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Antifungal Effects of *Bjerkandera Adusta* (Willd.) P. Karst. Against To The Plant Pathogens

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Abstract: In this study, antifungal activities of *Bjerkandera adusta* (Willd.) P. Karst. 1880 extracts with the help of hexane, methanol against to *Fusarium* species (*Fusarium inflexum* and *Fusarium heterosporium*) were investigated. The mushroom samples that were dried under aseptic conditions were put thru extraction for 12 hours in solvents. Than the extracts was filtered using Whatman filter paper No.1 and filtrate was evaporated in vacuous and dried using a rotary evaporator at 60°C and finally dried material stored at +4°C. Antifungal activities were measured by Disc Diffusion method. According to this method; the inoculums containing *Fusarium inflexum* and *Fusarium heterosporium* were spread on potato dextrose agar. The *Bjerkandera adusta* extracts to 6mm discs as 10 µl. For control water and only hexane, and methanol saturated discs were used. All these discs placed on the inoculated agar separately and incubated 30°C for 48 h. Antifungal activity experiments were made as three repetitions. All data were analyzed and treatments compared using the analysis of variance.

Key Words: Macrofungi, *Bjerkandera adusta, Fusarium,* antifungal activity

Bjerkandera adusta (Willd.) P. Karst. 'ın Bitki Patojenlerine Karşı Antifungal Etkisi

Özet: Bu çalışmada, *Bjerkandera adusta* (Willd.) P. Karst. 1880 ekstraktının antifungal etkisi, çözgen olarak kullanılan methanol ve hakzanol yardımı ile *Fusarium* türlerine (*Fusarium inflexum* ve *Fusarium heterosporium*) karşı araştırılmıştır. Mantar örnekleri aseptik şartlarda kurutularak 12 saat süre ile çözgenler içinde bırakıldı. Sonra ekstraktlar Whatman No:1 kağıdı kullanılarak süzüldü ve evaporatör yardımı ile 60°C'de kurutuldu ve kuru materyaller +4°C'de muhafaza edildi. Antifungal aktivite tayininde Disk Difüzyon metodu kullanıldı. Bu metoda göre *Fusarium inflexum* ve *Fusarium heterosporium* içeren inokulumlar patates dekstroz agar besiyeri yüzeyine yayıldı. *Bjerkandera adusta* ekstraktları 6mm çapındaki disklerde 10 µl olacak şekilde emdirildi ve besiyeri yüzeyine ayrı ayrı bırakıldı ve 30°C'de 48 saat inkübe edildi. Kontrol grubu olarak su ve sadece methanol ve hakzanol çözgenlerini içeren diskler kullanıldı. Antifungal aktivite deneyleri üç tekrarlı olarak hazırlandı. Tüm veriler analiz edildi ve denemeler varyans analizi kullanılarak karşılaştırıldı.

Anahtar Kelimeler: Makrofungi, *Bjerkandera adusta, Fusarium,* antifungal aktivite

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Introduction

Bjerkandera adusta that anamorphic fungus, morphologically similar to *Geotrichum candidum* Link ex Leman was isolated from a soil sample. The fungus was identified on the basis of the ITS region analysis as basidiomycete *Bjerkandera adusta* (Willd.) P. Karst. The strain exhibited interesting properties, in particular, the capacity for decomposing of anthracyclic antibiotics (daunomycin)-very uncommon feature among fungi (Komillowicz-Kowalska et al. 2006). Fungal strains, known for their ability to degrade lignocellulosic material or lignin derivatives, were screened for their potential to decolorize commercially used reactive textile dyes. From the 18 tested fungal strains only *Bjerkandera adusta*, *Trametes versicolor* and *Phanerochaete chrysosporium* were able to decolorize all the dyes tested (Heinfling et al. 1997). Ninety samples of wood-colonizing fungi including *Bjerkandera adusta* were cultivated on agar plates, and their extracts tested for antibacterial activity using the *Vibrio fischeri* bioluminescence test (Zrimec et al.2004). Studies on antimicrobial properties of white-root fungus were carried out (Fagadev and Oyelade 2009). The extracts of ethyl acetate, chloroform, acetone and ethyl alcohol of *Pleurotus ostreatus* (Jack ex Fr.) Kum var. *salignus*, *Pleurotus florida* Fovose, *Schizophyllum commune* Fr., *Helvella leucomelaena* (Pexs) Nannf. and *Amanita virosa* (Fr.) Bertillon were investigated in respect to their antimicrobial activities against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* and *Pseudomonas aureginosa* ATCC 27857 by Diffusion Method (Demirhan et al. 2007).

In this study, antifungal activities of *Bjerkandera adusta* (Willd.) P. Karst. 1880 extracts with the help of hexane, methanol against to *Fusarium* species (*Fusarium inflexum* and *Fusarium heterosporium*) were investigated.

Materials and Methods

Organism

Bjerkandera adusta was collected from Işık Mountain-Kızılcahamam-Ankara of Turkey and these mushrooms were collected and identified by Ilgaz Akata and all specimens have been deposited at the ANK-Herbarium of Ankara University, Turkey.

Test organisms

In this study, *Fusarium* spp. were used that *F.inflexum, F.heterosporium.* Test organisms were obtained Ministry of Agricultural 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 (PDA) and were incubated in the nutrient broth at 100 rpm for 48 hours and activated.

