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Trichoderma and Arbuscular Mycorrhizal Fungi Based Biocontrol of *Fusarium udum* Butler and Their Growth Promotion Effects on Pigeon Pea

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ABSTRACT

The aim of present study was to investigate the effect of individual and co-inoculation of *Trichoderma* species and arbuscular mycorrhizal fungi (AMF) on growth, mycorrhization, population of *Trichoderma,* and wilt disease severity in pigeon pea (*Cajanus cajan* L Millsp). Three species of *Trichoderma,* namely, *T. harzianum* (Th), *T. virens* (Tr), and *T. viride* (Tv) and consortium of AMF (Myc; mixture of *Funneliformis mosseae*, *Glomus cerebriforme*, *Rhizophagus irregularis*) individually (Th, Tr, Tv and Myc) and in different combinations (Th+Myc, Tr+Myc and Tv+Myc) were tested. Among all the treatments, co-inoculation of Th and Myc gave highest growth and reduced severity of wilt disease of pigeon pea significantly (P< 0.05). Myc alone was sufficient for growth promotion but it was effective in terms of disease suppression when inoculated before pathogen. *Fusarium* reduced the shoot length, dry weight, phosphorus (P) uptake of plants, AMF colonization, spore density, and population of *Trichoderma*. Results clearly showed that different species of *Trichoderma* produced varied results with Myc.

Keywords: Colony forming unit, Hyperparasitism, Phytoparasitism.

INTRODUCTION

Fusarium species are common population of soil. *Fusarium* survives in plant roots, soil, and dead plant debris for several years (Saremi and Burgess, 2000). It infects through roots and penetrates into the vascular system of plants, causing wilt and substantially reducing yields. *Fusarium udum* (Fu) Butler is a wilt causing agent of pigeon pea (*Cajanus cajan* L. Millsp.). Globally, pigeon pea is cultivated on 4.64 M ha, with an annual production of 3.43 million tons (http://faostat.fao.org). The loss due to wilt disease in pigeon pea was estimated to be approximately 97,000 tons per year (Saxena *et al.,* 2010). In order to keep pace with the food requirement of ever increasing population, world is facing a constant pressure on crop production from available cultivable land. To achieve this, intensive agricultural practices incorporating the uses of chemical pesticides have increased. Such increase in the use of pesticides helps in disease reduction, but their injudicious applications adversely affects the natural balance of soil ecosystems, pollutes soil and favors the development of resistance in pathogens (Vosátka and Albrechtova, 2009).

Disease management with biocontrol agents offers a great promise (Prashar *et al.,* 2013, Singh *et al.,* 2009). These agents are vital component of sustainable agriculture (Xu *et al*., 2011), which colonize the rhizosphere (the site requiring protection) and leave no toxic residues as opposed to chemicals (Dubey *et al*., 2007). *Trichoderma* and AMF are most promising biocontrol

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agents (Amer and Abou-El-Seoud II, 2008). *Trichoderma* species are useful avirulent saprophytes that act as biocontrol agents against phytopathogenic fungi by various mechanisms such as rhizosphere competition, mycoparasitism, antibiotic and enzyme production, and induced resistance. Growth promotion activity of *Trichoderma* has also been reported (Cumagun, 2012, Harman *et al*., 2004). Arbuscular MF-mediated bioprotection has been exploited and accepted as a key practice for disease control (Garcia-Garrido, 2009). According to Linderman (2000), induced systemic resistance (ISR) is primary phenomenon which involves phytoprotection by AMF. The mechanism of ISR by AM fungi focuses on nutritional effects, competition for infection sites, morphological changes in roots and root tissues, changes in chemical constituents in plant tissue, and microbial changes in mycorrhizosphere (Hause and Fester, 2005).

Interaction between *Trichoderma* and AMF has been studied to stimulate or inhibit the population/colonization of each species (Brimner and Boland, 2003). However, fewer reports are available on the development of microbial consortium involving *Trichoderma* and AMF. Information regarding mutually interactive species of *Trichoderma* and AMF might be beneficial for development of successful biocontrol strategy which will overcome harmful effects associated with the use of chemical pesticides and fertilizers. Thus, the purpose of the present study was to develop a biocontrol strategy by studying the interaction among wilt causal pathogen ***F. udum***, *Trichoderma,* and AMF isolated from rhizosphere of *C. cajan* on growth promotion and wilt disease reduction of the same.

MATERIALS AND METHODS

Plant Material

Seeds of *C. cajan* (cv. Bahar) procured from National Research Centre for Agroforestry, Jhansi, were surface sterilized in 0.1% sodium hypochlorite for two minutes, washed several times in distilled water, and germinated on sterilized sand.

