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 requirement of ever increasing food 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., Arbuscular 2004). MF-mediated bioprotection exploited has been 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+1°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 NH₄NO₃, 0.9 g Na₂H₂PO₄, 0.2 g $MgSO_4.7H_2O, 0.15$ g KC1, 20 mg FeS0₄.7H₂0, 20 mg ZnS0₄.7H₂O, 20 mg MnS0₄.H₂0, 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°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/).

Biocontrol of Fusarium Wilt of Pigeon Pea -

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 $25\pm2^{\circ}$ 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°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 $28\pm2^{\circ}$ C for 15 days. The colony forming units (CFU) of F. udum $(1.8 \times 10^3 \text{ per gram})$ substrate) and Trichoderma $(2.3 \times 10^3 \text{ per})$ 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, cerebriforme McGee, Glomus 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 fourdays 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°C. The per cent inhibition of F. udum was calculated according to the formula: L= $[(C-T)/C] \times 100$, 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 Experiment house conditions. 1 demonstrated the effect of individual and coinoculation 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

thoroughly and applied. Non-mycorrhizal treatments received the same amount of inoculum Myc. autoclaved of Pregerminated 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 KH₂PO₄, 0.51 g L⁻¹ KNO₃, 0.246 g L⁻¹ Ca(NO₃)₂, 0.245 g L⁻¹ MgSO₄.7H₂O, 1.43 g L^{-1} H₃BO₃, 0.91 g L^{-1} MnCl₂.7H₂O, 0.11 g L⁻¹ ZnSO₄.5H₂O, 0.04 g L⁻¹ CuSO₄.5H₂O and 0.04 g L⁻¹ H₂MoO₄ (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°C. 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 $\times 100$ 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 (Gerdemann method decanting 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 spoon from three sides sterile (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 $(25\pm2^{\circ}C)$ 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 vellowing 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 $P \le 0.05$. 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 Fugroup 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



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 \leq 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 Trichoderma recorded of was 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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lreatments	AMF root c	olonization	ANUVA	AMF spor	e density	AVOVIA	I LICHOMELIN	a population	ANUVA
	Fu-	Fu+		Fu-	Fu+		Fu-	Fu+	1
Control	Nd	PN		Nd	PN		PN	PN	1
Th	Nd	PN	ı	Nd	Nd		62.16±0.96a	61.16±0.62a	su
Tr	Nd	PN	ı	Nd	Nd	·	47.34±0.93c	34.23±0.81d	*
Tv	Nd	PN	ı	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	*	PN	PN	·
Th+Myc	37.88±1.53c	35.22±0.78b	su	45.53±0.80b	49.16±0.77a	su	60.19±0.58a	59.43±0.79a	su
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	su
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	*

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 development, crop root vield. 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 used mycorrhizal genera was as representative which could be the reason of

within the same row ($P \le 0.05$)



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 $P \le 0.05$). 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 coinoculation 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 coinoculation 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 antimicrobial is phytoalexin. On the other hand, spore density of Myc was increased when it was inoculated with T. harzianum in the presence Fusarium. This shows synergistic of 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 if AMF postulated that and saprophytic fungi were applied simultaneously to the plants, then, AMF was

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 coinoculated 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 symbiosis-mediated factors could AM Trichoderma suppress the colonies (Chandanie et al., 2009). On the other hand, CFU of T. virens increased when inoculated Myc. This showed synergistic with interaction between T. virens and AMF. We species different deduced that ofTrichoderma 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 was used spp. 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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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.

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

(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

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

inoculation

Furthermore,

harzianum

suggested that T.

reports

AMF.

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REFERENCES

- Amer, M. A. and Abou-El-Seoud, II. 2008. Mycorrhizal Fungi and *Trichoderma harzianum* as Biocontrol Agents for Suppression of *Rhizoctonia solani* Dampingoff Disease of Tomato. *Commun. Agric. Appl. Biol. Sci.*, **73(2)**: 217-32.
- Brimner, T. A. and Boland G. J. 2003. A Review of the Non-target Effects of Fungi Used to Biologically Controlled Plant Diseases. *Agric. Ecosyst. Environ.*, 100: 3– 16.
- 3. Calvet, C., Pera, J. and Barea, J. M. 1993. Growth Response of Marigold (*Tagetes erecta* L.) to Inoculation with *Glomus mosseae*, *Trichoderma aureoviride* and *Pythium ultimum* in a Peat-perlite Mixture. *Plant Soil*, **148**: 1–6.
- 4. Chandanie, W. A., Kubota, M. and Hyakumachi, M. 2009. Interactions between the Arbuscular Mycorrhizal Fungus *Glomus mosseae* and Plant Growth-promoting Fungi and Their Significance for Enhancing Plant Growth and Suppressing Damping-off of Cucumber (*Cucumis sativus* L.). *Appl. Soil Ecol.*, **41**: 336–341.
- 5. Cumagun, C. J. R. 2012. Managing Plant Diseases and Promoting Sustainability and Productivity with *Trichoderma*: The

