

Short Communication

Antifungal activities of *Fomitopsis pinicola* (Sw.:Fr) Karst and *Lactarius vellereus* (Pers.) Fr.

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In this study, antifungal activities of *Fomitopsis pinicola* (Sw.:Fr) Karst and *Lactarius vellereus* (Pers.) Fr. extracts with chloroform and ethanol against *Fusarium inflexum* and *Fusarium heterosporium* were investigated. Mycelial discs (8 mm Ø) taken from actively growing margin of 7-day-old culture of each species and were placed in each extract medium. All species were incubated for 3 days at 28°C in the dark. Antifungal activity was obtained by disc diffusion method.

Key words: *Fomitopsis pinicola*, *Lactarius vellereus*, antifungal activity, *Fusarium inflexum*, *Fusarium heterosporium*.

INTRODUCTION

Fusarium sp. are well known plant pathogens causing seed abortion, seed, root, stem and seeding roots, vascular wilt, damping-off, die back, stunting and reduction in growth in a variety of host plants (Sharfun-Nahar and Mushtaq, 2007). Seed-borne nature of *Fusarium* sp. in sunflower is well documented (Sharfun-Nahar et al., 2005). *Fusarium* is a large genus of filamentous fungi widely distributed in soil and in association with plants. *Fusarium* species especially *Fusarium oxysporum*, *Fusarium lycopersici* have the harmful effects upon plants, especially tomato plants (Chandler, 1978). *F. oxysporum* f.sp. *gladioli* is the most important pathogen of *Gladiolus*. Cultivation of both corms and flowers are hampered by this fungus (van Rijbroek et al., 1997). Many *Fusarium* head blight caused by *F. graminearum* and *F. culmorum* in both spring and winter wheat-*Triticum aestivum* L. (Haberle et al., 2007). Sorghum (*Sorghum bicolor*) is one of the major cereal crops, especially in hot and dry areas of the world but *Fusarium* sp. cause several diseases of sorghum, including root rots, seedling blights, stalk rot and grain mold (Huang and Backhouse, 2004).

Generally, mushrooms are used as food because of their good taste, appetizing aroma and nutrient contents (Jonathan and Fasidi, 2001; Gbolagade and Fasidi,

2005). Besides, mushrooms have been used as traditional medicine for curing various types of diseases. Mushrooms such as *Agaricus bisporus*, *Lentinula edodes*, *Aricularia auricula* and *Pleurotus* sp. have antagonistic effects against bacteria, fungi and viruses (Tochikura et al., 1988).

In this study, antifungal activities of *Fomitopsis pinicola* (Sw.:Fr) Karst and *Lactarius vellereus* (Pers.) Fr. extracts with the help of chloroform and ethanol against to *Fusarium* species (*F. inflexum* and *F. heterosporium*) were investigated.

MATERIALS AND METHODS

Organism

F. pinicola (Swartz:Fr) Karst and *L. vellereus* (Pers.) Fr used in our research were collected from the Işık Mountain-Kızılcahamam-Ankara of Middle Anatolia of Turkey. This mushroom was collected and identified by Ilgaz Akata and this specimens has been stored at the ANK-Herbarium of the Department of Biology, Ankara University, Turkey.

Test organisms

In this study, the *Fusarium* sp. were used that includes *F. inflexum*, *F. heterosporium*. Test organisms were obtained from Ministry of Agricultural and Rural Affairs (MARA)-Turkey. These fungal cultures were maintained in nutrient broth (Merck). Mycelial agar discs were taken from developed *Fusarium* spp. on the potato dextrose agar

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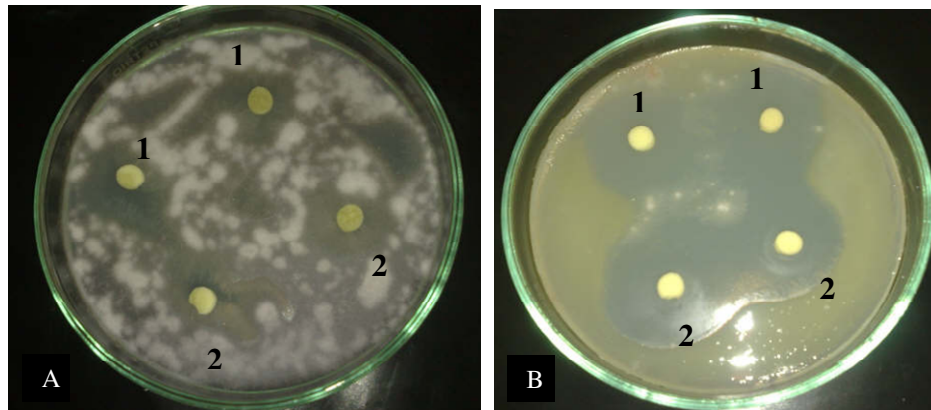


Figure 1. Antifungal activity of *F. pinicola* against *Fusarium* spp. A. *F. heterosporium*. B. *F. inflexum*. 1 = Ethanol, 2 = chloroform.

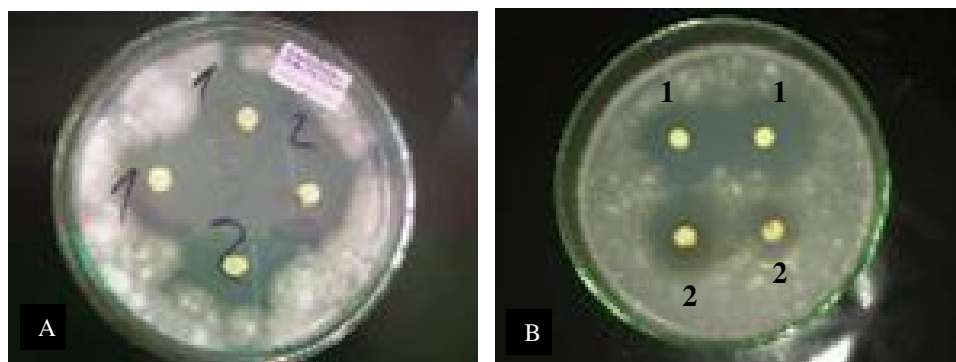


Figure 2. Antifungal activity of *L. vellereus* against *Fusarium* spp. A. *F. heterosporium*. B. *F. inflexum*. 1 = Ethanol, 2 = chloroform.

