

Antagonistic Potential of *Trichoderma viride* and *T. harzianum* Against Some Dermatophytic Fungi

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Received: 17.08.2020 | Revised: 24.09.2020 | Accepted: 1.10.2020

ABSTRACT

In this study, *Trichoderma viride* and *T. harzianum* were used as biological control agents to assess their antagonistic potential against some dermatophytic fungi (*Microsporum gypseum*, *M. fulvum*, *Trichophyton rubrum*, *T. interdigitale*) which causes ring worm in human. For this purpose dual culture method was used. *Trichoderma viride* and *T. harzianum* showed highest percentage inhibition of radial growth (PIRG) against *Trichophyton interdigitale* (92% & 60% respectively). Colony over growth was also observed with *Trichoderma viride*. The outcomes of the study indicate that *Trichoderma viride* was excellent antagonist to prevent the growth of dermatophytic fungi whereas *T. harzianum* exhibited moderate PIRG between 45-60% against *Microsporum gypseum*, *M. fulvum*, *Trichophyton rubrum*, *T. interdigitale*.

Keywords: *Trichoderma viride*, *T. harzianum*, Dermatophytic fungi, Inhibition, Dual culture

INTRODUCTION

Soils that are rich in keratinous materials are the most conducive for the growth and occurrence of keratinophilic fungi. Keratinophilic fungi along with dermatophytes are responsible for various cutaneous mycoses. Dermatophytoses allude to superficial fungal infection of keratinized tissues bring about keratinophilic dermatophytes. The infection is often called as ring worm or “tinea”. Biological control of pathogen by microorganisms is an alternative of chemical

treatment method. An interesting alternative approach to treat mycosis caused by dermatophytes such as *T. rubrum*, *T. interdigitale*, *Microsporum gypseum* and *M. fulvum*, may be the use of antagonistic fungi such as *Trichoderma*. *Trichoderma* spp. is occurred worldwide in the soil (Domsch et al., 1980; Christensen, 1981). Its antagonistic activity against plant pathogen has been studied widely and it's extensively used as BCAs in the world (Khetan, 2001; Tronsmo & Hjeljord, 1998).

Cite this article: Bairwa, S., & Sharma, M. (2020). Antagonistic potential of *Trichoderma viride* and *T. harzianum* Against Some Dermatophytic Fungi, *Ind. J. Pure App. Biosci.* 8(5), 132-137. doi: <http://dx.doi.org/10.18782/2582-2845.8345>

The success of *Trichoderma* strains as BCAs is due to their high reproductive capacity, ability to survive under very unfavorable conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi and efficiency in promoting plant growth and defense mechanisms. *Trichoderma viride* and *T. harzianum* were found to be an antagonist to many plant pathogens. Antagonists perform against pathogens through parasitism, antibiosis or competition. They produce toxic metabolites and inhibit pathogen by antibiosis (Dandurand & Knudsen, 1993).

MATERIALS AND METHODS

Isolation and identification of dermatophytic isolates:

Dermatophytic fungi were isolated from soil by using To.Ka.Va. hair baiting technique and cultured on Sabouraud Dextrose Agar(SDA) and Potato Dextrose Agar(PDA). *Microsporum gypseum*, *M. fulvum*, *Trichophyton rubrum*, *T. interdigitale* isolates were sub-cultured regularly.

$$\text{PIRG} = \frac{R - r}{R} \times 100$$

Where R - Indicates the radius of dermatophytic fungi in control plates

r - Indicates the radius of dermatophytic fungi in dual cultured plates

Investigation were continue to record the number of days needed for the colony overgrowth. In dual culture, assessment of colony interactions grading were done based on intermingling and inhibition zone

Screening by dual cultured method:

Trichoderma viride and *T. harzianum* obtained from Rajasthan Agriculture Research Institute (RARI) Durgapura, Jaipur and were used in present study for their antagonistic activity against *Microsporum gypseum*, *M. fulvum*, *Trichophyton rubrum* and *T. interdigitale*. For this purpose dual culture method was used which based on percentage inhibition of radial growth (PIRG). A 3mm diameter size were cut from the margins of 7 days old vigorously growing cultures of dermatophytic fungi and antagonistic fungi and placed on 1 cm away from the periphery of 9cm petri plates containing PDA medium on opposite side of each other on same petri plate. As a control dermatophytic fungi were similarly placed on PDA medium without *Trichoderma* spp. These petri plates were incubated at 28°C for 7 days. Antagonistic activity of *Trichoderma* spp. were determined by measuring the percentage inhibition of radial growth (PIRG) of dermatophytic fungi using the formula (Edgington, et al., 1971).