of

Philippine Experience J. Agr. Sci. Tech., 14: 699-714

- Dennis, C. and Webster, J. 1971. Antagonism Properties of Species Groups of *Trichoderma*. III. Hyphal Interaction. *Trans. Br. Mycol. Soc.*, 57: 363-369.
- Dubey, S. C., Suresh, M. and Singh, B. 2007. Evaluation of *Trichoderma* Species against *Fusarium oxysporum* f. sp. *ciceris* for Integrated Management of Chickpea Wilt. *Biol. Control.*, 40(1): 118-127.
- 8. Elad, Y., Chet, I. and Henis, Y. 1981. A Selective Medium for Improving Quantitative Isolation of *Trichoderma* spp. from Soil. *Phytoparasitica*, **9(1)**: 59–67.
- 9. FAOSTAT. 2012. Food and Agriculture Organization of the United Nations (Updated 2012). Available from: http://faostat.fao.org
- Fracchia, S., Mujica, M. T., García-Romera, I., García-Garrido, J. M., Martín, J., Ocampo, J. A. and Godeas, A. 1998. Interactions between *Glomus mosseae* and Arbuscular Mycorrhizal Sporocarp Associated Saprophytic Fungi. *Plant Soil*, 200: 131–137.
- Garcia-Garrido, J. M. 2009. Arbuscular mycorrhizae as Defense against Pathogens. In: "Defensive Mutualism in Microbial Symbiosis", (Eds.): White J. F. and Torres M. S.. CRC Press, Boca Raton, Florida, PP. 183-198.
- 12. Gerdemann, J. W. and Nicolson, T. H. 1963. Spores of Mycorrhizal *Endogone* Species Extracted from Soil by Wet Sieving and Decanting. *Trans. Br. Mycol. Soc.*, **46**: 235-244.
- 13. Giovannetti, M. and Mosse, B. 1980. An Evaluation of Techniques for Measuring Vesicular Arbuscular Mycorrhizal Infection in Roots. *New Phytol.*, 84: 489-500.
- Green, H., Larsen, J., Olsson, P. A., Jensen, D. F., Jakobsen, I. 1999. Suppression of the Biocontrol Agent *Trichoderma harzianum* by Mycelium of the Arbuscular Mycorrhizal Fungus *Glomus intraradices*. *Appl. Environ. Microbiol.*, 65: 1428–1434.
- Harman, G. E. 2000. Myths and Dogmas of Biocontrol Changes in Perceptions Derived from Research on *Trichoderma harzianum* T-22. *Plant Dis.*, 84: 377-393.
- Harman, G. E., Howell, C. R., Viterbo, A., Chet, I. and Lorito, M. 2004. *Trichoderma* Species: Opportunistic, Avirulent Plant Symbionts. *Nat. Rev. Microbiol.*, 2: 43–56.

 Hause, B. and Fester T. 2005. Molecular and Cell Biology of Arbuscular Mycorrhizal Symbiosis. *Planta*, 221: 184-196

JAST

- Hoagland, D. R. and Arnon, D. I. 1938. The Water-culture Method for Growing Plants without Soil. *Calif. Agr. Exp. Sta. Cir.*, 347.
- International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (Updated 2013). Available from: http://www.invam.caf.wvu.edu
- Jackson, M. L. 1973. Soil Chemical Analysis. Prentice Hall of India, New Delhi, PP-1-498.
- 21. Linderman, R. G. 1992. Vesiculararbuscular Mycorrhizae and Soil Microbial "Mycorrhizae Interactions. In: in Agriculture", Sustainable (Eds.): Bethlenfalvay, G. J. and Linderman, R. G. ASA Special Publication No. 54, Madison, WI, PP. 45-70
- Linderman, R. G. 2000. Effects of Mycorrhizas on Plant Tolerances to Diseases. In: "Arbuscular Mycorrhizas: Physiology and Function", (Eds.): Kapulnik, Y. and Douds, D. D.. Kluwer, Dordrecht, PP. 345–365
- 23. López-Ráez, J. A., Flors, V., García, J. M. and Pozo, M. J. 2010. AM Symbiosis Alters Phenolic Acid Content in Tomato Roots. *Plant. SignaL. Behav.*, **5**: 1–3.
- Martinez-Medina, A., Pascual, J. A., Lloret, E. and Roldan, A. 2009. Interactions between Arbuscular Mycorrhizal Fungi and *Trichoderma harzianum* and Their Effects on *Fusarium* Wilt in Melon Plants Grown in Seedling Nurseries. J. Sci. Food. Agric., 89(11): 1843-1850.
- 25. Martínez-Medina, A., Roldan, A., Albacete, A. and Pascual, J. A. 2010. The Interaction with Arbuscular Mycorrhizal Fungi or *Trichoderma harzianum* Alters the Shoot Hormonal Profile in Melon Plants. *Phytochem.*, **72**: 223-229
- 26. McAllister, C. B., Garcia-Romera, I., Godeas, A. and Ocampo, J. A. 1994. Interactions between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae*: Effects on Plant Growth, Arbuscular Mycorrhizas and the Saprophyte Inoculants. *Soil Biol. Biochem.*, 26: 1363–1367.
- McAllister, C. B., Garcia-Garrido, J. M., Garcia-Romera, I., Godeas, A. and Ocampo, J. A. 1997. Interaction between *Alternaria alternata* or *Fusarium equiseti* and *Glomus*