Preparation of crude extracts

Bjerkandera adusta were dried at aseptic conditions and were cut into bits. Dried mushrooms were pulverized in a blender and 50g each of the powdered samples were soaked separately in 300ml of 95% methanol and hexane until complete exhaustion in an Erlenmayer 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* spp. were poured into potato dextrose agar (PDA) separately as 500µl. and were spread with spatula and dried as aseptic. They were settled inside the prepared methanol and hexane 6mm extracts for 10 seconds and than left into the *Fusarium* inoculated agar medium. The antifungal effects of the extracts were determined disc diffusion method (Stoke and Ridgway 1980). They were incubated at 28ºC in order for their development can be monitored. At the end of the incubation the sizes of the formed inhibition zones were measured as

milimetric and their photos were taken. Also the methanol and hexane sucked discs which were used as solvents in the study tried in the *Fusarium* inhibition and comparisons are made. The sterile distilled water used in the dilution of solid mushrooms extracts were used as the control. All the tests were carried out in triplicates.

Statistical analysis

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

Results and Discussion

In this study; antagonistic effects of *Bjerkandera adusta* was found against *Fusarium inflexum, Fusarium heterosporium.* This was obtained by the clear zone of inhibition produced by the fungi around the tested mushroom extracts. An antifungal activity of *Bjerkandera adusta* extracts was high at the *F. inflexum* than *F. heterosporium.* All of antifungal activities of mushrooms were shown at Table 1.

Table 1. Antifungal activity of *Bjerkandera adusta*

	Inhibition zone diameter (mm)		
		Solvents	
Microorganisms	**Control***	**1**	**2**
Fusarium heterosporium	0	29	33
Fusarium inflexum	0	27	25.5

Control*=Distilled Water; 1-Methanol; 2-Hexane

As can be seen in Table 1 *Bjerkandera adusta* has shown the most activity towards *F. heterosporium* in methanol .The values of methanol and hexane as 21 mm and 37 mm (average, 29 mm) ($p>0.05$) and as 24 mm and 42 mm (average 25.5 mm) ($p>0.05$) at the *F. heterosporium* respectively are higher than the *F. inflexum*. At the *F. heteosporium* antifungal effect of *Bjerkandera adusta,* is higher in the hexane than methanol.

The values are in the methanol and hexane as 24 mm and 42 mm (average, 33 mm) ($p>0.05$) and as 31 mm and 35 mm (average 33 mm) ($p>0.05$) respectively (Fig.1).

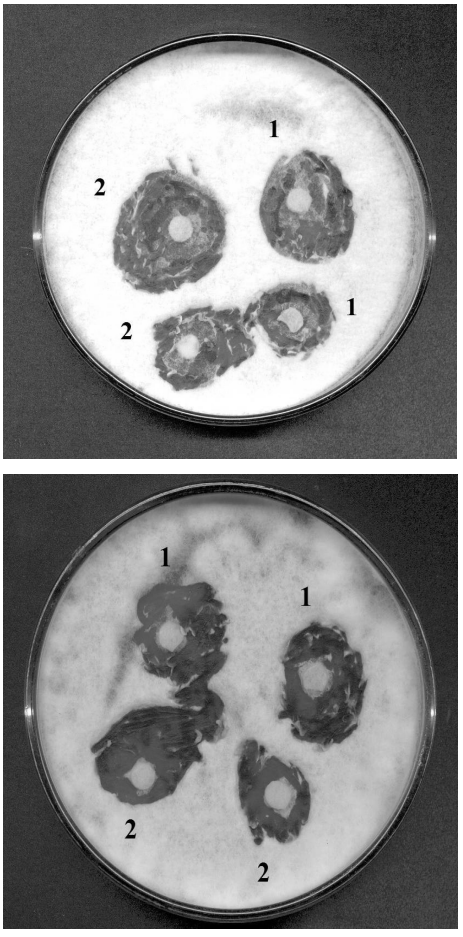

Figure 1. Antifungal activity of *Bjerkandera adusta* against *Fusarium* spp.
A-*F.heterosporium* B-*F.inflexum* 1- Methanol, 2-Hexane

In our study; ethanol and chloroform discs without mushroom were either weakly inhibit or not inhibited of growth of *F. heterosporium* and *F.inflexum* (Fig.2).

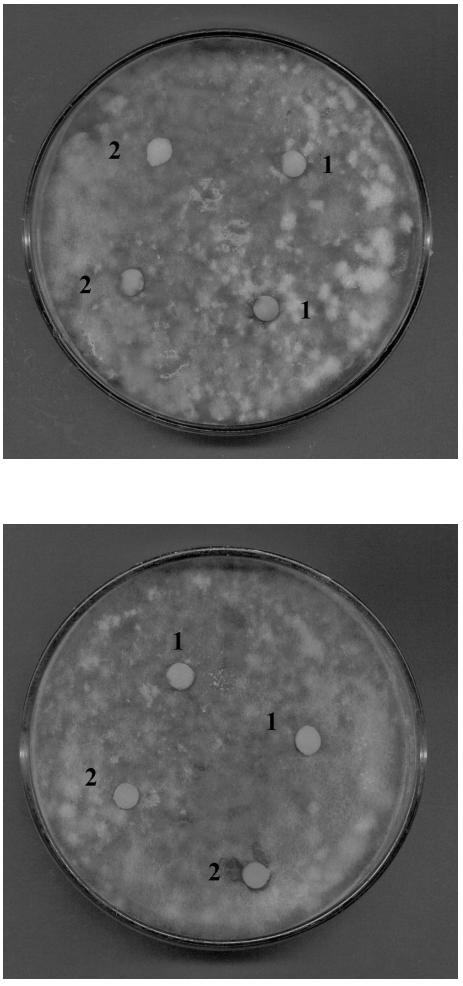

Figure 2. Antifungal activity of *Bjerkandera adusta* against *Fusarium* spp.
A-*F.heterosporium* B-*F.inflexum* 1- Methanol, 2-Hexane

As conclusion we suggested that extracts of *Bjerkandera adusta* may be effective antifungal agents

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