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IN VITRO SCREENING OF *TRICHODERMA* SPECIES AGAINST *MACROPHOMINA PHASEOLINA* AND *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*

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A B S T R A C T

In vitro bioassays were carried out to evaluate antagonistic behavior of seven species of *Trichoderma* namely *T. pseudokoningii*, *T. harzianum, T. reesei, T. koningii, T. hanatus, T. viridi* and *T. aureoviridi,* against two highly problematic soil-borne plant pathogenic fungi viz. *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hansn and *Macrophomina phaseolina* (Tassi) Goid*.* All the *Trichoderma* species exhibited pronounced antagonistic behavior against the target fungal pathogens in dual cultures resulting in 45–65% and 59–74% reduction in radial growth of *M. phaseolina* and *F. oxysporum* f. sp. *lycopersici*, respectively*. T. harzianum* was found to be the most effective biocontrol agent against both the fungal pathogens followed by *T. aureoviridi* and *T. hanatus*.

Keywords: Biological control, *Fusarium oxysporum* f. sp. *lycopersici, Macrophomina phaseolina, Trichodema*.

INTRODUCTION

Soil-borne fungal phytopathogens are causal agents of many diseases of economic importance such as root rot, seedling damping-off and vascular wilts (Lichtenzveig *et al*., 2006). These pathogens are often challenging due to their several years survival in soil in the form of scleroses, chlamydospores, or others (Agrios 1997). In this regard, *Macrophomina phaseolina*, is anamorphic ascomycete soil- and seed-borne notorious pathogen that causes diseases in more than 500 plant species in the tropics and subtropics (Khan *et al*., 2007; Rayatpanah *et al*., 2012; Ijaz *et al*., 2013). The fungus is characterized by the production of both pycnidia and sclerotia in host tissues and in soil. This fungus infects plants at almost all growth stages and symptoms range from leaf yellowing to plant death due to obstruction of xylem vessels (Khan *et al*., 2007). Presence of the pathogen in seed poses a serious risk to some overseas sprouting seed markets because disease may cause up to 100% yield losses (Iqbal *et al*., 2010). Another important highly prevalent and destructive soil-borne ascomycetous fungus is *Fusarium oxysporum* f. sp. *lycopersici* (Sudhamoy *et al.,* 2009). It causes wilt disease both in green house and field grown tomatoes thus responsible for significant losses in tomato production globally in warmer areas (Nusret and Steven, 2004). Seedlings infected by the wilt fungus show yellowing of the lower leaves, often only on one side of the plant succeeded by reduced growth and eventually death of entire plant (Kirankumar *et al.,* 2008).

With the purpose of reducing the economic losses caused by soil-borne diseases, generally utilization of chemical fungicides is considered as easy and attractive approach for the farmers. Due to their relatively low cost, ease of use, and effectiveness, fungicides have become the primary means to combat fungal diseases (Vinale *et al*., 2008; Sharma, 2011; Dias, 2012). However, intensified utilization of fungicides has resulted in harmful effect on non-target organisms, the development of resistance races of the pathogens, and the possible carcinogenicity (Vinale *et al*., 2008; Doley and Jite, 2012). Antagonistic microbes are an attractive, environmental friendly and economic alternatives option over chemical fungicides (El-Bramawy and El-Sarag, 2012). Amongst the range of antagonists, species of *Trichoderma* of phylum Ascomycota are most frequently isolated soil fungi that exist in plant root ecosystem (Harman *et al*., 2004). These fungi are opportunistic, avirulent plant symbionts with

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considerable antifungal activity protecting plants against notorious soil-borne pathogens e.g. *Fusarium*, *Phytophthora*, *Rhizoctonia*, *Botrytis*, *Sclerotinia* and *Pythium* (Benítez *et al*., 2004; Vinale *et al*., 2008). There are about 89 species of *Trichoderma* and more than 50% have been explored for their antifungal activity during the last 70 years (Hjeljord and Tronsmo, 1998). Three well-known mechanism i.e. competition for nutrients, antibiosis, and myco-parasitism generally have been documented for *Trichoderma* to act against fungal pathogens (Gajera *et al*., 2012). Depending on species, use of *Trichoderma* can provide several advantages in agriculture. So far, some species of *Trichderma* have been reported to enhance root system and increase in biological nitrogen fixation besides controlling soil-borne pathogens (John *et al*., 2010). Many *Trichoderma* species are regarded as growth promoter of plants by increasing fresh weight, height and flowering in plants while potentially inhibiting pathogen growth (Sharma, 2011). Hence, *Trichoderma* spp. are extensively exploiting and seeking attention of scientists from all over the world, and are also being commercially marketed as biopesticides, biofertilizers and soil amendments (Harman *et al*., 2004; Vinale *et al*., 2008; Sharma, 2011; El-Bramawy and El-Sarag, 2012). The current study was conducted *in vitro* to assess the antagonistic activity of seven species of *Trichoderma* viz., *T. pseudokoningii*, *T. harzianum, T. reesei, T. koningii, T. hanatus, T. viridi* and *T. aureoviridi* against *F. oxysporum* f. sp. *lycopersici* and *M. phaseolina.*

MATERIALS AND METHODS

Pure cultures of target fungi viz. *M. phaseolina* and *F. oxysporum* f. sp. *lycopersici* were procured from Biofertilizers and Biopesticides Laboratory of Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. Seven species of *Trichoderma* namely *T. pseudokoningii*, *T. harzianum, T. reesei, T. koningii, T. hanatus, T. viridi* and *T. aureoviridi* were obtained from First Fungal Culture Bank of Pakistan, University of the Punjab Lahore, Pakistan. Sub-culturing of the fungi was done on 2% malt extract agar medium and cultures were stored at 4 °C.