mosseae and Its Effects on Plant Growth. Biol. Fertility Soils, 24: 301-305

- Phillips, J. M. and Hayman, D. S. 1970. Improved Procedure for Clearing Roots and Staining Parasitic and Vesicular Arbuscular Mycorrhizal Fungi for Rapid Assessment of Infection. *Trans. Br. Mycol. Soc.*, 55: 158-161.
- Prashar, P., Kapoor, N. and Sachdeva, S. 2013. Isolation and Characterization of *Bacillus sp.* with *In-vitro* Antagonistic Activity against *Fusarium oxysporum* from Rhizosphere of Tomato. J. Agr. Sci. Tech., 15: 1501-1512.
- Rai, B. and Upadhyay, R. S. 1982. Gibberella indica: The Perfect State of Fusarium udum. Mycologia, 74: 343-346
- Rifai, M. A. 1969. A Revision of the Genus Trichoderma. Mycol. Papers, 116: 1–56.
- 32. Rosendahl, C. N. and Rosendahl, S. 1990. The Role of Vesicular-arbuscular Mycorrhiza in Controlling Damping-off and Growth Reduction in Cucumber Caused by *Pythium ultimum. Symbiosis*, **9**: 363–366.
- Rousseau, A., Benhamou, N., Chet, I. and Piché, Y. 1996. Mycoparasitism of the Extramatrical Phase of *Glomus intraradices* by *Trichoderma harzianum*. *Phytopathol.*, 86: 434–443
- Saremi, H. and Burgess, L. W. 2000. Effect of Soil Temperature on Distribution and Population Dynamics of *Fusarium* Species. *J. Agr. Sci. Tech.*, 2: 119-125
- 35. Saxena, R. K., Saxena, K. B., Kumar, R. V., Hoisington, D. A. and Varshney, R. K. 2010. Simple Sequence Repeat-based Diversity in Elite Pigeonpea Genotypes for Developing Mapping Populations to Map Resistance to *Fusarium* Wilt and Sterility Mosaic Disease. *Plant Breed.*, **129**: 135-141.
- 36. Shoresh, M. I, Yedidia, I. and Chet, I. 2005. Involvement of Jasmonic Acid/Ethylene Signaling Pathway in the Systemic Resistance Induced in Cucumber by *Trichoderma asperellum* T203. *Phytopathol.*, **95**: 76–84.
- Shoresh, M., Harman, G. E. and Mastouri, F. 2010. Induced Systemic Resistance and Plant Responses to Fungal Biocontrol Agents. *Annu. Rev. Phytopathol.*, 48: 21–43.
- Shukla, A., Kumar, A., Jha, A., Ajit, and Rao, D. V. K. N. 2012. Phosphorus Threshold for Arbuscular Mycorrhizal Colonization of Crops and Tree Seedlings. *Biol. Fertility Soils*, 48: 109–116.