Isolation and Identification of Fungi

Infected roots and rhizosphere soil samples were collected from diseased as well as healthy pigeon pea plans growing on selected study sites. Roots were washed with tap water to remove the soil particles and cut into small pieces (1 cm) and surface sterilized in 0.2% mercuric chloride solution for 1 minute and washed several times with distilled water. Pieces of roots were placed on filter paper to remove excess water; and then on potato dextrose agar (PDA), to isolate *F. udum.* The plates were incubated at 28$\pm$1^{o}C for seven days, and the pathogen was purified by hyphal tip culture technique. ***F. udum*** isolates were identified according to Rai and Upadhyay (1982). To isolate *Tricoderma* species, different dilutions (10^{-3} to 10^{-5}) of soil samples were spread on plates containing *Trichoderma* selective agar medium (TSM; Elad *et al.,* 1981). The composition of TSM was as follows: 3 g glucose, 1 g NH_4NO_3, 0.9 g $Na_2H_2P0_4$, 0.2 g $MgS0_4.7H_20$, 0.15 g KC1, 20 mg $FeS0_4.7H_20$, 20 mg $ZnS0_4.7H_2O$, 20 mg $MnS0_4.H_20$, 30 µg rose bengal and 1,000 mL distilled water, amended with 50 mg streptomycin sulfate, 50 mg chloramphenicol, 10 mg metalaxyl and 10 mg pentachloronitro benzene (PCNB). The inoculated plates were incubated for five days at 28^{o}C. Colonies of *Trichoderma* were isolated, purified and then maintained on PDA and identified according to Rifai (1969). Species of AMF were isolated from the soil samples according to the Gerdeman and Nicolson (1963) and propagated as pure culture on *Zea mays* L. for five months in sterilized sand. Taxonomic identification of these was matched with the description provided by the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (http://www.invam.caf.wvu.edu/).

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Preparation of Inocula

The purified culture of *F. udum* grown on PDA was multiplied on sand-maize flour medium. Fifteen grams maize flour and 85 g autoclaved sand were mixed thoroughly in 500 mL flasks (100 g flask^{-1}) and autoclaved at 15 Lbs for 30 minutes. Then, each flask was inoculated with pure culture of *F. udum* and incubated at 25±2^{0}C for two weeks. 10 g wheat bran and 90 g sand mixed thoroughly then moistened with 25 mL mineral salt solution (pH: 5 to 5.2) was taken in 500 mL flask and sterilized (121^{0}C and 15 Lbs for 30 minutes) in autoclave. These flasks were inoculated with the mycelial disc (4 mm diameter) of *Trichoderma* and incubated at 28±2^{0}C for 15 days. The colony forming units (CFU) of *F. udum* (1.8×10^3 per gram substrate) and *Trichoderma* (2.3×10^3 per gram substrate) were determined by serial dilution technique before being used. Arbuscular-MF inocula (Myc) comprised of equal parts in weight of three indigenous AM species, namely, *Funneliformis mosseae* (Nicolson and Gerd.), Walker and Schubler, *Glomus cerebriforme* McGee, and *Rhizophagus irregularis* (Blaszk, Wubet, Renker and Buscot). Walker and Schubler was used in both experiments which consisted of sand with chopped root bits, spores (average 82 spores 50 g^{-1} sand) and extramatrical mycelia. The above mentioned AM species were very common and often co-occurring in selected study sites.

Pathogenicity Assay of *F. udum*

The pathogenicity of *F. udum* was tested under greenhouse conditions. The dried soil was sterilized for three successive days, which was inoculated with (5% W/W) of *F. udum* inoculum, three days before seed sowing. Three seeds (pre-germinated) were sown in each pot. Pathogenicity of *F. udum* was confirmed after re-isolating the pathogen from diseased plants showing wilting symptoms. Colonies of *F. udum* were purified in PDA slants and stored at 4^{0}C.

In vitro Antagonistic Activity of *Trichoderma* against *F. udum*

Trichoderma isolates were screened for their antagonistic activities against *F. udum*, using dual culture technique (Dennis and Webster, 1971). The mycelial discs (5 mm) of *Trichoderma* and *F. udum* from a four-days old culture were placed at 3 cm away from each other on either side of freshly prepared PDA plates. Control plates contained only *F. udum* disc in the centre of the plate. There were five replications for the treatments as well as for the control. The plates were then incubated at 28±1^{0}C. The per cent inhibition of *F. udum* was calculated according to the formula: $L= [(C–T)/C]\times100$, where L is the per cent inhibition of radial mycelial growth; C is the radial growth measurement of the pathogen in the control; and T is the radial growth of the pathogen in presence of *Trichoderma*.