Antifungal activity of all the species of *Trichoderma* against the target fungal pathogens were assessed *in vitro* conditions following the dual culture technique of Rini and Sulochana (2007). Petri plates filled with malt extract agar medium amended with streptomycin were inoculated with plugs (2 mm) of *F. oxysporum* f. sp. *lycopersici* and each of the seven species of *Trichoderma* (*T. pseudokoningii*, *T. koningii, T. hanatus, T. viridi, T. aureoviridi, T. harzianum* and *T. ressei*) were placed in Petri plates with a distance of 2 cm between the plugs. Similarly, another set was arranged to study dual culture interactions for *Trichoderma* and *M. phaseolina*. In control treatments, only the pathogenic fungal species were inoculated. Petri plates were incubated at 27±2 °C for 5 days. Five replicates of each treatment were made and increase in the diameter of both fungi was recorded after 5 days of incubation. The colony diameter in each plate was measured at three places and average was calculated.

Percentage inhibition in the radial growth of *F. oxysporum* f. sp. *lycopersici* and *M. phaseolina* by different *Trichoderma* species was calculated as follows:

$$\text{Growth inhibition\%} = \frac{\text{C} - \text{T}}{\text{C}} \times 100$$

Where;

C = Radial growth of pathogen in control plates (mm)

T = Radial growth of pathogen in dual culture (mm)

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

RESULTS

Data presented in Figure 1 indicates that all the seven species of *Trichoderma* tested *in vitro* were effective in suppressing the growth of *F. oxysporum* f. sp. *lycopersici*. Among the seven *Trichoderma* species, maximum inhibitory effect of 74% was recorded due to *T. harzianum* against radial growth of the fungus after five days of inoculation. *T. aureoviridi* was the second best antagonistic fungus resulting in 70% inhibition in growth of the target fungal pathogen. Other *Trichoderma* spp. namely *T. hanatus*, *T. viridi, T. ressei*, *T. koninjii* resulted in 67%, 65%, 60% and 60% inhibition in growth of the target fungal pathogen. Least inhibition of 59% in growth of the fungus was recorded due to *T. pseudokoninjii.*

Data illustrated in Figure 2 demonstrates that *T. harzianum* showed the highest inhibition (65%) in growth of *M. phaseolina* followed by *T. aureoviridi* (60%). *T. ressei, T. viridi* and *T. pseudokoninjii* also showed pronounced antagonistic effect resulting in 55% suppression in growth of the target fungal pathogen. Likewise, *T. koninjii* reduced fungal growth by 48%. *T. hanatus* showed the least antagonistic behaviour showing only 45% reduction in growth of the pathogen fungal species.

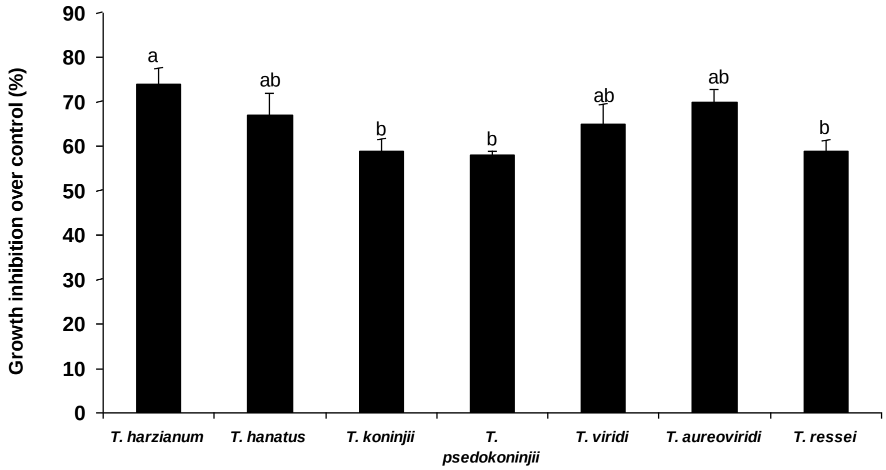

Figure 1. Growth inhibition of *Fusarium oxysporum* f. sp. *lycopersici* due to interactions with different *Trichoderma* species. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

