Mycopath (2015) 13(2): 93-96 **Research Article**

Screening of *Trichoderma* species for their biological control potential against *Sclerotium rolfsii,* the cause of collar rot disease of chickpea

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Abstract

In the present study, *in vitro* antagonistic potential of seven species of genus *Trichoderma* namely *T. viride* Pers., *T. harzianum* Rifai, *T. koningii* Oudem, *T. pseudokoningii* Oudem, *T. aureoviride* Pers., *T. hamatum* Bain and *T. reesei* Rifai was assessed against a destructive soil-borne plant pathogen *Sclerotium rolfsii* Sacc., the cause of collar rot disease of chickpea (*Cicer arietinum* L.). *Trichoderma* species were screened by dual culture method in which cultures of the pathogenic and antagonistic fungi were grown side by side on the growth medium in the same Petri plate. *T. viride*, *T. harzianum*, *T. koningii* and *T. pseudokoningii* exhibited pronounced antagonistic activity against the pathogenic fungal species resulting up to 40-68% reduction in its growth. *T. viride* showed the best antagonistic behavior approximately followed by *T. harzianum* causing 68% and 57% reduction in pathogen growth. Besides, the percentage reduction in number of sclerotia of *S. rolfsii* due to interactions with different *Trichoderma* species was also recorded. The present study concludes that *T.* v*iride* has the best antagonistic potential against *S. rolfsii* followed by *T. harzianum.*

Keywords: Antagonism, biological control, collar rot of chickpea, *Sclerotium rolfsii*, *Trichoderma* spp.

Introduction

S. rolfsii is a destructive soil-borne pathogen in warm and moist climates worldwide, which attacks over 500 plant species. The first report of *S. rolfsii* from Pakistan was given by Ahmed *et al.* (1984) on maize (*Zea mays* L.) and consequently revealed in different field crops (Yaqub and Saleem, 2005). Biocontrol of plant pathogens can be impressive especially with hyperparasitizing possibilities of antagonists on pathogenic fungi. Biocontrol agents may create competition against pathogens and induce resistance in plant by producing different hydrolytic enzymes. Bosah *et al.* (2010) reported that chitinase and β-1, 3 glucanase are well known fungus-controlling enzymes to break two essential cell walls components: chitin and β-1, 3 glucan. Besides many chemical fungicides are frequently use to control the plant diseases. However, their detrimental effect on our environment or wildlife also arise. Therefore, the use of organism as biological control agents has provided a very appealing substitute and less dangerous method for plant disease management. Biocontrol of plant pathogens is a potential non-chemical means for plant disease management and can serve as a substitute for costly chemical treatment (Omorusi *et al.,* 2007). Different species of *Trichoderma* are known to be extremely efficient against various pathogenic fungi (Yaqub and Saleem, 2010; Doley and Jite, 2012). *Trichoderma* spp. are able to produce unpredictable antibiotics in agar and their culture filtrates can also be used for control of fungi (Rekha *et al.,* 2012). The aim of the current investigation was to examine the antagonistic potential of seven *Trichoderma* species for biocontrol of *S. rolfsii*.

Materials and Methods

Procurement of fungal species

Culture of the target fungus *S. rolfsii* was procured from Biofertilizers and Biopesticides Laboratory of Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. Seven species of *Trichoderma* namely *T. pseudokoningii* (FCBP-213), *T. harzianum* (FCBP-732), *T. reesei* (FCBP-364), *T. koningii* (FCBP-951), *T. hamatum* (FCBP-907), *T. viridi* (FCBP-1123) and *T. aureoviridi* (FCBP-691) were acquired from First Fungal Culture Bank of Pakistan, University of the Punjab Lahore, Pakistan. Individual colonies of each species were sub-cultured on malt extract agar medium and stored in a refrigerator at 4 °C.