(Skidmore & Dickinson 1976) identified 5 separate modes of interaction colony overgrowth and were assigned values on 0-5 scale for each type of interaction where '0' indicates no inhibition.

Modes of interaction	Grade	Value
Homogeneous	A	1
Mycoparasitism	B	2
Overlapping growth	C	3
Inhibition at line of contact	D	4
Aversion	E	5
No inhibition	-	0

RESULTS

Results from dual culture method indicate that both the species of *Trichoderma* inhibited the radial growth of dermatophytic fungi with

varying efficiencies (Table 1 & Fig.1). The percentage inhibition radial growth values ranged from 45 to 92. It was observed that *Trichoderma viride* was inhibited the radial

growth of *Microsporium gypseum*, *M. fulvum*, *Trichophyton rubrum* and *T. interdigitale* more than *T. harzianum*. Maximum radial growth inhibition showed by *Trichoderma viride* against all the pathogenic fungi *M. gypseum* (77.78%), *M. fulvum* (77.5%), *Trichophyton rubrum* (73.33%), and *T. interdigitale* (92%) whereas *T. harzianum* showed highest PIRG against *T. interdigitale* (60%). Colony overgrowth time was varying 7 to 10 days. *Trichoderma viride* showed tremendous

overgrowth whereas *T. harzianum* exhibited minor overgrowth.

In present study, antagonistic relationships among *Trichoderma* spp. and dermatophytic fungi ranged from grade 2 – 4 (Table 1& Fig. 2). However, grade 3 was observed as the most commonly experienced type of colony interaction, followed by grade 4. *Trichoderma harzianum* showed grade 2 interaction against *M. fulvum*.

Table 1: Showing antagonistic activity against dermatophytic fungi in form of PIRG, Grade and Value

S.No	Dermatophytic fungi	Radial growth in control (cm)	Antagonistic fungi					
			<i>Trichoderma viride</i>			<i>T. harzianum</i>		
			RG in dual culture (cm)	PIRG (%)	Grade/ Value	RG in dual culture (cm)	PIRG (%)	Grade/ Value
1	<i>Trichophyton rubrum</i>	3.0	0.8	73.33	C/ 3	1.50	50	D/ 4
2	<i>Microsporium gypseum</i>	4.50	1.00	77.78	D/ 4	1.90	57.78	D/ 4
3	<i>Microsporium fulvum</i>	4.00	0.9	77.5	C/ 3	2.20	45	B/ 2
4	<i>Trichophyton interdigitale</i>	5.00	0.4	92	C/ 3	2.00	60	C/ 3

