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BIOLOGICAL POTENTIAL OF TRICHODERMA SPECIES IN THE CONTROL OF SOME PHYTOPATHOGENIC FUNGI

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A B S T R A C T

Trichoderma are soilborne, green-spore ascomycetes that can be found all over the world. They are well known for their antagonistic ability towards the control of plant pathogenic fungi. Current research reveals the potential of Trichoderma species against fiveplant pathogenic fungi viz.,*Ceratocystis fimbriata,Colletotrichum gleosporiodes,Alternaria solani, Fusarium solani* and *Verticillium* spp*Trichoderma* species viz., *Trichoderma harzianum,Trichoderma viridae* and *Trichoderma harzianum* (AT strain) were evaluated under laboratory conditions for their antagonistic ability towards test fungi.All the Trichoderma isolates had marked statistical inhibitory effect on mycelial growth of test fungi over control. Among these*T. harzianum* (AT) gave better results showing 85.19% radial growth inhibition of *C. fimbriata*with the mean radial growth of 1.79 cm. followed by 83.09, 79.69, 76.66 and 73.66 percent growth inhibition of *F. solani, A. solani, C. gleosporioides* and *Verticillium* spp respectively. Topsin-M was used as positive control that gave maximum growth reduction of all tested fungal species over negative control. Under glasshouse conditions *T. harzianum* (AT) when tested against *C. fimbriata*also gave good results by showing reduced colonization (52.55%) of pathogen in the soil. For the management of mango sudden death syndrome (MSDS) affected plants in the field,*T. harzianum* (AT) showed significant response for the control of the disease among all the treatments. These investigations constitute a strong base in future for use of Trichoderma species as a potential biological agent for the management of various diseases of plants.

Keywords: Trichoderma spp**,** biological potential,fungal pathogens.

INTRODUCTION

The genus, Trichoderma is a common soilborne, green-spored ascomycetes (imperfect fungi) that can be found all over the world. Trichoderma being associated with roots, soil, debris has been studied with respect to various characteristics and applications and are known as successful colonizers, efficiently fighting their competitors (Howell *et al.,* 2003). Once established, Trichoderma introducestrong degradative mechanism for decomposition of the assorted substrate. Trichoderma are one of the best studied fungi because the efficient biocontrol strains of the genus are being developed as promising biological fungicides due to presence of secondary metabolites with potential applications as novel antibiotics (Howell et al., 2003; Ha, 2010). The rapid growth of Trichoderma has an advantage to compete with plant pathogens for the space and nutrients. This mycoparasitic ability of various species of Trichoderma against large number of economically important plant pathogens have been studied by many scientists (Freeman et al., 2004; Kratzer et al. 2006; Dubey *et al.*, 2007; Hajieghrari *et al.*, 2008). Strains of *Trichoderma* can produce antifungal metabolites which check the growth of various fungi, act as competitors and promote plant growth. Volatile compounds released by these bioagents have also been found effective against different pathogens (Hatvani e*t al.*, 2007). Kumar and Dubey (2012) reported that

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Trichoderma harzianum showed superiority over other isolates in inhibiting mycelial growth of *Fusarium solani* f. sp. *pisi* causing collar rot of pea through the production of volatile and non-volatile antibiotics. *T. harizanum, T. viridae, T. virens, T. hamatum, T. roseum and T. koningii* etc are the species that most often used for biological control of pathogens. Currently most of these species are available in the markets of Europe and Asia as commercial products.

In this study, in vitro biological potential of Trichoderma species were evaluated against some important plant pathogenic fungi including *Ceratocystis fimbriata, Colletotrichum gleosporiodes, Alternaria solani, Fusarium solani* and Verticillium spp. Further, highly effective strain and most potential fungus was selected for bio-control studies in green house and field. The objective of this study was to evaluate Trichoderma species as potential bio-control agents to reduce the impact of the disease.

MATERIALS AND METHODS

Experimental Area: The study regarding biological potential of *Trichoderma* isolates against various plant pathogenic fungi was carried out at the Department of Plant Pathology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan.

Fungal Pathogens and Trichoderma Species: Fungal pathogens viz; *Ceratocystis fimbriata, Colletotrichum gleosporiodes, Alternaria solani, Fusarium solani* and Verticillium spp., and Trichoderma isolates were procured from Fungal Culture bank of "Fungal Ecology and Bio-control Laboratory" in Department of Plant Pathology, Faculty of Agricultural Science and Technology, Bahauddin Zakariya University, Multan. Pakistan. The isolates were maintained on potato dextrose agar medium at 4_{o}C for further use.