Experimental Design

Two experiments were conducted in completely randomized design under green house conditions. Experiment 1 demonstrated the effect of individual and co-inoculation of *Trichoderma* spp and Myc on growth, AMF root colonization, spore density, and population of *Trichoderma*. Experiment 2 demonstrated the effect of different treatments on disease reduction under pre and simultaneous inoculation of *Trichoderma* and Myc with the pathogen. All three inocula i.e. Myc, *Trichoderma T. harzianum*, *T. virens* and *T. viride* and *F. udum* (5 g each) were applied to plastic pots (Size: 24×16 cm), filled with 3 kg potting substrate i.e. mixture of autoclaved soil and sand (1:1; by volume). Inocula were placed in the cavity made in potting substrate, as per the treatments. In co-inoculated pots, Myc, *Trichoderma* and *F. udum* were mixed

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thoroughly and applied. Non-mycorrhizal treatments received the same amount of autoclaved inoculum of Myc. Pre-germinated seedlings of similar sizes were transplanted into pots and watered as required. Half strength Hoagland's solution in de-ionized water was applied at weekly intervals. The composition of the solution was: 0.03 g L^{-1} KH_2PO_4, 0.51 g L^{-1} KNO_3, 0.246 g L^{-1} $Ca(NO_3)_2$, 0.245 g L^{-1} $MgSO_4.7H_2O$, 1.43 g L^{-1} H_3BO_3, 0.91 g L^{-1} $MnCl_2.7H_2O$, 0.11 g L^{-1} $ZnSO_4.5H_2O$, 0.04 g L^{-1} $CuSO_4.5H_2O$ and 0.04 g L^{-1} H_2MoO_4 (Hoagland and Arnon, 1938).

Experiment 1

Effect on Growth, P Uptake, AMF Colonization, Spore Density and *Trichoderma* Population

This experiment had eight treatments as follows: Control, *T. harzianum* (Th), *T. virens* (Tr), *T. viride* (Tv), *Myc, T. harzianum*+Myc (Th+Myc), *T. virens*+Myc (Tr+Myc) and *T. viride*+Myc (Tv+Myc). It was conducted in two groups i.e. with (*F. udum*+) and without (*F. udum*-) *F. udum.* In *F. udum*- (Fu-) group, un-inoculated pots served as the control, whereas in *F. udum*+ (Fu+) group, *F. udum* inoculated pots (individual inoculation) served as the control. All treatments were replicated five times. Thus, a total of 80 pots were employed in this experiment.

Plant Analysis

Seedlings were harvested after six months growth and observations on shoot length (cm) was taken by standard methods. For dry weight (g $plant^{-1}$) estimation, harvested samples were dried in sun and, then, in hot air oven at 70^0C. The dried samples were ground to pass through 1 mm sieve and stored in polyethylene bags (composite of all plant parts) for phosphorus (P) analysis. Phosphorus content of plant was estimated using vanado-molybdo phosphoric yellow color method (Jackson, 1973) and expressed in mg $plant^{-1}$ on the basis of dry weight.

AMF Colonization and Spore Density

AMF and root colonization and spore density (SD, 50 g^{-1} potting mixture) were determined in Myc inoculated pots. Fine roots were cleared with 10% potassium hydroxide and stained with acid fuchsin (0.01% in lacto-glycerol) following the method of Phillips and Hayman (1970). Colonization in cleared root parts was determined with a microscope (Nikon Eclipse 400) at ×100 using the grid line intersect method (Giovannetti and Mosse, 1980). Briefly, 50 g potting mixture was collected from each pot and AMF spores were extracted using wet sieving and decanting method (Gerdemann and Nicolson, 1963). Collected samples were taken in substantial amount of water (1 L) and decanted through a series of sieves (mesh size: 250, 150 and 53 µ). For observation under stereomicroscope (Nikon SMZ 800), the sievings were transferred on to a gridded Petri dish (11 cm). Then, we isolated the AMF spores and calculated the spore density (number of spores in 50 g potting mixture).

Trichoderma Population Density

To determine *Trichoderma* population density (CFU), 1.5 g soil was taken. Sampling was done by removing top layer (10 cm below) of potting mixture with a sterile spoon from three sides (approximately 0.5 g from each side) of the seedling. Collected samples were mixed thoroughly to make a composite sample and 1 g sample was taken for further study. Then, a 10 fold aqueous dilution series (10^{-1} to 10^{-4}) was prepared. One mL from 10^{-3} dilution was poured on Petri dishes containing TSM and incubated (25 ± 2^0C) for

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one week, then, CFU was calculated and expressed as per g of potting mixture.

