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Screening of *Trichoderma species* for Their Biological Control Potential against *Sclerotium rolfsii* (The Cause of Collar Rot of Chickpea)

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A B S T R A C T

In the present study in-vitro antagonistic effect of four species of *Trichoderma. T. harzianum* (FCBP-732), *T.viridi* (FCBP-1123), *T. koningii* (FCBP-951), *T. aureoviridie* (FCBP-691) was studied (assessed) against a destructive soil-borne plant pathogen S*clerotium rolfsii* Trichoderma species were screened by dual culture method. In this method antagonistic and pathogenic fungi were grown side by side on the growth media (i.e., potato dextrose agar) in the same petridish. Trichoderma shows antagonistic effect against sclerotia species resulting up to 100-95 % reduction in its growth *T.harzianum* showed the best (results) antagonistic effect followed by *Trichoderma viridi* causing 95-90% reduction in pathogen growth. The percentage reduction in number of sclerotia of *S.rolfsii* is due to the interaction with different Trichoderma spp. was also recorded the present study showed that *T. harzianum* has the best antagonistic result against *S.rolfsii* followed by *T. viridi*

Introduction

Sclerotia rolfsii is a soil-borne pathogen in warm and most climate which attacks nearly 500 species of plant. The first report of *S. rolfsii* from Pakistan was given by Ahmed *et al.*, (1984) on maize (*Zea mays* L). Biocontrol of plant pathogen can by hyper parasitizing possibilities of antagonistic on pathogenic fungi Bio-control agent may create competition against pathogens and may induce resistance in plant by producing different-hydrolytic enzymes. Bosah *et al.*, (2010) reported that chinase and β-1, 3glucan. The use of organism as biological control agents has provided a very appealing substitute and less dangerous method for plant disease management. Bio-control of plant pathogens is a potential non chemical means for plant disease management and can serve as a substitute for costly chemical treatment are known to be extremely efficient against various pathogenic fungi Yaqub and Saleem (2010). The aim of the current investigation four *Trichoderma* species for Bio-control of *S. rolfsii*.

Materials and Methods

The present study Screening of "*Trichoderma* species for their biological control potential against S*clerotium rolfsii* (the cause of collar rot of chickpea)" carried out at Department of Plant Biotechnology SDMVM's College of Agricultural Biotechnology, Georai Tanda, Paithan Road, Aurangabad (M.S.), 431001, during Nov -2017 to Jan -2018, with objects to Culture of target fungus *S.rolfsii* was procured form Biofertilizer and Biopesticides for four species of *Trichoderma* namely *T. harzianum* (FCBP-732), *T. viridi* (FCBP-1123),

T. koningii (FCBP-951), *T. aureoviridie* (FCBP-691). Individual colonies of each species were sub cultured on malt extract agar medium and stored in refrigerator at 4^0C.

Antagonistic activity

Four different species of *Trichoderma* were tested for in vitro antagonistic activity against the target pathogen by using the dual culture method Javed *et al.,* (2014).

Malt extract agar supplemented with streptomycin were autoclaved and poured in sterilized petriplates and inoculated with 2mm plugs fungi with the help of cork borer one isolate of *Trichoderma* species and *S. rolsfii* were placed simultaneously on opposite sides of each petriplate 5cm part.

In control treatment only the pathogenic fungus was inoculated plates were incubated at 27^0 C for 5 days, five replicates of each treatment were made and colony diameter of both the fungal species in a petriplate was recorded after 5 days of incubation.

The colony diameter in each plate was measured and average was taken percentage inhibition of mycelia growth of *S.rolfsii* in the presence of *Trichoderma* spp. was measured.

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

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

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

Reduction in number of *S.rolfsii* was determine according to the method of Mishra (2010) by counting the number of *Sclerotia* in treated and control plates.