In vitro studies

Dual culture technique: Three Trichoderma species *viz*., *Trichoderma harzianum*, *Trichoderma viridae* and *Trichoderma- strainAT* were evaluated under *in vitro* conditions for their antagonistic potential against *Colletotrichum gleosporiodes*, *Ceratocystis fimbriata*, *Verticillium* spp, *Fusarium solani* and *Alternaria solani*, through dual culture technique (Morton and Strouble, 1955; Skidmore and Dickinson, 1976). The sterilized potato dextrose agar medium was poured in to Petri plates (9cm) and allowed to solidify. After solidification, mycelial discs of 0.4 cm diameter from the margins of 7 days-old *Trichoderma* spp. cultures and test fungus were separately placed approximately 5 cm apart at equal distance from the periphery. The experiment was conducted with completely randomized design having four Petri dishes for each treatment. In control plates (without *Trichoderma*) the discs of all the test fungal cultures including *Colletotrichum gleosporiodes*, *Ceratocystis fimbriata*, *Verticillium* spp, *Fusarium* solani and *Alternaria solani,* were placed alone in the center. Topsin-M was used as positive control. After inoculation, the plates were incubated at 26 ± 2 °C and colony growth of both bio-control agents and pathogens were recorded daily up to five days. Percent inhibition of average radial growth was calculated using formula given by Edington *et al.,* (1971) and compared with negative control.

$$L = \frac{(C - T)}{C} \times 100$$

L = Inhibition of radial mycelial growth.

C = Pathogen radial growth measurement in control.

T = Pathogen radial growth in the presence of *Trichoderma* spp.

Glass house studies: Glass house experiment was conducted specifically to observe the potential of selected *Trichoderma* isolates that gave statistically best response under*in-vitro* conditions against best inhibited fungus out of all tested fungi. Fungus *Ceratocystis fimbriata* was selected as test organism. For green house experiment five mango plants of 2 years oldwere collected from local nursery of Multan and planted in the earthen pots (9x9.5x11"). Inoculum of *Ceratocystis fimbriata* was applied to mango seedlings @ $2x10_{6}$ spores per ml and plants were covered for 24 hrs with polythene bags to create humidity. After removal of polythene bags spore suspension of biocontrol agent-*T. harzianum* strain AT was inter-mixed in soils present in pots. The untreated earthen pot was kept as control. Disease severity data egarding the Mango sudden death syndrome was calculated using the scale (0-7) and formula given by (Masood *et al*., 2010).

$$\text{Disease severity} = \frac{\text{Infected tissue area}}{\text{Total tissue area}} \times 100$$

Field Studies: For the management of naturally infected mango plants with MSDS, the experiment was laid out on diseased plants showing typical symptoms of MSDS in the experimental orchard with following treatments (Table 2). Two fungicides *viz*., Topsin-M and Score were selected for application in the field. The experiment was conducted in complete randomized

block design with three replications having total number nine plants in the experiment.20 years old plants of Chaunsa were selected for this experiment. The orchard soil type was sandy loam. The chemical fertilizer N.P.K (17:17:17) was applied 5kg/plant. Pruning and fungicide spray were kept as a constant factor in all treatments. Pruning of dried and diseased branches was done followed by three consecutive sprays of Topsin- M @ 2g/liter and Score @ 2.5g/liter of water. Soil amendment was done with the removal of soil under the tree canopy up-to 9" depth and then refilling with canal silt, recommended dose of N.P.K and Farmyard Manure was added at the time of treatment (Malik *et al.,* 2001).

Table 1. Layout plan for the field experiment

Treatments	Practices
T_1	Soil amendment with *T.harzianum* strain-AT+ Foliar spray of Topsin-M (Thiophanate methyl)
T_2	Soil amendment with *T.harzianum* strain-AT + Foliar spray of Difenoconazole (Score)
T_3	Soil amendment with application of Difenoconazole + Foliar spray of Topsin-M
T_4	Pruning + Foliar spray of Topsin-M (Thiophanate methyl)
T_5	Control

The data on the basis of decrease percentage in disease over control were calculated taking defoliation, drying of branches, gummosis and bark splitting as main symptoms of die back. Severity of each symptoms expression was recorded with rating scale (Malik *et al.,* 2005) as: 0 = no symptom, 1 = light symptom, 2 = moderate symptom, 3 = severe symptoms and then disease severity was calculated with the formula mentioned earlier.