Experiment 2

Effect on Wilt Disease Severity

In this experiment, eight treatments i.e. Control, *T. harzianum*, *T. virens*, *T. viride,* Myc, *T. harzianum*+Myc*, T. virens*+Myc, and *T. viride*+Myc were employed. This experiment was also carried out in two sets. One set was inoculated with *Trichoderma* and Myc (according to treatments) ten days before inoculation of *F. udum* (prior inoculation), however, in the other set, *Trichoderma* and Myc were inoculated at the time of *F. udum* inoculation (simultaneous inoculation). Five pots per treatments (five replicates) were maintained. Thus, a total of 80 pots (40 per set) were maintained in this experiment. Five plants per pot were maintained, which were harvested after one month. Disease severity index was determined according to Siddiqui and Mahmood (1999) with some modifications using 0-5 point scale; 0= No wilting, 1= Very mild wilting (approximately 10% of leaves showing yellowing), 2= Mild wilting (approximately 25% of leaves showing yellowing), 3= Moderate wilting (50% of leaves showing yellowing as well as shoot stunted or wilted), 4= Moderately high wilting (75% of leaves showing wilting as well as shoot stunted or wilted), 5= Severe wilting (resulting in death of the plants).

Statistical Analysis

Data on AMF root colonization was arcsine transformed to normalize their distributions. The effects of each factor and their interactions on the measured parameters were tested by two way analysis of variance. For each factor analyzed, the means of the different treatments were compared and ranked using Duncan multiple range test at $P \leq 0.05$. All the statistical analysis and graph preparation was done using SPSS version 16.

RESULTS

Treatments and *Fusarium* inoculation significantly affected the growth and P uptake. Within Fu-group, highest shoot length was recorded in Th+Myc (37.43 cm) followed by Th (32.33 cm)/Myc alone (34.41) treated pots and least in the control pots (Figure 1-a). Dry weight was significantly highest in Th+Myc (36.85 g)/Myc (30.33 g) alone treated pots. However, within Fu+ group, Th+Myc (36.78 cm) treated pots showed highest shoot length followed by Myc alone (31.89 cm) and least in the control (*Fusarium* only) pots (Figure 1-a). Dry weight was least in the control pots (18.57g) and highest in Th+Myc (36.50 g) treated pots among all treatments (Figure 1-b). Further, P uptake was highest in Th+Myc (60.218mg), followed by Myc alone (56.206 mg) treated pots, and least in the control (46.044 mg) pots within Fu- group treatments. Remaining treatments showed values comparable to each other. More or less similar trend of P uptake was observed in Fu+ group (Figure 1-c). Interactions between various inoculants and *F. udum* inoculation could not be made because the interactive values were statistically not significant ($P<0.05$). The observations on root colonization index and spore density 50 g^{-1} soil were recorded from Myc inoculated pots (Myc, Th+Myc, Tr+Myc and Tv+Myc), in the absence and presence of *Fusarium* (Table 1). Differences were found significant ($P< 0.05$) among treatments. Root colonization was highest in Myc alone followed within both Fu- and Fu+ group. Further, within Fu- group, spore density was highest in Myc alone but within Fu+ group, it was highest in Th+Myc followed by Myc alone treated pots. When comparison was made between *Fusarium* uninoculated and inoculated counterparts of treatments, results suggested that *Fusarium*