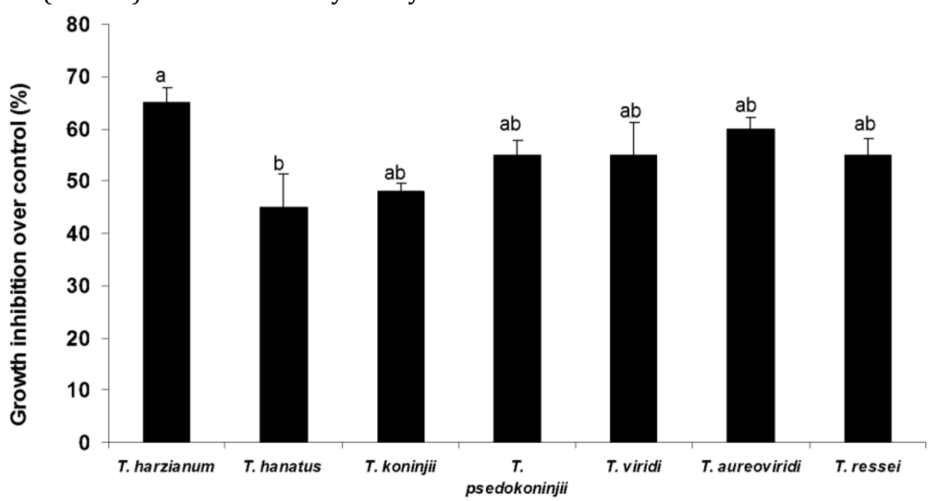

Figure 2. Growth inhibition of *M. phaseolina* due to interactions with different *Trichoderma* species. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

DISCUSSION

All the *Trichoderma* species exhibited considerable antagonistic potential against the *M. phaseolina* and *F. oxysporum* f. sp. *lycopersici* in dual cultures. Presently reported antagonistic activity of *Trichoderma* was in line with many previous findings against *M. phaseolina* (Aly *et al.,* 2007; Doley and Jite, 2012; Gajera *et al*., 2012) and *F. oxysporum* f. sp. *lycopersici* (Ramezani, 2010; Sharma, 2011; Devi *et al*., 2013). The occurrence of inhibition zones in dual cultures between pathogenic fungi and *Trichoderma* spp. suggested secretion of diffusible non-volatile and volatile inhibitory metabolites e.g. harzianic acids, tricholin, viridian, glisoprenins, massoilactone gliovir and heptelidic acid etc. in the growth medium that probably hinder the pathogen colonization (Rini and Sulochana, 2007). Comparatively much faster growth of *Trichoderma* spp. than the pathogenic fungi might be indication of competition efficiencies of antagonists for space and nutrients (Devi *et al*., 2013). During competition, *Trichoderma* may display diverse methods of parasitism against phytopathogenic fungi including competition, mycoparasitism, antibiosis, induced resistance and inactivation of pathogen's enzyme. The direct mycoparasitic activity of *Trichoderma* species has been proposed as one of the major mechanism for their antagonistic activity against phytopathogenic fungi (Doley and Jite, 2012). Therefore, it seems that *Trichoderma* parasitizes *M. phaseolina* and *F. oxysporum* f. sp. *lycopersici* by growing towards and coiling about the hyphae of pathogenic fungi and degrade their cell walls components glucan, proteins and chitin by the secretion of mostly glucanases, proteases and chitinases (Almeida *et al.*, 2006).

Overall, considerable variations in the inhibitory properties of different species of *Trichoderma* species were discernible. *T. harzianum* was found to be the most effective biocontrol agent against both the fungal pathogens followed by *T. aureoviridi* and *T. hanatus*. Ramezani (2001) also documented that *T. viride* and *T. harzianum* as potential inhibitor of *M. phaseolina* as compared to *T. hamatum*. Sharma (2011) documented that *T. atroviride* and *T. harzianum* as best antagonist against *F. oxysporum* f. sp. *pisi*. Gajera *et al*. (2012) showed *T. koningi* and *T. harzianum* have a better growth inhibition of *M. phaseolina* compared to *T. viride*. Meraj-ul-Haque and Nandkar (2012) found *T. virens* as strongest inhibitor of the growth of *F. oxysporum* f. sp. *lycopersic* amongst the *T. harzianum*, *T. hamatum*, *T. longibrachiatum*, *T. atroviride*, *T. viride*, *T. ressei* and *T. virens*.Such variations in the inhibitory potential of *Trichderma* species could be attributed to the morphological and genetic variability that possibly resulted in quantitative and qualitative variation in production of inhibitory substances (volatile and non-volatile) with varying degree of mycoparasitism (Rini and Sulochana, 2007).

The inhibitory action of *Trichoderma* species were more pronounced against *F. oxysporum* f. sp. *lycopersici* as compared to *M. phaseolina.* It might be due to varying type of cell well composition, defense system and enzymatic activity of each pathogenic fungus, suggesting their differential reaction against antagonist action. The present study concludes that *T. harzianum* is the best biocontrol agent against both the test fungal pathogens followed by *T. aureoviridi* and *T. hanatus*.

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