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MANAGEMENT OF CHICKPEA SEEDLING DISEASES THROUGH SOIL AMENDMENT WITH TRICHO-COMPOST

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ABSTRACT

Performance of different Tricho-inocula (*Trichoderma harzianum* inocula), Tricho-compost (*T. harzianum* compost) and Provax 200 WP (Carboxin + Thiram group fungicide) for controlling seedling mortality of chickpea was investigated under *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *ciceri* inoculated soil in two different agro-ecological zones viz. Bangladesh Agricultural Research Institute (BARI), Gazipur and Pulses Research Station, Madaripur of Bangladesh. Tricho-compost was prepared with a mixed substrate of cow dung, rice husk and poultry refuse colonized by *T. harzianum*. The seedling mortality of chickpea was significantly reduced by the Tricho-inocula, Tricho-compost and Provax 200 WP where Tricho-compost was found superior with respect to reduction of seedling mortality and accelerating plant growth with increasing grain yield of chickpea under pot culture as well as field conditions in two different agro-ecological zones of Bangladesh.

Keywords: *Trichoderma harzianum, Sclerotium rolfsii, Fusarium oxysporum f. sp. ciceri, Cicer arietinum, chickpea*.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important legume crop in the semi-arid tropics of the world and it is the third major pulse crop in Bangladesh (Hasanuzzaman et al., 2007). The chickpea yield in Bangladesh is very low as compared to other chickpea growing countries due to many biotic and a abiotic factors. Among the biotic factors foot and root rot (*S. rolfsii* Sacc.) and wilt (*F. oxysporum* f. sp. *ciceri*) are the two major diseases contributing 55-95% seedling mortality of chickpea (Hussain et al., 2006). In Bangladesh chemical fungicides are only the way to combat these diseases but major limitation is the requirement of a large amount of chemicals which are expensive and hazardous to human health as well as environment. So, environment and user friendly biological control measure may be an alternative method of controlling these pathogens in large fields. The use of antagonistic microorganisms is an alternative and contemporary way of controlling the pathogens *S. rolfsii* and *F. oxysporum* f. sp. *ciceri* in the crop field (Chaverri & Samuels, 2002). The effect of *T. harzianum* as bio-agent against *S. rolfsii* and *F. oxysporum* f. sp. *ciceri* is reported by many investigators (Góes et al., 2002, Cherif & Benhamou, 1990) (Shalini *et. al.* 2006). Different species of the genus *Trichoderma* are being used as bio-control agents successfully against many plant pathogens throughout the world (Sivan et al., 1984). *T. harzianum* is commercially used as preventive measure for several soil borne plant pathogenic fungi (Harman, 2006)(Shalini *et. al.* 2006). The *Trichoderma*-based biocontrol agents (BCAs) possessed better ability to promote plant growth and soil remediation activity (Esposito & Silva, 1998, Lorito et al., 1993). Like other fungal BCAs, conidial mass of *Trichoderma* is the most proficient propagule, which tolerates downstream processing like air drying (Amsellem et al., 1999) thus it has gained wide acceptance as effective BCAs against several plant pathogens (Whipps & Lumsden, 2001) because of their multiple BCA characteristics, namely, antagonism and plant-growth stimulation (Punja & Utkhede, 2003). Despite the advantages, mass

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production of *Trichoderma* BCAs is less prevalent because of high-cost raw materials like Mendel's medium, molasses, corn steep liquor and other (Verma et al., 2005). The conventional component of synthetic media like glucose, cellulose, soluble starch, and molasses to produce *Trichoderma* BCAs are costly (Lewis & Papavizas, 1983, Gupta et al., 1997). For mass production of *Trichoderma,* many researchers have successfully used cost effective substrates like wheat bran, rice bran, maize bran, sawdust (Das *et al*. 1997); rice straw, chickpea bran, grass pea bran, rice course powder, black gram bran (Shamsuzzaman et al., 2003); cow dung, poultry manure, ground nut shell, black ash, coir waste, spent straw from mushroom bed, talc, vermiculite (Rettinassababady & Ramadoss, 2000), sewage sludge compost (Cotxarrera et al., 2002). So, mass production of *T. harzianum* on comparatively cheap, stable and easily available substrate is essential. Therefore, the present study was aimed to find out a suitability of solid substrate for mass production of Tricho-compost with *T. harzianum* and also its inocula for seed treatment to reduce seedling mortality of chickpea caused by *S. rolfsii* Sacc. and *F. oxysporum* f. sp. *ciceri*.

