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ANTAGONISTIC ACTIVITIES OF *TRICHODERMA* SPP. ON DERMATOPHYTES AND OTHER RELATED FUNGI

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Dermatophytosis - the fungal infection of the keratinized tissue hair, nail and stratum corneum of the skin is difficult to eradicate with drug treatment. The increasing resistance to antifungal compounds and the reduced number of available drugs let us search for alternative approach. An alternative approach to treat dermatophytosis may be possible by the application of a biological control agent against the pathogen. In analogy with the success of biocontrol of phytopathogenic fungi, screening of *Trichoderma* spp. for potential antagonism between *Trichoderma* spp. and dermatophytes *(T. rubrum, T. mentagrophytes, M. gypseum* and *C. tropicum)* was undertaken. A wide spectrum of antagonism capacity with effective overgrowth on dermatophytes was found, with *T.viride* being the most effective against tested dermatophytes. Growth of *Trichoderma* spp. in poor medium also resulted in secretion of antibiotics active in arresting the growth of dermatophytes inoculum. The findings may open new direction for the treatment of dermatophytosis, either in combination with known medication or as a new "natural" route.

Keywords: Antagonism; Dermatophytes; Mycosis; *Trichoderma.*

Introduction

The major cause of mycoses - is the dermatophytic infection by members of the genera *Trichophyton, Microsporum,* and *Epidermophyton.* Dermatophytosis of the nails are particularly difficult to eradicate with drug treatments mainly due to the protective nail plate, sequestration of the pathogens between the nail bed and plate and the slow growth of the nail[1]. The most frequently employed anti-mycotic agents used to treat onychomycosis are based on allylamine and azole derivatives, orally administered for long periods of time (several months), with potential harmful side effects on liver functions[2]. Whenever applied persistently, these treatments are usually effective. It appears, however, that 20-40% of treated patients do not respond to these treatments and many others avoid them due to suspected side effects. Alternatively, local external treatment of mycoses with fungicide formulations may be applied. The efficacy of this approach depends, however, on effective penetration of the fungicide to reach the dermatophytes growing areas. An alternative approach to treat mycoses caused by dermatophytes, may possibly be the application of biological control agents against the pathogen. Biological control is defined as the use of biological processes to lower inoculum density of the pathogen, with the aim of reducing its disease-producing activities. Antagonistic interactions among microorganisms differ in their nature and include parasitism or lysis, antibiosis and competition. *Trichoderma* spp. are well-known to antagonize and control a wide range of economically important plant pathogenic fungi. The finding that some *Trichoderma* spp. are capable of producing antibiotics[3], extracellular lytic enzymes[4] or both, has provided essential information on the nature of the molecular events associated with antagonism. *Trichoderma* spp. secretes hydrolytic enzymes such as β-1, 3-glucanases, β-1, 6-glucanases, chitinases and proteases, considered to aid penetration of the host cell walls and the utilization of its cellular contents as a source of nutrients[5]. *Trichoderma* spp. are producers of a variety of antibiotics among them "Peptaibols" that generally exhibit anti-microbial activity against gram positive bacteria and fungi[6]. Their biological activities are thought to arise from their membrane modifying properties and their ability to form trans-membrane voltage-dependent channels. Peptaibols are thought to act on the membrane of the target fungus by means of inhibition of the membrane-associated enzymes involved in cell wall synthesis[7]. The secretion of hydrolytic enzymes or

antibiotics is considered to be important components of the antagonistic process by which *Trichoderma* spp. attack the host fungi. While the interaction of *Trichoderma* spp. with plant pathogens has been intensively investigated and practiced, there is less information of its antagonistic properties toward human pathogenic fungus such as the dermatophyte, *T. rubrum, T. mentagrophytes, M. gypseum* and *C. tropicum* the most common agent of dermatomycoses.