Antagonistic activity

Seven different species of *Trichoderma* were tested for *in vitro* antagonistic activity against the target pathogen by using the dual culture method of Javaid *et al.* (2014). Petri plates containing autoclaved malt extract agar medium supplemented with streptomycin were inoculated with 2 mm plugs of fungi. One isolate of *Trichoderma* species and *S. rolfsii* were placed simultaneously on opposite sides of each dish, 5 cm apart. In control treatment, only the pathogenic fungus was inoculated. Incubation of the plates was carried out at 27 °C for 5 days. Five replicates of each treatment were made and colony diameter of both the fungal species in a Petri plate was recorded after 5 days of incubation. The colony diameter in each plate was measured at three places and average was calculated. Percent inhibition of mycelial growth of *S. rolfsii* in the presence of *Trichoderma* spp. was measured as follows:

$$\text{Growth inhibition (\%)} = \frac{\text{Control - Treatment}}{\text{Control}} \times 100$$

Where

Control = Radial growth (mm) of *S. rolfsii* in control.

Treatment = Radial growth (mm) of *S. rolfsii* inin the presence of a *Trichoderma* species.

Similarly, reduction in number of sclerotia of *S. rolfsii* was also determined according to the method of Mishra (2010) by counting the number of sclerotia in treated and control plates.

Statistical Analysis

The experiment was performed in a completely randomized design with five replicates. All the data from different treatments were analyzed through analysis of variance followed by Tukey's HSD test ($P \leq 0.05$) using computer software Statistix 8.1.

Results and Discussion

Antagonistic activity of seven different species of *Trichoderma* was exhibited with measuring the pathogen growth by dual culture technique. The radial growth of *S. rolfsii* restricted by antagonistic strains of *Trichoderma* spp. and showed significant inhibition (Fig. 1). In the present study seven *Trichoderma* spp. were selected for screening against *S. rolfsii* of chickpea. It was observed that *T. viridi* showed excellent antagonistic activity against pathogen with 68% of inhibition besides *T. harzianum* reduced the growth by 57%. However least inhibition was observed in case of *T. reesei* (14%), *T. aureoviride* (33%) and *T. hamatum* (36%) in dual culture method over control. The observation revealed that *T. koningii* and *T. pseudokoningii* were moderately inhibited the pathogen with 43% and 53% of inhibition, respectively. Data illustrated in Fig. 2 demonstrates that *T. viridi* and *T. harzianum* also showed the highest percentage of reduction of sclerotial bodies in experimental plates.

It has been extensively recorded that *Trichoderma* species are mycoparasite (Doley and Jite, 2012) and are commonly used as antimicrobial agents. Several *Trichoderma* species are viewed to be antagonistic to other plant pathogenic fungi (Bosah *et al.,* 2010). Their different ways of parasitism to other plant pathogenic fungi happens by various systems i.e. competition, antibiosis, myco-parasitism, induce resistance and inactivation of pathogen's compounds such as enzymes. The direct mycoparasitic action of *Trichoderma* species has been suggested as one of the significant procedure for their antimicrobial activity against plant pathogenic fungus (Mishra *et al.,* 2011). Previously, different *Trichoderma* species have been recorded to produce a variety of antibiotics such as trichodermin, trichodermol, harzianum A and harzianolide (Mishra, 2010), which may help in decreasing ill effects of dangerous pathogens. Different scientists revealed antagonistic activity of *Trichoderma* species against numerous phyto-pathogens (Howell, 2002; Mishra, 2010; Bosah *et al.,* 2010; Mishra *et al.,* 2011; Doley and Jite, 2012). Besides, dual culture technique is widely used in antagonistic studies by different scientist Khattabi *et al.* (2004) and Rekha *et al.* (2012). In dual culture a clear zone of inhibition was observed exhibiting antibiosis between pathogen and antagonist. The degree of inhibition varied from one species to another. In the same way, isolates of different *Trichoderma* species to management *S. rolfsii* have been revealed to vary in their potential (Omar and Maha, 2010). Moreover, *T. viride* exhibited 68% reduction in growth of the pathogen. Formation of inhibition zone at the contact between *Trichoderma* and *S. rolfsii* could be explained on the basis of production of volatile and nonvolatile metabolites as well as the production of extracellular hydrolytic enzymes by *Trichoderma* (El-Katatny, 2001). Aly *et al.* (2007) observed bio-control activity of *Trichoderma* spp. against *Macrophomina phaseolina in vitro* and Mishra *et al.* (2011) revealed that *Trichoderma* species are typical inhabitant of rhizosphere and manage many soil-borne plant diseases caused by fungi. Rekha *et*