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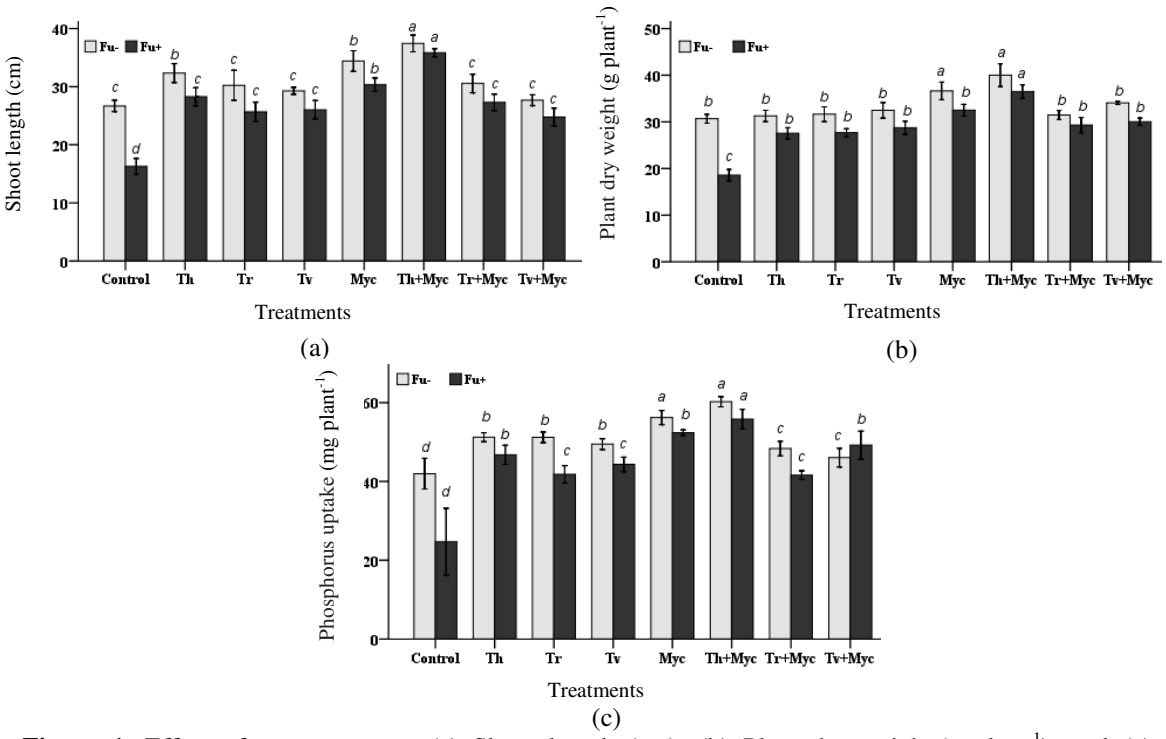

Figure 1. Effect of treatments on: (a) Shoot length (cm); (b) Plant dry weight (g plant^{-1}), and (c) Phosphors uptake of *Cajanus cajan* (cv. Bahar) after six months of sowing. Bars with same color and with letter(s) in common are not significantly different according to *post hoc* comparison (Duncan's multiple range Test at P≤ 0.05). Each bar is the mean of five replicates. Each error bars indicate standard error. Fu-= *Fusarium udum* uninoculated; Fu+= *F. udum* inoculated; Th= *Trichoderma harzianum*; Tr= *T. virens*; Tv= *T. viride*; Myc= *Mycorrhizae consortium,* Th+Myc= *T. harzianum* and Myc*;* Tr+Myc= *T. viride,* and Myc*,* Tv+Myc= *T. virens* and Myc.

inoculated counterparts of all treatments, except Th+Myc, showed significantly less root colonization and spore density than their *Fusarium* un-inoculated counterparts (Table 1). Root colonization and spore density were not affected in Th+Myc treated pots in the absence and presence of *Fusarium.* The population density i.e. CFU of *Trichoderma* was recorded from *Trichoderma* inoculated pots (Th, Tr, Tv, Th+Myc, Tr+Myc and Tv+Myc); hence, differences were found significant among various treatments (Table 1). Within Fu- and Fu+ group, highest CFU was recorded in Th/Th+Myc treated pots. When *Fusarium* un-inoculated and inoculated counterparts of treatments were compared with each other, results suggested that *Fusarium* inoculated counterparts of Tr and Tv+Myc treated pots showed significantly less CFU as compared to their *Fusarium* uninoculated counterparts. On the other hand, CFU was significantly higher in *Fusarium* inoculated counterparts of Tv treated pots. Differences between *Fusarium* un-inoculated and inoculated counterparts of Th, Th+Myc and Tr+Myc treated pots were not significant regarding CFU. Irrespective of treatments, CFU was significantly less in Fu+ group. Interaction between different treatments and inoculation of *F. udum* was not significant (Table 1).

Data obtained from the wilt disease severity index depicted in Figure 2 suggested that different treatments and time of inoculation significantly affected the disease severity, however, interaction between treatments and time of inoculation was not significant. Th+Myc treated pots showed least disease severity index among all treatments under both prior (0.41) and

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Table 1. AM fungi root colonization, spore density (50 g^{-1} potting mixture), and *Trichoderma* population (Colony forming units$\times 10^3$ g^{-1} of potting mixture) at the time of harvesting, in the absence and presence of *Fusarium udum*.[a]