Material and Method

Fungal cultures -The *Trichoderma* spp. were obtained from the Durgaura Agriculture Research Institute, Jaipur: *Trichoderma harzianum* and *Trichoderma viride*. Four species of fungi were selected for the biological control activity *viz. Trichophyton rubrum, Trichophyton mentagrophytes, Microsporoum gypseum* and *Chrisosporum tropicum.*

Dual culture test -Hyphal interaction between *Trichoderma* spp. and dermatophytes were investigated according to the following procedure: the agar disc of 3 mm diameter size were cut from the margins of three days old vigorously growing cultures of antagonistic and test fungi and were inoculated 3 cm apart in petri dishes containing 15 ml each of PDA medium and incubated for 5 days at 28° C. Interaction between the two fungal colonies was examined with the help of microscope. The comparisons were made with control and percent inhibition of fungi was calculated by the following formula.

Percent Inhibiton (I) = C-T /C X 100

C=Growth in control; T=Growth in treatment (mm); I=Inhibition of fungal growth

Evaluation of antibiosis potential- For evaluation of antibiosis potential *Trichoderma* spp. were grown for 3 days in 100 ml SM medium - supplemented with 1% sucrose or in 100 ml SDB medium, in shake flasks incubated at 150 rpm and 30°C. Supernatant samples collected after 3 days were incubated for 10 min at 90°C to eliminate enzymatic activities and diluted x 0, 2, 4, 8, and 16 times. One ml samples of these serial dilutions were incubated with dermatophytes homogenate for 24 h at 30°C and 40 µl from each dilution were placed as innoculum on SDA medium plates. The plates were incubated at 30°C for 6 days and the emerged colonies counted.

Results and Discussion

Trichoderma spp. are known for their potential as a biocontrol agents against plant pathogenic fungi. The study is aimed to verify potential antagonism of *Trichoderma* spp. to the dermatophytes (*T. rubrum, T. mentagrophytes, M. gypseum* and *C. tropicum*). A two-stage procedure was adopted : first, a series of available *Trichoderma* strains were confronted with a series of available dermatophytic strains, in a dual culture test for the visual identification of antagonistic overgrowth capability. Secondly, *Trichoderma* strains exhibiting overgrowth were also tested for antibiotic secretion and its inhibitory impact on dermatophytes growth.

A dual culture antagonism test was performed to test antagonistic activity of *Trichoderma* spp. against dermatophytes. *T.viride* was found to be most effective as compaire to *T. harzianum.* In the present study it was found that both antagonistic fungi (*T. viride* and *T. harzianum*) showed more than 66% inhibition of growth against all test fungi. Maximum inhibition of mycelial growth of *C. tropicum* was obtained with *T. viride* (100%) and 97.78% by *T. harzianum.* Minimum inhibition of mycelial growth of *M.gypseum* was obtained with *T.viride* (72.23%) and (66.67%) by *T. harzianum* (Table 1). Sharma and Pareek[8] also obtained similar resulte with test fungi of otomycosis. They reported maximum growth inhibition of *C. albicans* (93.3%) followed by *A. fumigatus* (70%) and *A. niger* (67.7%) for *T. viride.* This visually observed antagonism may lead to its application as a whole viable mycoparasitic organism or alternatively using the accompanying secreted products such as hydrolytic enzymes and antibiotics compounds to affect lysis and growth arrest.

As antibiosis is another expected characteristic of the antagonism process, the production of secreted dermatophyte growth inhibitors by *Trichoderma* spp. were investigated. The data incorporated in Table 2 strongly indicates that *Trichoderma* spp. was capable of secreting and affecting growth arrest or growth inhibition of dermatophytes. The intensity of these effects, however strongly depends on the composition of the growth medium employed. *Trichoderma* spp. grown in rich culture medium (SDB) exhibited poor to mild dermatophytic hyphae growth inhibition, while growth in synthetic medium (poor medium) supplemented with 1% sucrose revealed highly effective growth arrest. The data of table also indicate that *T. viride* shows more growth arrest or growth inhibition of dermatophytes compaired to *T. harzianum.* Maximal specific production of secreted compounds has also been observed for *Trichoderma* spp. grown in synthetic poor medium. For *T. viride* maximum inhibition of growth observed for *C. tropicum* followed by *T. mentagrophytes, M. gypseum* and *T. rubrum* in SDB medium. *C. tropicum* was found more susceptible and *T. rubrum* was most resistant pathogenic fungi. Likewise in synthetic medium maximum inhibition of growth was observed for *C. tropicum* followed by *T. mentagrophytes, T. rubrum* and

Table 1. Biological control of dermatophytes by antagonistic fungi.