STATISTICAL ANALYSIS

The collected data was statistically analyzed using Fischer's ANOVA (Analysis of Variance) by employing least significant test, treatment means compared at 0.05 probability level through SAS 8.1.

RESULTS

Trichoderma species *viz*., *Trichoderma harzianum* ,*Trichoderma viridae* and *Trichoderma harzianum* (AT) evaluated under laboratory conditions for their antagonistic ability towards five fungal phyto-pathogens. Among the three tested Trichoderma isolates and five fungal pathogens, *T. harzianum* (strain-AT) gave best results against *C. fimbriata* showing 85.19% radial growth inhibition of pathogen with the mean radial growth of 1.79 cm in three replicates. Similarly, in the case of *F.solani* , *Trichoderma harzianum* (AT) also gave better results showing 83.09% radial growth inhibition of pathogen with the mean radial growth of 2.49 cm. Likewise towards A. solani (79.69%) followed by *C. gleosporioides* and Verticillium spp (76.66% and 73.66%) over negative control. (Table 2). Other Trichoderma isolates also significantly reduced mycelial growth of all tested fungi.

Table 2. Percent inhibition and mean mycellial growth of *Trichoderma* spp. against various plant pathogenic fungi

Sr. No.	Treatments	*Colletotrichum gleosporiodes*		*Verticillium* spp.		*Alternaria solani*		*Ceratocystis fimbriata*		*Fusarium solani*	
		Mean radial growth	Percent inhibition	Mean radial growth	Percent inhibition	Mean radial growth	Percent inhibition	Mean radial growth	Percent inhibition	Mean radial growth	Percent inhibition
1.	T1	2.66 ± 1.01	71.34 ± 2.12	3.01 ± 1.01	68.34 ± 2.12	2.99 ± 2.01	74.41 ± 2.12	2.11 ± 1.31	81.03 ± 1.12	3.01 ± 0.33	74.66 ± 0.11
2.	T2	3.33 ± 0.56	59.89 ± 1.99	3.43 ± 0.56	57.01 ± 1.99	3.73 ± 1.56	61.22 ± 1.99	2.77 ± 2.16	77.13 ± 2.99	3.99 ± 1.17	71.07 ± 2.99
3.	T3	2.01 ± 1.67	76.66 ± 1.78	2.66 ± 1.67	73.66 ± 1.78	2.16 ± 1.33	79.69 ± 1.78	1.79 ± 1.66	85.19 ± 3.48	2.49 ± 2.06	83.09 ± 3.48
4.	T4	1.99 ± 0.89	76.66 ± 1.78	2.32 ± 0.89	76.66 ± 1.78	1.32 ± 0.89	88.66 ± 1.78	1.23 ± 1.89	91.33 ± 2.18	2.13 ± 2.99	81.03 ± 1.18
5.	T5	8.88 ± 0.45	---	7.99 ± 0.45	---	8.23 ± 0.99	---	7.23 ± 1.39	---	8.13 ± 0.39	---

T1= *Trichoderma harzianum*, T2, *Trichoderma viridae*, T3, *Trichoderma harzianum* (AT), T4= Control positive (Topsin-M), T5= Control Untreated(Respective fungi)

Trichoderma isolate *viz*., *T.harzianum* (strain-AT) was selected on the basis of its efficient response in laboratory conditions against all the evaluated mycoflora for their antagonistic ability. *Trichoderma harzianum* (AT) gave good results in glass house also by showing reduced colonization (52.55%) of pathogen (*C. fimbriata*) in the soil and reasonable control of the disease under glass house conditions (Table 3).

Table 3. Response of mango plants towards biological mediated management through *Trichoderma harzianum*strain-AT in glass house

Sr. No.	Treatment	Disease severity (%)	Decrease reduction (%)
T_1	*Ceratocystis fimbriata* + *T. harzianum* strain AT	41.3331.33	36.9016.33
T_2	*Ceratocystis fimbriata* + *T. harzianum* strain AT	43.33	34.3721.55
T_3	*Ceratocystis fimbriata* + *T. harzianum*strain-AT	31.3341.66	52.5522.66
T_4	*Ceratocystis fimbriata* + Topsin-M	23.33	64.6633.22
Plant T_5	*Ceratocystis fimbriata* (Untreated Control)	66.03	---

In field experiment from all the four treatments T_1 gave the significant response for the control of the disease among all the treatments. Application of T_1 gave the best response with the 59.41 suppression of diseases severity and 19.65 percent decrease over control followed by the T_2 with 61.09 suppression of disease severity and 16.59 percent decrease over control (Table4).