Statistical analysis

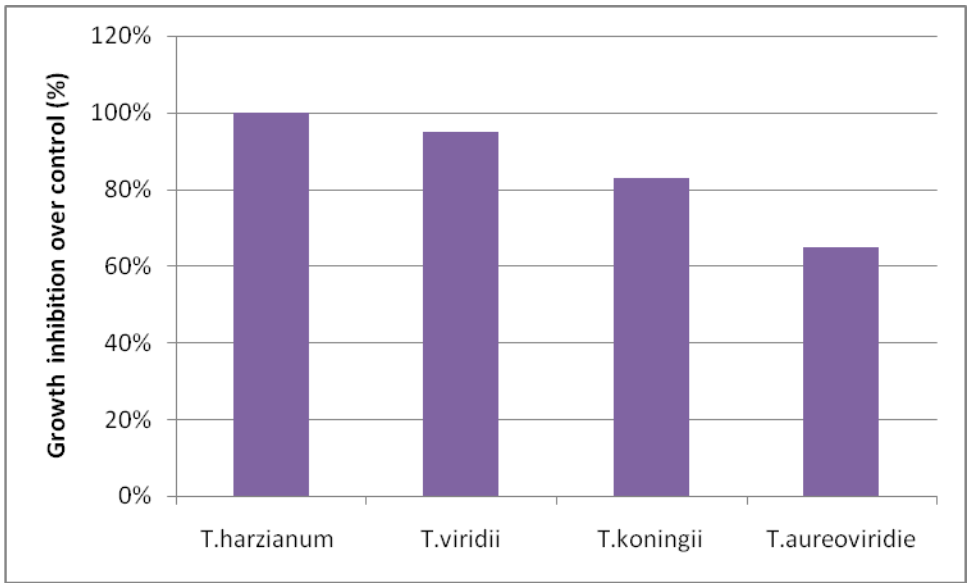
The experiment was performed in completely randomized block design with five replicates the data from different treatments were analyzed through analysis of variance followed by turkeys HSD test ($P \leq 0.5$) using computer software statistics

Results and Discussion

Antagonistic activity of four different species of *Trichoderma* was shown with measuring the pathogen growth by dual culture techniques. The growth of *S.rolfsii* restricted by antagonistic strains of *Trichoderma* spp. and showed significance inhibition. In the present study four *Trichoderma* spp. were selected for screening against *S.rolfsii* of chickpea.

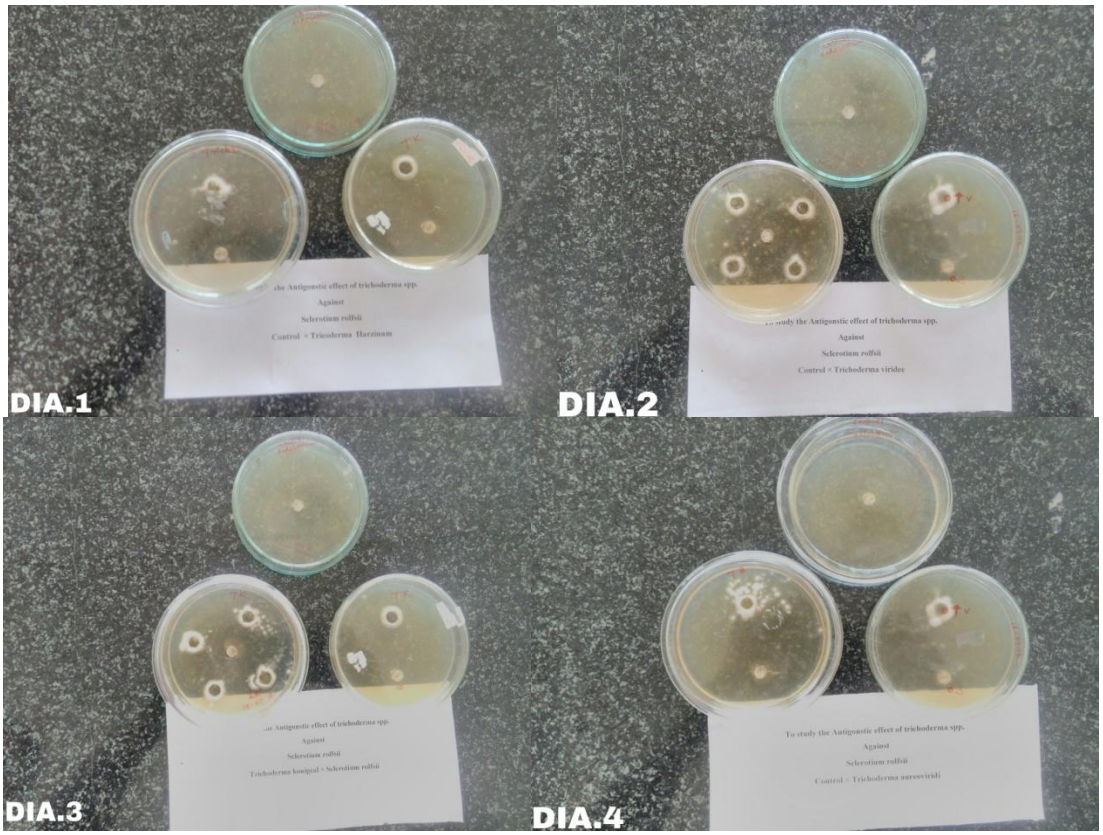
It was observed that *T. harzianum* showed excellent antagonistic activity against pathogen with 100-95% of inhibition besides *T. viridi* reduces the growth by 95-90% followed by *T. koningii* 83-83% lastly by T. aureoviridi 65-60% in dual culture method over control. Data illustrated showed that *T. viridi*i and *T. harzainum* showed the highest % of reduction of sclerotial bodies in experimental plates (Fig. 1).

