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IN VITRO SENSITIVITY OF DERMATOPHYTE FUNGUS *MICROSPORUM AUDOUINII* TO FUNGAL FILTRATE OF *PLEUROTUS OSTREATUS* AND *TRICHODERMA HARZIANUM*

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Abstract

Biological control represents an important approach for controlling many dermatophyte fungi. *Trichoderma* spp. and *Pleurotus* spp. are the most promising and effective bioagents against many pathogenic fungi. In this paper *Trichoderma harzianum* and *Pleurotus ostreatus* were screened for their efficacy against *Microsporum audouinii*. The results had been revealed that the culture filtrate of the bioagents *Trichoderma harzianum was* and *Pleurotus ostreatus* affected the radial growth of the dermatophyte fungus *Microsporum audouinii*. Fugal filtrates of test fungi at all test concentrations had inhibitory effect on the radial growth of *Microsporum audouinii*. The results had been revealed also that *Pleurotus ostreatus* antifungal activity was more than the activity of *Trichoderma harzianum* but lower than the effectivity of the antifungal drug clotrimazole .

Keywords: Biocontrol, *Microsporum audouinii*, *Pleurotus ostreatus*, *Trichoderma harzianum*.

Introduction

Dermatophytes are keratinophilic fungi that cause infections in nails, skin and hair. They are including three genera *Microsporum, Trichophyton* and *Epidermophyton*, each genus containing several species that may be of anthropophilic, zoophilic or geophilic origin. These genera cause superficial infections which are named according to the body location: *Tinea unguium, Tinea capitis, Tinea barbae, Tinea corporis* and *Tinea cruris*. *Tinea capitis* causes hair loss, scaling, erythema and impetigo-like lesions (Ellabib *et al*., 2002 ; Woldeamanuel *et al*., 2005).

The epidemiology of *Tinea capitis* that cause by *Microsporum* species varies within different geographical regions. It can be sporadic or epidemic and during the last two decades an increase of this pathology has been observed worldwide. (Ginter-Hanselmayer *et al*., 2007).

Different biological and chemical compounds had been used to control *Microsporum* infections such as antifungal drugs (Hsiao *et al*., 2018), fungi (Jasmina *et al*., 2015) and bacteria (Guo *et al*., 2012).

Despite the introduction of new antifungal medications, antifungal resistance continues to grow and evolve and makes patient management harder so the efforts of researchers will continued to develop new antifungal drugs (Pfaller *et al*., 2005).

It is now widely acknowledged that there is a need to develop novel antimicrobial agents to minimize the threat of further antimicrobial resistance and the side effect. So different studies were conducted to examine the antimicrobial properties of different biocontrol agents such as fungal metabolite (Rungsaiwattana, 2011) and bacteria (Guo *et al*., 2012).

Issa, (2016) study the antifungal activity of *T. harzianum* against different dermatophytes which include *Microsporum canis, M. gypseum, Trichophyton mentagrophytes* and *T. rubrum* and revealed that crude chitinase production from *T. harzianum* has different antifungal activity on different dermatophytes.

Aasi and Al-Aaraji (2018) confirmed the antifungal activity of *T. harzianum* CA-07 crude extract against *Microsporium canis* from patients with dermatophytosis and the result revealed that the crude extract of *Trichoderma* exhibited significantly high antifungal activity against *M. canis*.

Numerous studies have shown that mycelia and basidiocarps of Oyster (*Pleurotus ostreatus*) are sources of active compound that have antimicrobial and antifungal activities against pathogenic microorganisms (Hearst *et al*., 2009; Oztὺrk *et al*., 2011).

Recent had been revealed that the pathogenic fungi *C. albicans* and *C. parapsilosis* (ATCC 90018) were found to be susceptible to indigenous wild mushrooms (Gebreselema *et al*., 2019).

This study has been conducted to evaluate the antifungal activity of *Trichoderma harzianum* and *Pleurotus ostreatus* against the pathogenic fungus *Microsporum audouinii*.

Materials and Methods

Source of *Microsporum audouinii* isolate

Specimens were collected from patients in Alzahraa Hospital and medical clinic. Specimens then cultured on SDA medium and identified morphologically with the corporation of central health laboratory- Baghdad.

Source of *Trichoderma harzianum* and *Pleurotus ostreatus* isolates

Trichoderma harzianum and *Pleurotus ostreatus* isolates were obtained from the Ministry of science and Technology-Directorate of Agriculture Research-Department of Biotechnology.

Source of antifungal drug

The antifungal drug (Clotrimazole) obtain as a standard solution from nation pharmacy.