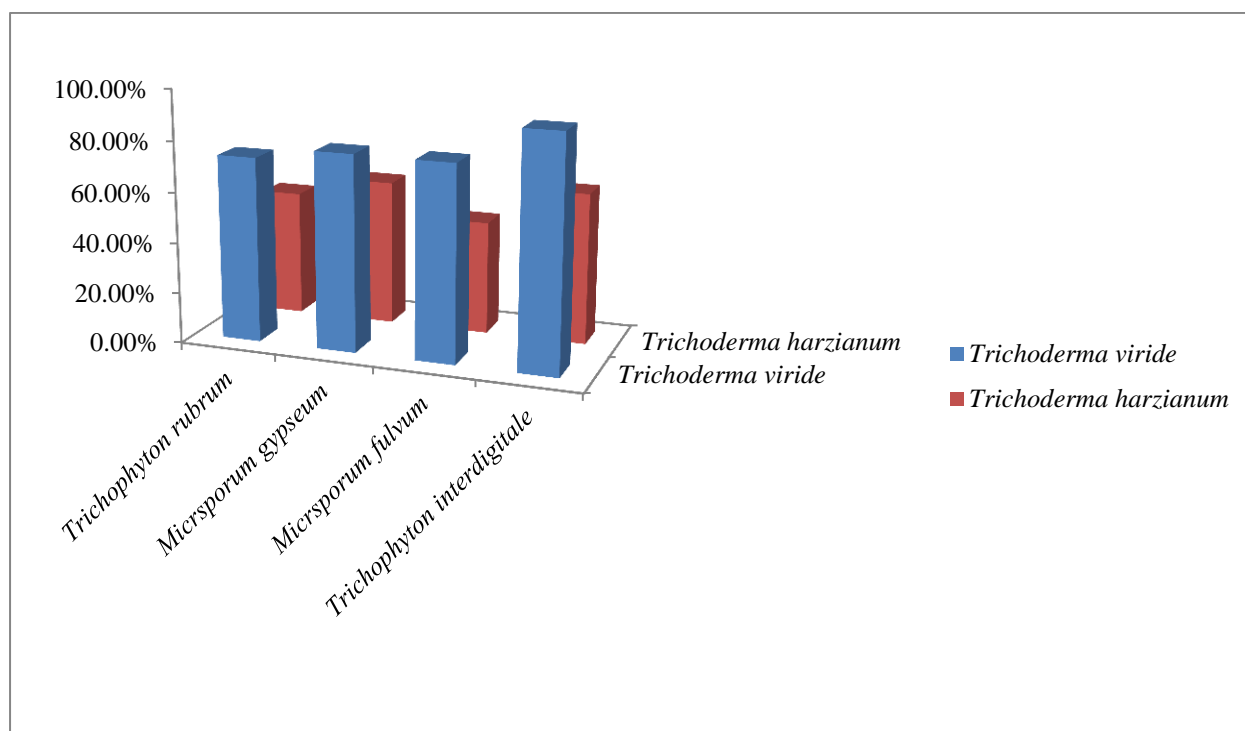


Fig. 1: Graphical representation of PIRG against dermatophytic fungi

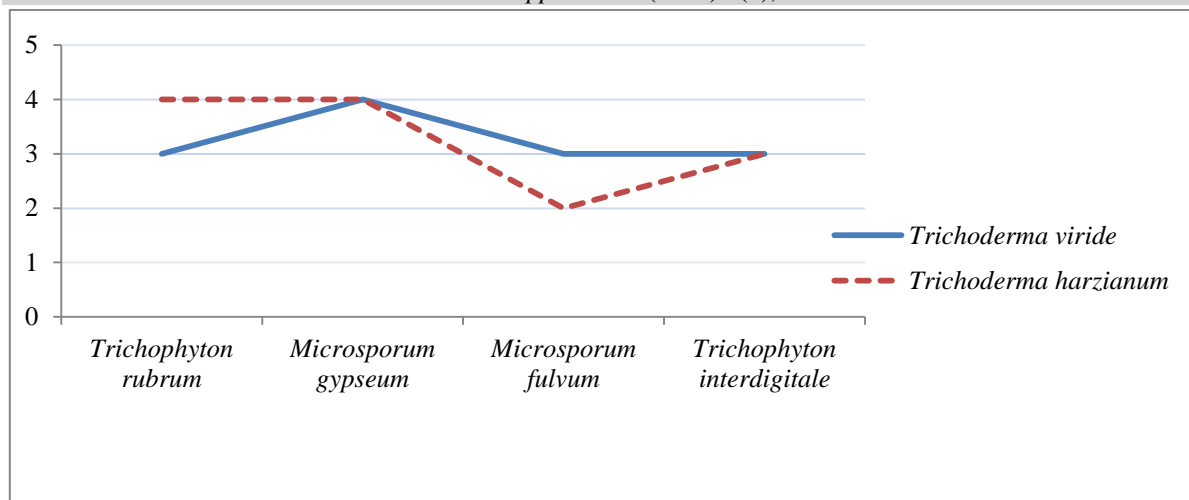


Fig. 2: Showing antagonistic activity against dermatophytic fungi

DISCUSSION

Trichoderma has been reported as potential biocontrol agent due to their ability to inhibit the prevalence of diseases caused by soil borne pathogens (Calvet et al., 1990; Elad et al., 1993; Ashrafizadeh et al., 2005; Dubey & Suresh, 2007). In present study, two isolates of *Trichoderma* were assessed in vitro for screening antagonistic potential against *Microsporium gypseum*, *M. fulvum*, *Trichophyton rubrum* and *T. interdigitale*. The result revealed that *Trichoderma viride* demonstrated strongest antagonistic activity to inhibit the growth of above mention dermatophytic fungi. Begum et al. (2008) observed that *Trichoderma virens* and *Trichoderma harzianum* inhibit the growth of *Colletotrichum truncatum*. The study was based on culture filtrate test and high PIRG value in dual culture method. *Trichoderma harzianum* exhibited different isolates and abilities to attack *Sclerotium rofsii* (Jinantara, 1995; Henis et al., 1983). *Trichoderma viride* was found best antagonist based on two criteria high PIRG value and minimum colony overgrowth time. Etabarian (2006) observed decreased/ minimized the colony area of *Macrophomia phaseoli* by using *Trichoderma viridie* (MO) as antagonistic in dual culture and cellophane method. Omero et al. (2004) investigated that *Trichoderma virens* NRRL 26672 was the most effective against *T. rubrum* NCPF118. *T. virens* NRRL 26672

developed with *T. rubrum* NCPF118 hyphae as a carbon source, showed upgraded discharge of active extracellular chitinases and b-glucosidases which affecting sporulation and lysis on *T. rubrum* NCPF118 hyphae. Rahman et al. (2009) were found that highest PIRG value with *T. harzianum* IMI-392432 using dual culture method as compare to *T. virens* IMI-392430, *T. pseudokoningii* IMI-392431 and *T. harzianum* IMI-392433. Cherif et al. (2009) investigated that *Pseudomonas aeruginosa* and *P. fluorescens* were effective against *Trichophyton rubrum*, *T. interdigitale* and *Microsporium canis*. Sharma (2010) reported that *T. viride* was more potent as compare to *T. harzianum* against *Trichophyton rubrum*, *T. mentagrophytes*, *M. gypseum* and *E. floccosum*.