Treatments	AMF root colonization		ANOVA	AMF spore density		ANOVA	*Trichoderma* population		ANOVA
	Fu-	Fu+		Fu-	Fu+		Fu-	Fu+	
Control	Nd	Nd	-	Nd	Nd	-	Nd	Nd	-
Th	Nd	Nd	-	Nd	Nd	-	62.16±0.96a	61.16±0.62a	ns
Tr	Nd	Nd	-	Nd	Nd	-	47.34±0.93c	34.23±0.81d	*
Tv	Nd	Nd	-	Nd	Nd	-	42.17±0.93d	46.96±0.06b	*
Myc	45.22±0.98a	39.19±1.09a	*	51.24±0.54a	36.22±0.76b	*	Nd	Nd	-
Th+Myc	37.88±1.53c	35.22±0.78b	ns	45.53±0.80b	49.16±0.77a	ns	60.19±0.58a	59.43±0.79a	ns
Tr+Myc	42.20±1.09b	34.48±0.71b	*	42.26±0.59b	28.25±0.60c	*	40.86±0.43d	37.22±0.71d	ns
Tv+Myc	37.93±1.27c	33.03±0.68b	*	38.35±0.91c	33.39±0.88c	*	53.23±1.09b	41.59±0.89c	*

[a] Data are means±standard deviation of five replicates. Values in the same column with the same letters represent no significant difference between treatments according to *post hoc* comparison (Duncan's multiple range Test at $P \leq 0.05$). *= Significant differences within the same row and ns= Not significant differences within the same row ($P \leq 0.05$).

simultaneous (1.01) inoculated conditions. Th-treated pots showed also significantly reduced severity of wilt disease under simultaneous (1.79) or prior (0.92) inoculation condition. Under simultaneous inoculation, Th was more effective among *Trichoderma* species. Myc was efficient in disease reduction when it was inoculated prior (0.89) to the pathogen. However, the disease severity under prior inoculation (1.7) was significantly lower than those observed under simultaneous inoculation (2.6). Significantly higher disease severity was recorded in pots where *F. udum* was inoculated alone under both prior (4.05) and simultaneous (5.47) inoculated conditions (Figure 2).

DISCUSSION

Present study demonstrated the effect of three different spp. of *Trichoderma* and mycorrhizal consortium individually (Th, Tr, Tv and Myc) and in combinations (Th+Myc, Tr+Myc and Tv+Myc) on growth response and wilt disease severity of *C. cajan*. Results suggested that different treatments and *Fusarium* inoculation significantly affected the observed parameters. *Trichoderma harzianum* exhibited better results with reference to growth and disease reduction among the *Trichoderma* spp taken in the study. Further, *T. harzianum* treated seedlings showed the highest CFU among all treatments. We deduced that growth promotion activities of *Trichoderma* are direct consequence of colonization which induces positive interactions between fungi and plant, and increased nutrient uptake by plant. Harman (2000) reported that *Trichoderma* increases root development, crop yield, the proliferation of secondary roots, and seedling fresh weight. Myc alone treated seedlings also showed highest colonization and spore density among all treatments. In the present study, consortium of AMF genera was used as mycorrhizal representative which could be the reason of

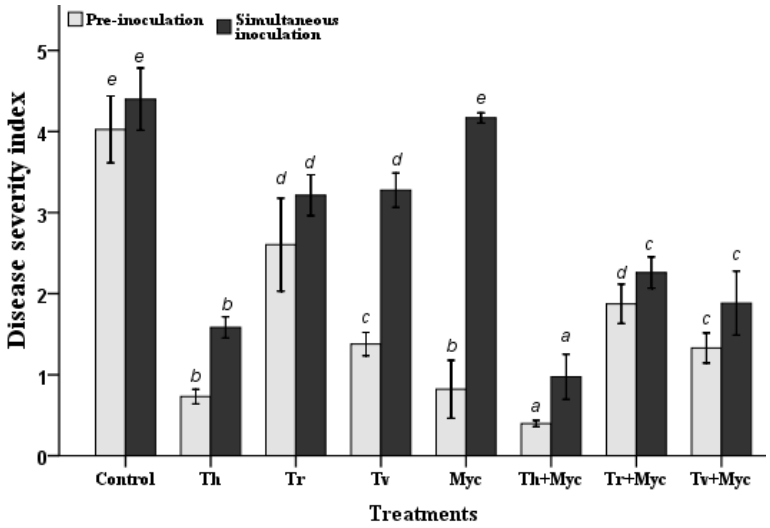

Figure 2. Effect of treatments on wilt disease severity of *Cajanus cajan* (cv. Bahar) after one month of sowing. Bars with same color and with letter/s in common are not significantly different according to *post hoc* comparison (Duncan's multiple range Test at P≤ 0.05). Each bar is the mean of five replicates. Each error bars indicate standard error. Treatments symbols as in Figure 1.