Effect of test fungi on *Microsporum audouinii* in Dual culture

The dual culture technique was used to test the antagonistic ability of *T. harzianum* against *M. audouinii*. The pathogenic fungus and *T. harzianum* were grown on SDA for a week at 28 ± 2°C. 5mm disc of the target fungus cut from the periphery of pure culture was transferred to the Petri dish previously poured with SDA. *T. harzianum* was transferred in the same plate of opposite end of the plate at equal distance and was incubated at 28 ± 2°C for 7 days. In control plates (without *Trichoderma*), a sterile agar disc was placed at opposite side of the *Microsporum* agar disc. The experimental design used was a completely randomized design (CRD) with three replicates for each treatment. Radial colony growth of *Microsporum* was measured after three and seven days. The percentage of inhibition of the mycelial growth of the test fungus was calculated using the formula by Philippe *et al*. (2012). Inhibition of mycelial growth (%) = (dc-dt)/dc × 100 where dc is mean diameter of colony in the control sample and dt is mean diameter of colony in the treated sample. The antagonistic activity of *Pleurotus ostreatus* against *M. audouinii* was tested as above.

Effect of fungal Filtrate of on growth of *M. audouinii*

The effects of the *T. harzianum* filtrate on mycelia growth of the *M. audouinii* was determine by remove three discs of mycelial agar plugs (5 mm diameter) from the edge of the young culture of *T. harzianum* and inoculated in 100 ml sterilized PDB in 250 ml conical flasks and incubated at 28 ± 2°C.The culture was filtered through Millipore filter and then sterilized through 0.2 µm pore biological membrane filter. Different volumes of fungal filtrates were added to the molten SDA medium to obtain final concentrations of 50, 25 and 10% (v/v). The control set was made by pouring 20 ml of SDA medium only in sterilized Petri-plate. The medium was placed (10ml) in Petri-plate and inoculated with 5 mm diameter mycelial disc of 7 days-old culture of *M. audouinii* in the center of the plates and incubated at 28 ± 2°C until the colony reached the plate edge (Rashmi, *et al*. 2016). There were 3 replicates for each treatment. Radial growths of the *M. audouinii* were recorded. Inhibition percent (%) of mycelial growth of *M. canis* was calculated as follows: L = [(C – T)/C] x 100 Where L is inhibition (%) of radial mycelial growth; C is radial growth measurement of the *M. audouinii* in control; T is radial growth of the *M. canis* in the presence of *Trichoderma* Filtrate (Edington *et al*., 1971).

The effects of the *P. ostreatus* filtrate on mycelia growth of the *M. audouinii* was determine as above.

Effect of the antifungal drug Clotrimazole on growth of *M. audouinii*

The antifungal drug Clotrimazole was obtained as standard solution in a concentration 10 µg/ml from nation pharmacy and consider as a stock solutions (S). Stock solution was mixed with melted SDA medium to obtain different concentrations 50, 25 and 10%. 10ml of each concentration were poured in petri-plate (9cm). Each petri-plate was inoculated with 5mm discs from 7 days old culture of *M. audouinii*. Control petri-plates without Clotrimazole were inoculated also. After 7 days of inoculation colony diameter were recorded and percent of inhibition was calculated according to Edington etal.,1971.

Results

Effect of *Trichoderma harzianum* on *Microsporum audouinii* growth in Dual culture

The results that obtained from this study are shown in the Table (1) and Fig (1). In dual culture assay it observed that the *Trichoderma harzianum* inhibited the radial mycelial growth of *Microsporum audouinii* significantly as compared with the control. The Mean of radial growth of *Microsporum audouinii* after 3 days and 7 days of treatment was 2.16 cm , 2.53 cm respectively as compared with the control which were 5.10cm and 6.83cm respectively. The percentages of inhibition of radial growth (PIRG) values after 3 and 7 days were 57.64% and 62.95% respectively.

The fungus *Trichoderma harzianum* recorded high antagonistic activity and completely overgrew the test pathogen *Microsporum audouinii* and grow on the entire surface of petri-plate.

Effect of *Pleurotus ostreatus* on *Microsporum audouinii* growth in Dual culture

The antagonistic activity of *Pleurotus ostreatus* against *Microsporum* was observed in dual culture. The percent of inhibition of *Microsporum* growth by *Pleurotus ostreatus* mycelia in Dual culture is presented in Table (2).

The results of in vitro dual culture interactions between *Pleurotus ostreatus* and *Microsporum* showed that *Pleurotus ostreatus* inhibited the growth of *Microsporum* significantly ($P \leq 0.05$) as compared with the control. The radial growth of of *Microsporum audouinii* was 2.03 cm after three and seven days of treatment as compared with the control which were 5.2 cm and 7.6 cm respectively.

The results of dual culture demonstrated that *Pleurotus ostreatus* exhibited high percentage of growth inhibition of *Microsporum* (60.96 %) after 3 days and (73.28) after 7 days of treatment.