- Shukla, A., Dehariya, K., Vyas, D. and Jha, A. 2014. Interactions between Arbuscular Mycorrhizae and *Fusarium oxysporum* f. sp. *ciceris*: Effects on Fungal Development, Seedling Growth and Wilt Disease Suppression in *Cicer arietinum* L. Arch. *Phytopathol. Plant Prot.*, DOI: 10.1080/03235408.2014.884831
- 40. Siddiqui, Z. A. and Mahmood, I., 1999. The Effect of Inoculations of *Heterodera cajani* and *Meladoidogyne incognita* with *Fusarium udum* and *Bradyrhizobium japonicum* on the Wilt Disease Complex of Pigeonpea. *Indian Phytopathol.*, **52**: 66-70
- Singh, P. K., Mishra, M. and Vyas, D. 2007. Efficacy of *Trichoderma* spp. Strains in the Control of *Fusarium* Wilt of Tomato. *J. Mycol. Plant Pathol.*, **37**: 105-107.
- 42. Singh, P. K. and Vyas, D. 2009. Biocontrol of Plant Disease and Sustainable Agriculture. *Proc. Nat. Acad. Sci. India Sec. B.*, **79**: 110–128.
- Singh, P. K., Mishra, M. and Vyas, D. 2010. Effect of Root Exudates of Mycorrhizal Tomato Plants on Microconidia Germination of *Fusarium oxysporum* f. sp. *lycopersici*. *Arch. Phytopathol. Plant Prot.*, 43: 1495– 1503.
- 44. Slezack, S., Dumas-Gaudot, E., Paynot., M. and Gianinazzi, S. 2000. Is a Fully Established Arbuscular Mycorrhizal Symbiosis Required for Bioprotection of *Pisum sativum* Roots against *Aphanomyces euteiches? Mol. Plant Microbe Interact.*, **13**: 238–241.
- 45. Smith S. E. and Smith, F. A. 2012. Fresh Perspectives on the Roles of Arbuscular Mycorrhizal Fungi in Plant Nutrition and Growth. *Mycologia.*, **104**: 1–13.
- Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Woo, S. L. and Lorito, M. 2008. *Trichoderma* Plant Pathogen Interactions. *Soil Biol. Biochem.*, 40: 1-10
- Vosátka, M. and Albrechtová, J. 2009. Benefits of Arbuscular Mycorrhizal Fungi to Sustainable Crop Production. In: "*Microbial Strategies for Crop Improvement*", (Eds.): Khan, M., Zaidi, A. and Musarrat, J.. ISBN 978-3-642-01987, Springer, 4: 205-225.
- Vyas, D., Singh, P. K., Mishra, M. and Gupta, R. K. 2010. Phytoprotection by Arbuscular Mycorrhizae. In: *Bioinoculants* for Integrated Plant Growth", (Ed.):

Lakshman, H. C. MD Publication Pvt Ltd, New Delhi, PP. 374-420.

- 49. Wehner, J. P., Antunes, M., Powell, J. R., Mazukatow, J. and Rillig, M. C. 2010. Plant Pathogen Protection by Arbuscular Mycorrhizas: A Role for Fungal Diversity? *Pedobiologia*, **53(3):** 197-2011.
- 50. Wyss, P., Boller, T. H. and Wiemken, A. 1992. Testing the Effect of Biological

Control Agents on the Formation of Vesicular Arbuscular Mycorrhiza. *Plant Soil*, **147**: 159–162.

51. Xu, X. M., Jeffries, P., Pautasso, M. and Jeger, M. J. 2011. Combined Use of Biocontrol Agents to Manage Plant Diseases in Theory and Practice: A Review. *Phytopathol.*, **101**: 1024–1031.

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

ک. دهاریا، ا. شوکلا، ی. ا. شیخ، و د. ویاس

چکیدہ

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 ر شد، تشکیل میکوریز، جمعت تریکودرما شامل (T. virens (Tr) (T. harzianum (Th) بود. سه گونه تریکودرما شامل (T) (T) معرفی (T) viride (Tv) (T) و به نمای مخلوطی از قارچ های میکوریز آرباسکولا به نام Myc (سیز (T) مای ر T) و به نسبت های مخلوطی از قارچ های میکوریز آرباسکولا به نام Myc (T) معرفی (Myc ، T) و به نسبت های مخلوطی از قارچ های میکوریز آرباسکولا به نام Myc (Nyc ، Tr ، T) و به نسبت های مخلوطی از قارچ های میکوریز آرباسکولا به نام Myc (T) معرفی (T) مای و به نسبت های مخلوطی از قارچ های میکوریز آرباسکولا به نام Myc (T) معرفی تمام تیمارها، مایه زنی همزمان Th و مخلوفی ایم (T) معند (مین تمام تیمارها، مایه زنی همزمان M و به نسبت های مخلف (T) می ر می دو این کاهش شدند. از میان تمام تیمارها، مایه زنی همزمان M و به نسبت های Myc بیشترین ر شد و نیز کاهش شدت بیماری پژمردگی را نشان داد (معنی دار در سطح ۵ درصد). معرفی (T) می می می مور مای می مای مایه زنی همزمان آر 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 میلاند.