higher growth and P uptake due to enhanced root colonization and spore density (Shukla *et al*., 2012). According to Wehner *et al.* (2010), increased richness of AMF taxa can be responsible for extensive mycorrhization as compared to individual AMF inoculants. Smith and Smith (2012) suggested that plants with thriving mycorrhizal root systems are better able to grow/survive in comparison to their un-inoculated counterparts. Also, results suggested that co-inoculation of *T. harzianum* and Myc (Th+Myc) had additive effects on seedlings growth and P uptake as compared to their individual inoculation. It might be due to the positive interaction between AMF and *T. harzianum*. The proposed mechanism of growth stimulation of *T. harzianum* in the presence of Myc could be the release of volatile compounds by *T. harzianum*, which stimulate the formation of auxiliary cells of AMF (Fracchia *et al.,* 1998) which leads to the enhanced growth of plants. Calvet *et al.* (1993) has also reported a synergistic effect on the growth of marigold and percentage of AMF root colonization as a result of co-inoculation of *T. aureoviride* and *G. mosseae*. Colonization by AMF and spore density was reduced in Tv+Myc and Tr+Myc treated pots as compared to Myc alone treated pots. Our results corroborated with the earlier reports on the potential of *Trichoderma* spp to cause adverse effects on AMF (McAllister *et al*., 1994). According to Rousseau *et al.* (1996), antagonistic activity of *Trichoderma* against AMF is a complex mechanism involving production of antibiotic substances and cell wall degrading enzymes followed by AMF spore wall penetration. Wyss *et al.* (1992) reported that *Trichoderma* decreased colonization of soybean roots by *G. mosseae* due to elevated levels of the plant defence compound glyceollin, which is antimicrobial phytoalexin. On the other hand, spore density of Myc was increased when it was inoculated with *T. harzianum* in the presence of *Fusarium*. This shows synergistic interaction between *T. harzianum* and AMF. However, colonization by AMF and its spore density were reduced in the presence of *Fusarium* in all treatments except Th+Myc treated pots. McAllister *et al.* (1997) also observed the negative effect of *F. equiseti* on development of *G. mosseae*. They postulated that if AMF and saprophytic fungi were applied simultaneously to the plants, then, AMF was

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adversely affected by the latter, although when AMF was well established in the roots it was less affected by saprophytic fungus. On the other hand, there was no effect of *Fusarium* on root colonization and spore density of AMF when Myc was co-inoculated with *T. harzianum*. Further, CFU of *T. harzianum* was not affected by the presence of Myc and *Fusarium*. We deduced that *T. harzianum* was the significant factor which not only overcame the pathogenic effects of *Fusarium* but also interacted synergistically with AMF in the present study. Furthermore, CFU of *T. viride* was not affected by *Fusarium,* but decreased when inoculated with Myc. Green *et al*. (1999) also reported the detrimental effects of AMF on *Trichoderma*. Martínez-Medina *et al.* (2009) studied the interactions between four *Glomus* species and *T. harzianum* and found that, except *G. intraradices*, all *Glomus* species decreased the CFU of *T. harzianum*. Such results suggested that direct competition between extraradical mycelia of the AMF and other rhizosphere fungi for the colonization sites and nutrients have occurred. Besides, direct or indirect AM symbiosis-mediated factors could suppress the *Trichoderma* colonies (Chandanie *et al*., 2009). On the other hand, CFU of *T. virens* increased when inoculated with Myc. This showed synergistic interaction between *T. virens* and AMF. We deduced that different species of *Trichoderma* produced different response with AMF. Establishment of AM symbiosis in plants is known to change the physiological and biochemical properties of the host plant and these changes may alter the composition of root exudates which play a key role in the modification of the microbial population qualitatively and quantitatively in the mycorrhizosphere (Linderman, 1992).

Results obtained from disease severity index revealed that it was lower under prior inoculation than those observed under simultaneous inoculation. Individual inoculation of *T. harzianum* was effective in disease suppression under both prior and simultaneous inoculation when compared with the control, however, extent of disease reduction was more effective in prior (76.29%) as compared to simultaneous (59.96%) inoculation (Figure 2). Similar results obtained by Singh *et al.* (2007) suggested that plants pretreated with *Trichoderma* spp. showed increased resistance to pathogens attack. *Trichoderma* species strongly antagonize rhizosphere against pathogenic population (Chandanie *et al*., 2009). It functions as antagonist for many phytopathogenic fungi, thus protecting plants from diseases (Vinale *et al.,* 2008) by means of induced systemic resistance or localized resistance (Harman *et al.*, 2004). *Trichoderma* also induced production of phytoharmones such as jasmonic acid, ethylene, and salicylic acid, which play major role in resistance (Martinez-Medina *et al.,* 2010). Our further results suggested that Myc alone inoculation was effective in disease reduction when it was inoculated prior (78.27%) to the pathogen (Figure 2). According to Slezack *et al*. (2000), only well-established AM symbiosis could reduce damage caused by the root pathogens (Vyas *et al.,* 2010). Extent of disease reduction depends on the time taken for their establishment. Earlier, Singh *et al.* (2010) also observed that pre-inoculation of AMF had greater potential as biocontrol agent in *Lycopersicon esculentum*. Pre-transplant inoculation with mycorrhizal fungi can be a way to give efficient strains an immediate spatial advantage over the indigenous fungi which have to compete for root space (Shukla *et al.*, 2014). However, few researchers have reported that the simultaneous addition of AMF with pathogen could also reduce severity of some root diseases (Rosendahl and Rosendahl, 1990). As we mentioned, consortium of three *Glomus* spp. was used as representative of AMF, which might be responsible for effective disease reduction. Wehner *et al.* (2010) compared the effectiveness of single and multiple AMF species on plant protection and pointed out that the assemblages of multiple AMF

