Materials and Methods. In this study we investigated the antifungal activity of 4 strains of *T. gibbosa* having different origin. *T. gibbosa* is widely distributed in Armenia in all floristic regions. It is habitat on dead deciduous trees and cause white-rod [12, 13]. Carpophores are semicircular often with a hump, single or in groups, upper surface downy at first later smooth, greyish-white, sometimes greenish due to the growth of algae among the surface hairs [13].

Table 1

Studied collections of T. gibbosa and filamentous fungi

Species	Strains	Origin	Substrate
T. gibbosa	Tg-I-1	France, 2002	oak
	Tg-1020	Iran, 2008	beech
	Tg-2	Armenia, 2014	oak stamp
	Tg-3	Armenia, 2015	ash stamp
Filamentous fungi			
Chrysosporium keratinophilum	Chk-1430	Armenia, 2000	soil
Microsporum gypseum	Mg-842	Armenia, 2001	soil
Trichophyton terrestre	Trt-931	Armenia, 2000	soil
Penicillium griseofulvum	Peg-1	Armenia, 2000	soil

Four species (*Chrysosporium keratinophilum, Microsporum gypseum, Trichophyton terrestre* and *Penicillium griseofulvum*) of potentially pathogenic for humans and animals filamentous fungi isolated from soils of Armenia were tested [14] (Tab. 1).

AFA was evaluated by agar (potato dextrose agar, PDA) diffusion method by 3 approaches: in mutual growing, using samples of culture liquid (CL) and mycelial extract (ME).

The dynamics of the relationships of interacted organisms were evaluated by contacting the developed scale, which includes 3 types and 4 subtypes of reactions [15, 16]: A and B are mutual deadlock at the contact and at the distance, C is overgrowth without deadlock; C_{A1} and C_{A2} are partial and complete overgrowth after deadlock at the mycelial contact; C_{B1} and C_{B2} are partial and complete overgrowth after initial deadlock at a distance. The morphological changes (pigmentation of mycelia and agar, formation of demarcation line, secretion of exudate drops at the contact, etc.) in interacted colonies were described as well.

To study the AFA of CL samples, they were added in the PDA (1/1v/v) and were spilled into Petri dishes (25 *mL* on each). Then test fungi were inoculated and during 5 days the growth rate indicators of mycelia and morphological changes (present or absents of pigmentation, inhibition of sporulation, etc.) of colonies were recorded.

AFA of ME samples were studied by agar diffusion method on PDA. Paper discs (5 *mm*) were wetted with 4.0% (diluted in DMSO) ME of *T. gibbosa* and were placed on agar, around already formed colonies of test fungi. AFA was evaluated by the degree of formation of the sterile zone or a rare growth area on the edge of colonies around the discs.

Diameter of the colony was measured daily until the interaction (dual culture) or during 7 days (under the influence of CL and ME). Mycelial growth rate (GR; millimeters per day) indicators were calculated according to the formula: $GR = D/T$, where D is the diameter of the colony during T time. Average GR indicators (GR_{avr}) were calculated from obtained GR data.

GR parameters were statistically analyzed using the SLOPE algorithm (Microsoft Excel; Microsoft Corp., Redmond, WA, USA).

Results and Discussion. In dual culture only overgrowth reactions by *T. gibbosa* were described (complete C_{A2} and partial C_{B1}). During interaction with *T. terrestre*, *M. gypseum* and *C. keratinophilum* all strains of *T. gibbosa* completely overgrowth on test fungi after mutual deadlock at the contact (type C_{A2}). The exception was the French strain Tg-I-1, which showed the reaction of partial overgrowth after deadlock at the distance (type C_{B1}), against *T. terrestre* and *C. keratinophilum*, while in interaction with *P. griseofulvum* all studied collection of *T. gibbosa* showed partial overgrew (type C_{B1}) reaction (Tab. 2).

Thus, in 16 cases of interactions the reactions of complete overgrowth composed 62.5% and the reactions of partial overgrowth were 37.5%. During the experiment overgrowth by tested fungi was not seen.

Table 2

Type of antagonistic reactions during interaction with filamentous fungi

Strain	*T. terrestre*	*M. gypseum*	*C. keratinophilum*	*P. griseofulvum*
Tg-I-1	C_{B1}	C_{A2}	C_{B1}	C_{B1}
Tg-2	C_{A2}	C_{A2}	C_{A2}	C_{B1}
Tg-3	C_{A2}	C_{A2}	C_{A2}	C_{B1}
Tg-1020	C_{A2}	C_{A2}	C_{A2}	C_{B1}

During interactions the decreasing of growth rate indicators was detected in collection of *T. gibbosa* (up to 25.0%) and to a lesser extent in test fungi (up to 13.0%).