Effect of culture Filtrate of *T. harzianum* on growth of *M. audouinii*

The results of the study of the effect of *T. harzianum* culture filtrate in growth of *M. audouinii* showed that the culture filtrate of the fungus *T. harzianum* was affected the radial growth of the dermatophyte fungus *M. audouinii* significantly as compared with the control. The effect was increased with the increasing of the concentration of culture filtrate. The radial growth of *M. audouinii* at the concentrations 50%, 25% and 10% were 1.66, 2.03 and 2.73cm respectively comparing with the control 7.36cm (table 3). The highest percentage of inhibition was recorded in the case of the concentration 50% which was 77.44% and the lowest percentage of inhibition in the case of the concentration 10% which was 62.90%.

Effect of culture filtrate of *P. ostreatus* on growth of *M. audouinii*

The results of the antifungal activity of culture filtrate of *P. ostreatus* against *M. audouinii* are summarized in the table (4). From these results it observed that the culture filtrate exhibited variable degree of antifungal activity against the tested fungus according to the concentration. All tested concentration affected the growth of *Microsporum* significantly as compared with the control, and the effect was increased with the increasing of concentration. The radial

growth of *M. audouinii* was 1.26cm, 1.56cm and 2.46cm at the concentrations 50%, 25% and 10% respectively, comparing with the control 7.30cm.

The greatest percentage of inhibition was recorded in the concentration 50% which was 82.73% and the lowest percentage of inhibition in the case of the concentration 10% which was 66.30%.

Effect of antifungal drug Clotrimazole on growth of *M. audouinii*

The result of this study that presented in table (5) revealed that antifungal drug Clotrimazole affect the growth of *M. audouinii* and the radial growth were inhibited significantly as compared with the control. The lowest radial growth was recorded at the concentration 50% which was 0.46cm as compared with the control 7.33cm.

The effect of clotrimazole on growth was increased with increasing of the concentration of clotrimazole. The highest percentage of inhibition was recorded at the concentration 50% which was 93.72% and the lowest was recorded at the concentration 10% which was 65.07%.

Discussion

Antagonistic potential of *Trichoderma* species and *Pleurotus* species against different pathogenic fungi has been reported by several researchers (Patil and Prajapati, 2017; Owaid *et al*., 2017).

Mycelial interaction is a basic method to assess antagonistic properties of microorganisms. These results revealed that *Trichoderma harzianum* antagonized the test pathogen in high degree because it has different mechanisms to affect the pathogens specially the direct mycoparasitism and some of the extracellular lytic enzymes produced by this fungus are thought to play a role in mycoparasitism, due to their function in direct physical interactions. (Sharma *et al*., 2016; El-Katany *et al*., 2001).

The effect of culture filtrate was increased with increasing of concentrations of culture filtrate and this result was agree with the results of Mishra *et al*. (2011) who reported that more than 50% growth inhibition was found at 10% culture filtrate of T. *viride* against pathogens like *R. solani, S. rolfsii, M. phaseolina* and *C. capsici* while at 20% concentration 100 % mycelial growth inhibition was observed which suggest the inhibitory action of culture filtrate of *Trichoderma*.

Another study showed that the fungus *Trichoderma* sp. Strain MF106 produce two new antibiotic pyridines which called Trichodin A and Trichodin B and revealed that the trichodin B affect the growth of the dermatophyte fungus *Trichophyton rubrum* but there is no effect in the case of Trichodin A (Bin *et al*., 2014).

Pleurotus spp also produced active compounds that have Antibacterial and antifungal property include phenolic compounds like *p*-Anisaldehyde (Okamoto *et al*., 2002), or Terpenoids compounds like Terpene (Vatcharin *et al*., 2005) and Enzymes like Ribonuclease (Ngai and Ng, 2004).

Antimicrobial activity of four species of oyster mushrooms: *Pleurotus ostreatus* (grey and white strains), *Pleurotus cornucopiae* (yellow strain) and *Pleurotus salmoneostramineus* (pink strain) were investigated against five standard strains of pathogenic bacteria and yeast and the result revealed that the filtrate of *P. salmoneostramineus* was the best one compared with other filtrates against *Pseudomonas aeruginosa* and *Candida parapsilosis* and different sensitivities of tested pathogenic fungi were recorded (Owaid *et al*., 2015).

Owaid *et al*. (2017) investigated the antifungal activities of 4 *Pleurotus* spp. (oyster mushrooms) against pathogenic fungi, *Verticillium* sp., and *Pythium* sp. and showed that the fungus *Pleurotus ostreatus* grew over the mycelia of *Pythium* sp. in 5.33 days.

Clotrimazole was the most potent antifungal agent. Clotrimazole is one of the oldest antifungal drugs formulated as a topical for use against dermatophytosis. This antimycotic agent showed an excellent in-vitro potency against most dermatophyte fungi (Nweze *et al*., 2007).