al. (2012) studied that culture filtrates of *T. harzianum* restrict formation of zoospore and germ tube and mycelial growth of *S. rolfsii*. In another studies, culture filtrate of *T. viride* inhibited the mycelial growth of *Sclerotinia sclerotiorum* due to production of antibiotic like substance (Kapil and Kapoor, 2005). The above outcomes is convinced with Karthikeyan *et al.* (2006) who confirmed culture filtrates of *T. viride* suppressing the development of the pathogen growth as well as sclerotial germination. *T. viride* restricted the development of *S. rolfsii*, parasitized mycelium of *S. rolfsii* in dual culture assays noticed by Kapoor (2008). Besides other investigation, chitinases of *Trichoderma* spp., deteriorates the chitin in the cell walls of *S. rolfsii* that helps in penetration of *S. rolfsii* mycelium (Doley and Jite, 2012). Consequently, in recent study toxic and/or fungistatic metabolites may be produced by the *Trichoderma* species against *S. rolfsii*. Several researchers studied different species of *Trichoderma* as the best antimicrobial for growth inhibition of several plant pathogens related to seeds and soil (Mukherjee and Tripathi, 2000; Yaqub and Shahzad, 2005; Barakat *et al.,* 2006; Mishra *et al.,* 2011). Currently, the facts regarding the antagonistic potential of *T. reesei* is not significant. This study concludes that *T. viridi* and *T. harzianum* species had significant potential and effective antagonism against *S. rolfsii.*

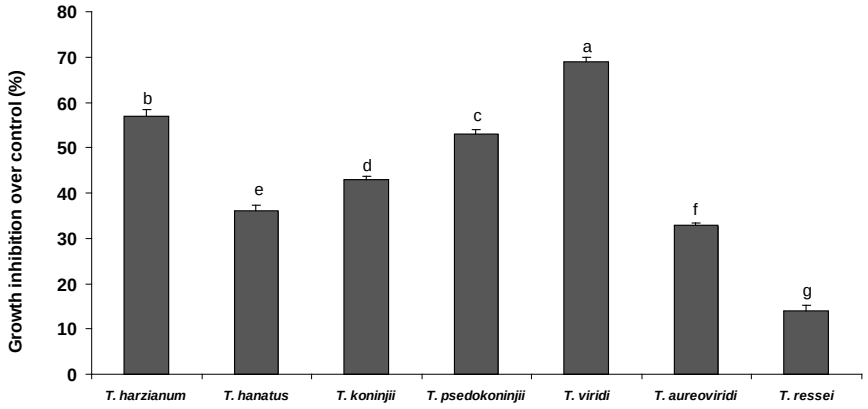

Fig. 1: Growth inhibition of *S. rolfsii* due to interactions with different *Trichoderma* species. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

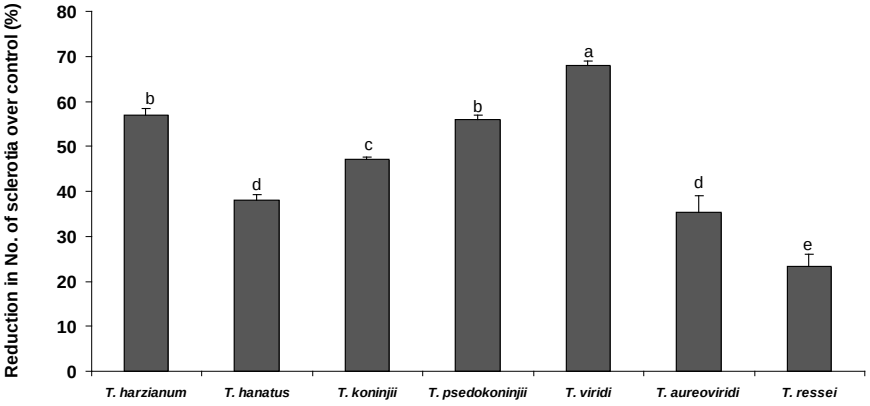

Fig. 2: Percentage reduction in number of sclerotia of *S. rolfsii* due to interactions with different *Trichoderma* species. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

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