(PDA) and were incubated in the nutrient broth at 100 rpm for 48 h and activated.

Preparation of crude extracts

F. pinicola and *L. vellereus* were dried at aseptic conditions and were cut into bits. Dried mushrooms were pulverized in a blender and 50 g each of the powdered samples were soaked separately in 300 ml of 95% ethanol and chloroform until the complete exhaustion in an Erlenmeyer flask. The flasks were covered with aluminum foil and allowed to stand for 7 days for extraction. These extracts were filtered through whatman filter paper no.1 and were evaporated in vacuous and dried using rotary evaporator at 40°C. The extracts were collected and dried (Jonathan and Fasidi, 2003).

Simple susceptibility screening

The activated test organisms of *Fusarium* sp. were poured into potato dextrose agar (PDA) separately as 500 µl and were spread with spatula and were dried as aseptic. They were settled inside the prepared ethanol and chloroform 6 mm extracts for 10 s and then left into the *Fusarium* inoculated agar medium. The antifungal effects of the extracts were determined by disc diffusion method (Stoke and Ridgway, 1980). They were incubated at 28°C in order for their development can be monitored. At the end of the

incubation the sizes of the formed inhibition zones were measured as millimetric and their photos were taken. Also the ethanol and chloroform sucked discs and trade antibiotics (amoxycillin and erythromycin) which were used as solvents in the study were tried in the *Fusarium* inhibition and comparisons were made. The sterile distilled water used in the dilution of solid mushroom's extracts which were used as the control. All the tests were carried out in triplicates.

Statistical analysis

The data were analyzed and treatments were compared using the analysis of variance (ANOVA) and LSD ($p > 0.05$)

RESULTS AND DISCUSSION

In this study, antagonistic effects of *F. pinicola* and *L. vellereus* were found against *F. inflexum* and *F. heterosporium*. This was obtained by the clear zone of inhibition produced by the fungi around the tested mushroom extracts. Antifungal activities of *F. pinicola* (Figure 1) and *L. vellereus* extracts were high (Figure 2). All the antifungal activities of mushrooms were shown at Table 1.

Table 1. Antifungal activity of *Fomitopsis pinicola* and *Lactarius vellereus*

Microorganisms	Inhibition zone diameter (mm)					
	<i>F. pinicola</i>			<i>L. vellereus</i>		
	Control*	Ethanol	Chloroform	Control*	Ethanol	Chloroform
<i>F. heterosporium</i>	0	25 ± 0.2	22 ± 0.4	0	35 ± 0.3	38 ± 0.2
<i>F. inflexum</i>	0	35 ± 0.3	28 ± 0.1	0	27 ± 0.2	33 ± 0.1

Control* = Distilled water.

As can be seen in Table 1 *F. pinicola* has shown the most activity towards *F. inflexum* in ethanol, on the other hand the effect of the *L. vellereus* towards *F. heterosporium* was the up most level.

The values of ethanol as 25 and 35 mm ($p > 0.05$) in *F. pinicola* are higher than the chloroform values in both *Fusarium* sp. On the other hand in *L. vellereus* contrary to *F. pinicola* the chloroform values of 38 and 33 mm ($p > 0.05$) are higher than the ethanol values in both *Fusarium*.

Table 1 shows that generally the antifungal activities of *L. vellereus* were higher than *F. pinicola*. The values of ethanol as 25 and 35 mm ($p > 0.05$) in *F. pinicola* are higher than the chloroform values in both *Fusarium* sp. On the other hand in *L. vellereus* contrary to *F. pinicola* the chloroform values of 38 and 33 mm ($p > 0.05$) are higher than the ethanol values in both *Fusarium*. The commercial antibiotics (amoxicillin and erythromycin) value is not shown in Table 1, because both amoxicillin and erythromycin were not effective on the development of *Fusarium* species.

Gbolagade and Fasidi (2005) found in their study that *Auricularia polytricha*, *Corilopsis occidentalis*, *Daldinia concentrica*, *Daedalea elegans* and *Tricholoma lobayensis* exhibited various degrees of antagonistic effects against the *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Microsporium bouldardii*.

In our study, ethanol and chloroform discs without mushroom weakly inhibited or not inhibit the growth of *F. heterosporium* and *F. inflexum* (Figure 3). This application showed that ethanol and chloroform were not effective on the formation of inhibition zone the both *Fusarium* sp.

Martin-Pinto et al. (2006) studied the interactions between the mycorrhizal fungi *Boletus edulis*, *Rhizopogon roseolus*, *Laccaria laccata* and *Lactarius deliciosus* and damping off pathogens (*F. oxysporum* and *F. moniliforme*) *in vitro*. They found that at the end of the assay, the inhibitory effect only could be observed in the *Lactarius deliciosus* treatment.

Dulger et al. (2004) aimed to determine the antimicrobial activity of the extracts of *Cantharellus cibarius* Fr. against various bacteria and filamentous fungi including *F. oxysporum*, found that all the extracts showed more antifungal activities than antibacterial activities.

In conclusion, the extracts of *F. pinicola* and *L. vellereus* may be effective antifungal agents.

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