Antagonistic fungi	Test fungi	Growth of test fungi in control	Growth of test fungi in treatment	Growth of antagonistic fungi in treatment	% Inhibition of growth
T. viride	*T. rubrum*	9.0	2.0	7.0	77.78%
	T. mentagrophytes	9.0	1.5	7.5	83.34%
	M. gypseum	9.0	2.5	6.5	72.23%
	C. tropicum	9.0	0.0	9.0	100%
T. harzianum	*T. rubrum*	9.0	2.2	6.8	75.56%
	T.mentagrophytes	9.0	0.5	8.5	94.45%
	M. gypseum	9.0	3.0	6.0	66.67%
	C. tropicum	9.0	0.2	8.8	97.8%

Table 2. Antibiosis of dermatophytes with non - enzymatic *Trichoderma* secretions.

Antagonistic fungi	Test fungi	Extract dilution											
		Trichoderma spp. growth in SDB						*Trichoderma* spp. growth in SM					
		0	2	4	8	16	Control	0	2	4	8	16	Control
T. viride	*T. rubrum*	120	136	150	174	218	243	0	1	6	8	32	235
	T. mentagrophytes	85	105	146	172	209	248	0	0	2	9	28	230
	M. gypseum	90	129	142	172	215	242	8	24	32	62	92	232
	C. tropicum	66	82	132	158	202	240	0	0	4	12	26	245
T. harzianum	*T. rubrum*	110	130	168	224	230	248	0	8	24	42	70	232
	T. mentagrophytes	102	124	154	192	210	245	0	5	18	31	62	233
	M. gypseum	103	126	158	194	220	246	16	36	49	71	98	240
	C. tropicum	72	91	138	189	202	245	0	6	12	24	32	240

SDB = Sabouraud Dextrose Broth
SM = Synthetic media

M. gypseum for non enzymatic secretions of *T. viride.* In these study *C. tropicum* was observed to be more susceptible and *M. gypseum* was more resistant pathogenic dermatophytic species. At zero dilution of antibiotics secretions 100% growth inhibition was observed for *T. rubrum, T. mentagrophytes* and *C. tropicum.*

Antagonistic activities of *Trichoderma* spp. have been detected by many workers. Omero *et al.*[9] observed that *T. virens* NRRL 26672 is capable of secreting and affecting growth arrest of *T. rubrum. Trichoderma virens* NRRL 26672 grown in rich culture medium (SDB) exhibited poor to mild *T. rubrum* hyphal growth inhibition, while grown in poor medium (SM) suppliemented with 1% sucrose revealed highly effective growth arrest. Tricholin a ribosome-inactivating protein isolated from the culture broth of *Trichoderma viride* has been shown to exert fungicidal effects on *Rhizoctonia solani*[10]. A protease produced by *T. harzianum* was purified and their antagonistic activity, against phytopathogenic fungi *Crinipellis perniciosa,* was studied[11]. Antagonistic activity of seven spp. of *Trichoderma viz., T. harzianum, T. viride, T.asperellum, T. aureoviride, T. koningii, T. longibrachiatum* and *T. virens* was studied on plant pathogen *Rhizoctonia solani.* All species showed inhibition of growth and sclerotial formation through the production of non-volatile antibiotics[12].

The observation that *Trichoderma* spp. are a potential antagonistic biocontrol agent against dermatophytic infection opens new opportunities and new approaches to treat mycoses, either as a new independent route or in combination with currently employed medications. This study also raises some interesting possibilities for future research.

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