During mutual growing at the contact zone in collection of *T. gibbosa* sealing of mycelia, formation of mycilial pillow/knoll and the presence of exudate drops were described, while in filamentous fungi only the lesion of colonies edges were seen.

Mushrooms need antibacterial and antifungal compounds to survive in natural environments. Thus antifungal compounds with different activities could be isolated from many mushrooms and could be beneficial for humans [4]. In dual culture condition studied collection of *T. gibbosa* show high antagonistic activity against tested filamentous fungi, which establish a base to continue researches for understanding the behavior of antifungal agents. For this purpose the CL and ME samples were tested.

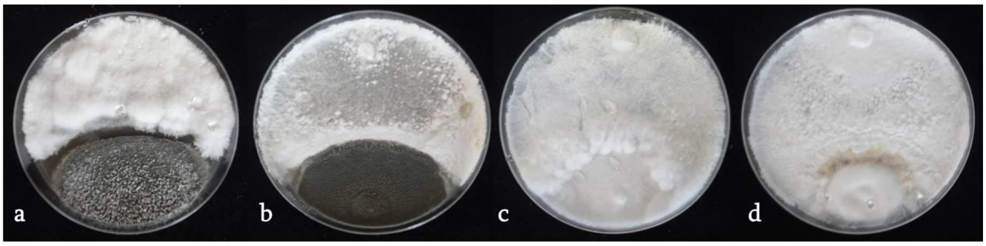

Fig. 1. Types of interaction between *T. gibbosa* and filamentous fungi in dual culture. Partial overgrowth after mutual deadlock at the distance (type C_{B1}) by Tg-1020 (a) and Tg-3 (b) in *P. griseofulvum.* Completely overgrowth after mutual deadlock at the contact (type C_{A2}) by Tg-1020 in *C. keratinophilum* (c) and by Tg-2 in *M. gypseum* (d).

CL samples inhibited the growth rate GR_{avr} of all tested fungi by different degrees. Thus, the growth rate indicators of *M. gypseum* were decreased from 12.5 to 27.3%, *T. terrestre* were decreased from 33.3 to 55.65%, *C. keratinophilum* were decreased from 13.3 to 45.3% and *P. griseofulvum* were decreased from 5 to 18.5% comparison with controls. All tested strains of *T. gibbosa* were active against *T. terrestre,* while against other test fungi higher activities show CL samples of Tg-2 and Tg-3 strains (Tab. 3). In all tested fungi the lesion of colonies edges were seen by the influence of CL samples of *T. gibbosa.*

Table 3

Inhibition effect of CL and ME samples of T. gibbosa on GR_{avr} indicators (%) of test fungi

Strains	*M. gypseum*	*T. terrestre*	*Ch. keratinophilum*	*P. griseofulvum*
Influence of CL samples				
Tg-I-1	13.6	33.3	36.8	14.8
Tg-2	27.3	42.6	47.4	18.5
Tg-3	27.3	55.6	47.4	14.8
Tg-1020	27.3	42.2	19.4	5.0
Influence of ME samples				
Tg-I-1	23.8	23.5	19.2	9.1
Tg-2	23.8	23.5	19.2	13.6
Tg-3	14.3	23.5	19.2	9.1
Tg-1020	9.5	23.5	19.2	13.6

ME samples of *T. gibbosa*, compared with controls, also inhibited the growth rate of all tested fungi from 9.1–23.8% (see Figure). In all tested filamentous fungi the lesion of colonies edges and in *P. griseofulvum* pronounced inhibition of sporulation were described.

It was shown that both CL and ME samples of *T. gibbosa* collection have inhibitory activity against tested fungal species, particularly against causing serious skin diseases *T. terrestre* and *C. keratinophilum*. Duse mycelia of *T. gibbosa* synthesize antifungal metabolites, which are mainly assembled in cultural broth.

Conclusion. Studied collection of *T. gibbosa,* especially CL samples, has a strong AFA against potentially pathogenic for humans and animals filamentous fungi. Mycelia of *T. gibbosa* synthesize both extra- and intracellular metabolites with antifungal action, and it is promising for further studies to provide antifungal agents.

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