Bakshi (2006) investigated antagonistic potency of three strains of *Trichoderma* and *Paecilomyces lilacinus* against *Chrysosporium indicum*, *Trichopyton mentagrophytes* and *T. simii*. *Trichoderma harzianum* and *T. reessii* were showed grade B antagonism against *T. mentagrophytes* and grade D antagonism against *T. simii* and *C. indicum*. *T. viride* showed overgrowth (grade B antagonism) against *T. simii* and *C. indicum* and grade D antagonism against *T. mentagrophytes*. *P. lilacinus* showed grade D antagonism against all the test dermatophytes. Aktar et al. (2014) examined the antagonistic potentials of seven rhizosphere soil fungi viz.

Aspergillus fumigatus Fresen., *A. terreus* Thom., *A. niger* Tiegh. *A. flavus* Link, *Trichoderma harzianum* Refat. *Penicillium* spp. and *T. viride* Pers. were tested against six pathogenic fungi isolated from different leaf spots and fruit rots of brinjal. They found antagonistic interactions among the soil fungi and test pathogens ranged from grade 2 - 4. Among the seven soil fungi *Trichoderma harzianum* exhibited grade 4 interaction against all the 6 test pathogens followed by *A. niger*. In the study of effects of volatile and non-volatile metabolites and colony interactions, *Trichoderma harzianum* was found most efficacious against all the test fungi.

CONCLUSION

Conclusively, *T. viride* was found to be more potent antagonist than *T. harzianum* against all the fungi tested. Our findings have led to the possibility that *Trichoderma* spp. might be suitable to control the activity of dermatophytic fungi.

Acknowledgement

The support provided by the University Grants Commission, New Delhi through Rajiv Gandhi National Fellowship to one of the authors Soniya is gratefully acknowledged. She is also thankful to the Head, Department of Botany for providing necessary facilities.

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