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species exhibited greater potential against pathogens than a single AMF species. Th+Myc treated pots showed significantly highest disease reduction under both prior (90.12%) and simultaneous inoculation (77.27%) among all treatments (Figure 2). This can be explained on the basis that *Trichoderma* fungi facilitated positive interactions (symbiosis with AM fungi and other beneficial microbes) in the rhizosphere which reduces biotic and abiotic damages (Shoresh *et al.,* 2010) caused by soil borne pathogens and even prohibit entry of pathogens in the rhizospheric zone. Earlier reports suggested that *T. harzianum* (Shoresh *et al.,* 2005) and AMF (López-Ráez *et al.,* 2010) establishment in the plant roots induce hormonal changes, which activate systemic defence responses (Vinale *et al.,* 2008). Further, as per our results, individual inoculation of *Fusarium* not only significantly reduced growth and P content of the plants (Figure 1) but also exhibited maximum severity of wilt disease under Prior to inoculation of Trichoderma (Figure 2), which could be a direct consequence of the action of pathogen (McAllister *et al.* 1994). *Fusarium* reduced colonization by AMF, spore density, and population density (CFU) of *Trichoderma* spp, except few cases. Thus, we deduced that activities of *Fusarium* directly affected the symbiotic relationship. However, *Trichoderma* and AMF develop a less favorable environment for *Fusarium* and reduce the harmful effects of this pathogen. On the basis of our results, it could be stated that combination of different groups of microorganisms activate several mechanisms such as competition, altered root exudations, anatomical and morphological changes in the root system, antibiosis, and induced plant defense systems in the presence of pathogen. Different species of *Trichoderma* interact in different ways with the same species of AMF. Furthermore, inoculation of biocontrol agents should be done prior to the transplantation of crop seedlings to the fields. However, extrapolation of the results to the real field conditions should be done with caution because of differences in growth condition and substrate used in the present study i.e. green house and sand, respectively. The information generated from this study can form the basis of further field/micro-plot experiments involving time effective *Trichoderma*/mycorrhizal inoculation to mitigate the losses due to *Fusarium* wilt of pigeon pea.

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کنترل بیولوژیک *Fusarium udum* Butler با استفاده از قارچهای تریکودرما و میکوریزهای آرباسکولار و اثرات تحریک کنندگی رشد روی نخود سودانی

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چکیده

هدف این مطالعه بررسی اثر مایه زنی همزمان گونه های تریکودرما و میکرویزهای آرباسکولار بر رشد، تشکیل میکوریز، جمعت تریکودرما و شدت بیماری پژمردگی در نخود سودانی(*Cajanus cajan* L Millsp) بود. سه گونه تریکودرما شامل *T. harzianum* (Th) ، *T. virens* (Tr)، *T. viride* (Tv)و مخلوطی از قارچ های میکوریز آرباسکولا به نام Myc (*Funneliformis mosseae*, *Glomus cerebriforme*, *Rhizophagus irregularis*) به صورت مجزا (Th، Tr، Tv، Myc) و به نسبت های مختلف (Th+Myc، Tr+Myc، Tv+Myc) آزمایش شدند. از میان تمام تیمارها، مایه زنی همزمان Th و Myc بیشترین رشد و نیز کاهش شدت بیماری پژمردگی را نشان داد (معنی دار در سطح ۵ درصد). Myc به تنهایی برای افزایش رشد کافی بود اما برای مهار بیماری زمانی موثر بود که قبل از بیمارگر مایه زنی می شد. فوزایوم طول ساقه، جذب فسفر در وزن خشک گیاهان، کلنیزه کردن قارچ میکوریز آرباسکولار، تراکم اسپور و جمعیت تریکودرما را کاهش داد. نتایج به روشنی نشان داد که گونه های مختلف تریکودرما در ترکیب با Myc نتایج متفاوتی دارند.

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