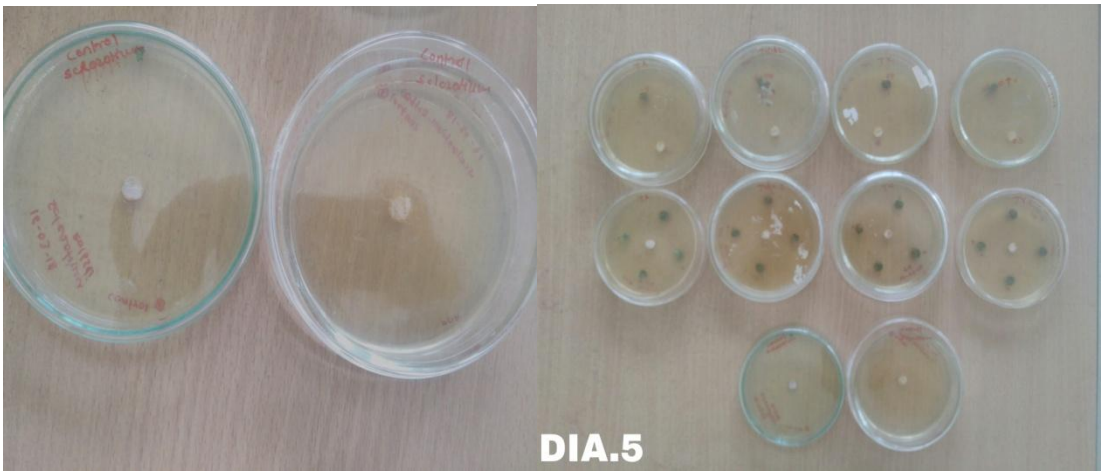
Fig.1 Growth inhibition of *S.rolfsii* due to interactions with different *Trichoderma* species. Vertical bars show standard errors of means of four replicates



Control *Sclerotium rolfsii*

Antagonsastic effect of all 4.spp of Trichoderma against *Sclerotium rolfsii*





Trichoderma species are mycoparsites Doley and Jite (2012) and are commonly used as antimicrobial agents. Several *Trichoderma* species are antagonistic to other plant pathogenic fungi Bosah *et al.,* (2010). The competition, antibiosis, myco-parasitism, induce resistance and inactivation of pathogens compound such as enzymes are some of their different ways of parasitism to other plant pathogenic fungi. The antimicrobial activity of *Trichoderma* against some plant pathogenic fungi Mishra *et al.,* (2011) has been suggested as one of the significance procedure.

Previously different *Trichoderma* species have been recorded to produce a variety of antibiotics such as Trichodermin, Trichodermol, Harizianum A, and Harizianolide Mishra (2010) which may help in decreasing ill effects of dangerous pathogens. Many scientists revelead anagonistic activity of *Trichoderma* species against many phaytopathogen (Howell (2002), mishra (2010), bosah *et al.,* (2010), mishra *et al.,* (2011), Doley and Jite (2012)) besides, dual culture techniques in widely used in antagonsitc studies by different scientist Khattabi *et al.,* (2004) and Rekha *et al.,* (2012). In dual culture technique a clear zone of inhibition and antagonist. The degree of inhibition varied from one species to another. The isolates of different *Trichoderma* species to management *S.rolfsii* have been revealed to vary in their potential Omar and Maha (2010). *Trichoderma harzianum* exhibited 100% 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 non-volatile metabolites as well as production of extracelluar 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 disease caused by fungi Rekha *et al.,* (2012) studied that culture filtrates of *T. harzianum* restrict formation of zoospore of germ tube is mycelial growth of *S. rolfsii*. In another studies, culture filtrate of *T. viride* inhibited the mycellal growth of *Sclerotinia sclerotiorum* due to production of antibiotic like substance Kapil and Kapoor (2005), Kartikeyan *et al.,* (2006) who confirmed culture filterates of *T. viridi* suppressing the development of the pathogen growth and sclerotial germination.

T. viride restricted the development of sclerotial germination parasitized mycelium

of *S.rolfsii* in dual culture assays noticed by Kapoor (2008) chitinase of *Trichoderma* spp. deteriorates the chitin in the cell walls of *S.rolfsii* helps in penetration of *S.rolfsii* mycelium (Doley and Jite, 2012).

In recent studies toxic 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 pathogen related to seeds and soil Mukherjee and Tripathi (2000) yaqub and shazad (2005), barakat *et al.*, (2006), Mishra *et al.*, (2011). Currently the facts regarding the antagonistic potential of *T. harzanium* and *T. viridi* species had significant potential and effective antagonism against *S.rolfsii*.

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