MATERIALS AND METHODS

The performance of Tricho-inocula (*T. harhianum*), Tricho-composts and Provax 200 WP (Carboxin + Thiram group fungicide) in controlling two soil borne pathogens namely *Sclerotium rolfsii* and *F. oxysporum* f. sp. *ciceri* of chickpea was investigated both in pot culture as well as in field experiments in two different agro-ecological zones of Bangladesh during the period from 2011 to 2013. The pot experiment was carried out at the Plant Pathology Division of Bangladesh Agricultural Research Institute (BARI), Gazipur while the field experiments were conducted in two locations namely BARI, Gazipur and Pulses Research Station (PRS), Madaripur.

Tricho-compost Preparation: Fungal bio-control agent *T. harzianum* was previously isolated from the soil. The Isolated *T. harzianum* was initially propagated on substrate containing a mixture of khesari bran, wheat bran and mustard oilcake to obtain a formulated *T. harzianum*. The formulated *T. harzianum* was used for mass multiplication in two different mixtures of cow dung based compost materials. One of those composts contained cow dung and rice husk and the other contained a mixture of cow dung, rice husk and poultry refuse. The formulated *Trichoderma* was added in between two layers of compost materials and kept for 45-50 days maintaining the moisture content approximately 60-70% for rapid propagation of *T. harzianum* in the compost materials. Based on compost materials these composts were designated as Trico-compost-1 and Trico-compost-2.

Pathogenic Fungal Inocula Preparation: The pure cultures of the pathogenic fungi *S. rolfsii* and *F. oxysporum* f. sp. *ciceri* were prepared on potato dextrose agar (PDA) medium. The inoculum of these soil borne pathogens were multiplied separately on a mixture of wheat bran, khesari bran and mustard oilcake (MOC).

Seed Treatment: The *T. harzianum* was cultured in potato dextrose agar (PDA) and potato dextrose broth (PDB) media and the spores were harvested from 10 days odl culture serpaately. The seeds of chickpea (var. BARI Chhola-5) were treated with the spore suspension of *T. harzianum* maintaining the approximate spore concentration of $1x10^8$/ml. Similarly another set of seeds were also treated with Provax 200 WP @ 2.5 g/kg seeds at the time of sowing.

Pot Experiment: The pot experiment was carried out in the pot green house of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur during cropping season of 2011-2012. There were six treatments viz. (i) seed treatment with Provax 200 WP (ii) seed treatment with *Trichoderma* spore suspension-1 (iii) seed treatment with *Trichoderma* spore conducted in suspension-2 (iv) soil amendment with Tricho-compost-1 (v with Tricho) soil amendment -compost-2 and (vi) untreated control. The pot experiment was completely randomized design (CRD) with 5 replications. The sterilized pot (20 cm) soil was inoculated with the *S. rolfsii* and *F. oxysporum* f. sp. *ciceri* colonized substrate composed of wheat bran, khesari bran and MOC @ 20g/kg soil. Inoculated soil was incubated for 10 days maintaining proper soil moisture then the soil was treated with formulated *T. harzianum* @ 20 g/kg soil and kept for 7 days with proper soil moisture. One hundred seeds of chickpea var. BARI Chhola-5 were sown in each pot and allowed to emerge the seedlings under congenial environment in the pot house.

Field Experiment: The field trials were conducted in the fields of Plant Pathology Division, BARI, Gazipur and Pulses Research station, Madaripur during 2011-12 and 2012-13 cropping years. The treatment combinations were similar as used in the pot experiment such as (i) Seed treatment with Provax 200 WP (ii) Seed treatment with *Trichoderma* spore suspension-1 (iii) Seed treatment with *Trichoderma* spore suspension-2 (iv) Soil amendment with Tricho-compost-1 (v) Soil amendment with Tricho-compost-2 and (vi) Untreated control. The field experiments were laid out in randomized complete block design (RCBD) with 3 replications. The unit plot size was 2.5 m x 3 m. The field soil was inoculated with *S. rolfsii* and *F. oxysporum* f. sp. *ciceri* colonized substrate consisting of khesari bran, wheat bran and mustard oilcake @ 100g/m^2 of soil and allowed the pathogen establishment in the soil for 10 days before seed sowing. The field soil was again treated with the Tricho-compost @ 3t/ha and kept for 7 days. The seeds of chickpea var. BARI Chhola-5 were sown in the experimental plots maintaining row to row distance of 20 cm. Proper intercultural operations were done for better growth of chickpea in the field.

Data were collected on seedling mortality, shoot height, shoot weight, root length, root weight and also the seed yield of chickpea. Data were analysis by using MSTATC program following the standard statistical procedures. Treatment means computed using least significant difference (LSD) test.

RESULTS

The performance of Tricho-inocula and Tricho-composts in controlling foot and root (*S. rolfsii*) and wilt (*F. oxysporum* f. sp. *ciceri*) diseases of chickpea was tested in pot as well as in field experiments during the period from 2011 to 2013. The results of one pot experiment and two field experiments were discussed individually.