Table 4. Effect of various treatments on the disease intensity of MSDS affected plants in field

Sr. No.	Treatments	Disease severity(%)	Decrease reduction (%)
T1	*Soil amendment + Foliar spray of Topsin M (Thiophanate methyl)	59.41	19.65
T2	Soil amendment + Foliar spray of Difenoconazole (Score)	61.09	16.56
T3	Soil amendment with application of Difenoconazole + Foliar spray of Topsin M	65.07	12.56
T4	Pruning + Foliar spray of Topsin M (Thiophanate methyl)	35.22	59.99
T5	Control	88.11	---

* Soil amendment with *Trichoderma harzianum*strain-AT

DISCUSSION

Plant pathogenic fungi is a widespread problem of economically important crops and use of synthetic chemicals over these crops is rejected by the population. No doubt, chemicals are giving complete remedy of pest and disease problems of plants but their indiscriminate use has rendered them unfit for both environment and health of humans and animals. Therefore, according to this study, biological control could be the best alternative. Trichoderma spp are common antagonistic fungi present in soil and rhizosphere of almost many plants. In present investigations all the isolates of antagonists in dual culture inhibited the mycelial growth of the test fungal pathogens. It was considered that the mechanism of inhibition may be competition for food and space, production of antibiotics and myco-parasitism. Trichoderma spp have been investigated for their potential bio-control agents because of their ability to reduce the incidence of disease caused by plant pathogenic fungi, particularly many common soil borne fungi (Ashrafizadeh *et al.,* 2005; Dubey et al., 2007; Hajieghrari *et al.,* 2008). The plant pathogenic mycoflora associated with mango, tomato and cotton crop diseases viz., *Colletotrichum gleosporiodes*, *Ceratocystis fimbriata*, *Alternaria solani, Verticillium* spp, and *Fusarium solani* were evaluated through antagonist, Trichoderma spp. in present study. Kumar and Dubby (2001) and Dubey *et al.* (2007) also found *T. harzianum* as the best antagonist for growth inhibition of *Fusarium oxysporum* f.sp. *pisi* and *F. oxysporum* f.sp. *ciceris*. Harman (1996) reported that Trichoderma sp. had a marked statistical inhibitory effect on the mycelial growth of F. graminearum by releasing volatile compound in dual culture. The growth inhibition of pathogen may be due to hyper-parasitism, antibiosis (Whipps and Lumsden, 2001) or production of chitinase and B-1,3-glucanase enzymes which degrade the cell wall of the pathogens (Behzad *et al.,*2008). Trichoderma generate many enzymes that are used against cell walls of fungi to utilize the fragment of pathogens. Trichoderma has attracted a substantial scientific attention as an intoxicating fungal bio-control agent against a wide range of plant pathogens (Obalu and Oti, 2007; Rajendiran *et al.,* 2010). Trichoderma spp. are well-known as biological myco-parasites, which are commercially used as bio-control against a variety of plant-pathogenic fungi such as Pythium, Fusarium and Rhizoctonia strains and also a product of ecological significance. (Meyer *et al.,* 1973). *Trichoderma harzianum,* and *Trichoderma viridae* are dynamic rhizosphere colonizers and can produce antibiotics like viridin, gliotoxin, cell wall degrading enzymes (Hajieghrari *et al.,* 2008). They can produce biologically

active metabolites and are involved in suppression of a particular disease and/or growth promotion of plant. Mango sudden death disease is an important disease of mango fruit crop. The infectious fungus *C. fimbriata* was isolated from leaves and twigs/branches, and isolates of Trichoderma spp were evaluated for its control. The results were in line with work ofJoshi *et al*., (2010) who evaluated 62 isolates of Trichoderma spp. from samples of different rhizospheric soils collected from various places located in Himalayas Western region and reported about 80% mycelial growth inhibition against *Rhizoctonia solani*, soil-borne pathogen. Khanzada *et al.* (2004) screened some fungal and bacterial bioagents in addition to actinomycetes isolate isolated from samples of soil rhizosphere of potato healthy plants for their aggressive potential against two plant pathogens, *Trichoderma harzianum* and Epicoccum spp. and suggested that biological control through *T. harzianum* and Epicoccum spp. expressed a good control of fungal pathogens.

CONCLUSION

Current research depicts that biological control a very promising alternative to chemical products because it can decrease environmental pollution as a consequence of using fungicides for controlling different plant diseases.

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