Another study which was conducted to evaluate the antifungal activity of clotrimazole and berberine against *Microsporum canis* showed that the clotrimazole was more effective with a minimum inhibitory concentrations 0.015 mg/mL as compared with berberine which was 1mg/mL and there is no significant difference was observed among the three groups before 18 h. (Chen-Wen *et al*., 2015).

Another study revealed that the miconazole is the most effective antifungal drugs against *Microsporum canis* followed by clotrimozazole (Sundar *et al*., 2017).

Present study was conducted by Prabha *et al*. (2019) to evaluate the efficacy of clotrimozazole in topical treatment of tinea corporis and tinea cruris and revealed that complete clearance was achieved by clotrimozazole when clotrimazole cream used twice daily for 4 weeks.

Table 1 : Effect of *Trichoderma harzianum* against *Microsporum audouinii* in dual culture.

Treatments	Radial growth (cm) of *M. audouinii* after 3 days	Inhibition (%)	Radial growth (cm) of *M. audouinii* after 7 days	Inhibition (%)	Over Growth after 8 days
Trichoderma harzianum	2.16	57.64	2.53	62.95	+++
Control	5.10	-	6.83	-	-
LSD(0.05)					

* Each value is a mean of 3 replicates. +++ = High antagonistic activity (61 – 75 PIRG) , PIRG percent of inhibition in radial growth (Soy tong 1988).

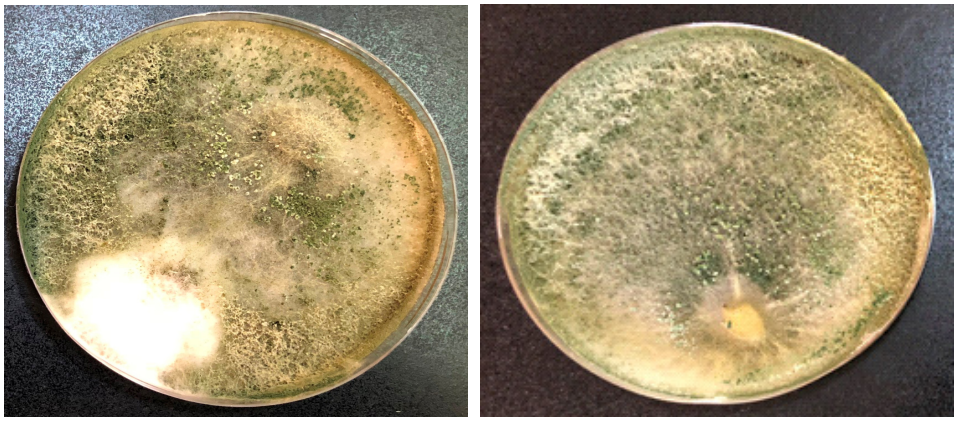

Fig. 1 : Effect of *T. harzianum* on *M. audouinii* in dual culture

Table 2 : Effect of *Pleurotus ostreatus* against *Microsporum audouinii* in dual culture

Treatments	Radial growth (cm) of *M. audouinii* after 3 days	Inhibition (%)	Radial growth (cm) of *M. audouinii* after 7 days	Inhibition (%)	Over Growth after 8 days
Pleurotus ostreatus	2.03	60.96	2.03	73.28	+++
Control	5.2	-	7.6	-	-
LSD(0.05)					

*Each value is a mean of 3 replicates. +++ = High antagonistic activity (61–75 PIRG), PIRG percent of inhibition in radial growth (Soy tong 1988).

Table 3 : Effect of T. *harzianum* culture filtrate on *Microsporum* by poisoned food technique

Treatments	Concentrations	Radial growth (cm) of *Microsporum*	% Inhibition
Culture filtrate of *T.harzianum*	50%	1.66a	77.44
	25%	2.03b	72.41
	10%	2.73c	62.90
Control	0	7.36d	-
LSD(0.05)	0.36		

*Each value is a mean of three replications.
Different letters refer to significant differences

Table 4 : Effect of *Pleurotus ostreatus* culture filtrate on *Microsporum* by poisoned food technique

Treatments	concentrations	Radial growth (cm) after 7 days	% inhibition
Culture filtrate of *Pleurotus ostreatus*	50%	1.26a	82.73
	25%	1.56a	78.63
	10%	2.46b	66.30
Control	0	7.30c	-
LSD(0.05)	0.44		

*Each value is a mean of three replications.
Different letters refer to significant differences

Table 5 : Effect of Clotrimazole on growth of *Microsporum* by poisoned food technique

Treatments	Concentrations	Radial growth (cm) of *M. audouinii*	% Inhibition
Clotrimazole	50%	0.46a	93.72
	25%	1.16b	84.17
	10%	2.56c	65.07
Control	0%	7.33d	-
LSD(0.05)	0.28		

*Each value is a mean of three replications.
Different letters refer to significant differences

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