Germination and growth of chickpea seedlings were significantly increased by the application of *T. harzianum* under pot culture conditions (Table 1). Tricho-composts, Tricho-inocula and Provax 200 WP were equally effective for enhancing seedling emergence of chickpea up to 96% as compared to untreated control (74.67%). Similarly, seedling mortality was considerably reduced by different formulations of *T. harzianum* (7.33-8.67%) and Provax 200 WP (6.33%) while it was higher in untreated control pot (32.10%). Root and shoot growth of chickpea seedlings were amplified to two folds over untreated control due to *T. harzianum* where Tricho-composts showed better effects in pot culture.

Chickpea shoot heights and shoot weights were significantly accelerated in both the locations viz. Gazipur and Madaripur districts due to the soil amendment with *T. harzianum* and also seed treatment with Provax 200 WP as compared to untreated control plots (Table 2). Among the treatments Tricho-compost-2 was found to be the best treatment in both locations for augmenting the chickpea shoot heights (25.60 cm and 42.63 cm) and shoot weights (11.90 g and 22.40 g) where untreated control showed inferior performance. Shoot growth of chickpea was better in Madaripur than Gazipur location.

Table 1. Role of *T. harzianum* on in reduction of seedling mortality and growth of chickpea under pot culture conditions during 2011-2012.

Treatments	Germination (%)	Seedling mortality (%)	Shoot length (cm)	Shoot weight (g/plant)	Root length (cm)	Root weight (g/plant)
Seed Treatment						
Provax 200 WP	95.00	6.33	8.83 c	3.70 c	5.77 c	0.34 c
Tricho-inocola-1	95.67	7.33	9.60 bc	4.73 b	6.40 b	0.43 b
Tricho-inocola-2	92.33	7.33	10.03 b	3.93 ab	6.48 b	0.44 ab
Soil amendment						
Tricho-compost-1	96.00	7.67	11.17 a	5.43 a	7.03 a	0.49 a
Tricho-compost-2	95.67	8.67	12.90 a	5.53 a	7.40 a	0.49 a
Untreated Control	74.67	32.10	7.20 d	2.61 d	4.57 d	0.24 d

Values in a column having same letter did not differ significantly (P=0.05) by LSD.

Root length of chickpea plants was significantly increased in both Gazipur and Madaripur locations due to the soil amendment with different formulation of *T. harzianum* and seed treatment with Provax 200 WP as compared to untreated control plots (Table 3). Besides, root weights were significantly increased only in Gazipur location due to the soil amendment and seed treatment. The Tricho-composts were found to be the better in both locations for enhancing chickpea root heights and root weights over the rest treatments. Better root growth of chickpea was observed in Madaripur as compared with Gazipur location.

Table 2. Effect of *T. harzianum* on shoot growth of chickpea under *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *ciceri* inoculated field soils during 2011-2012.

Treatments	Chickpea shoot height in two locations (cm)		Chickpea shoot weight in two locations (g/plant)	
	BARI, Gazipur	PRS, Madaripur	BARI, Gazipur	PRS, Madaripur
Seed treatment				
Provax 200 WP	22.23 bc	39.40 b	10.29 ab	21.37 a
Tricho-inocola-1	19.90 c	39.37 b	9.75 ab	20.07 ab
Tricho-inocola-2	19.93 c	39.03 b	8.43 bc	19.88 ab
Soil amendment				
Tricho-compost-1	22.40 b	39.66 b	10.08 ab	21.50 a
Tricho-compost-2	25.60 a	42.63 a	11.90 a	22.40 a
Untreated control	16.63 d	35.90 c	6.50 c	17.96 b

Values in a column having same letter did not differ significantly (P=0.05) by LSD.

Table 3. Effect of *T. harzianum* on root growth of chickpea under *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *ciceri* inoculated field soils during 2011-2012.

Treatments	Chickpea root length in two locations (cm)		Chickpea root weight in two locations (g/plant)	
	BARI, Gazipur	PRS, Madaripur	BARI, Gazipur	PRS, Madaripur
Seed treatment				
Provax 200 WP	9.97 ab	14.35 ab	3.54 b	5.97
Tricho-inocola-1	10.60 ab	14.11 b	3.57 b	5.57
Tricho-inocola-2	9.73 b	14.36 ab	3.52 b	5.47
Soil amendment				
Tricho-compost-1	10.87 a	14.43 ab	3.70 ab	5.92
Tricho-compost-2	11.43 a	15.13 a	3.80 a	6.03
Untreated control	8.27 c	13.08 c	2.38 c	4.87

Values in a column having same letter did not differ significantly (P=0.05) by LSD.

Seedling mortality of chickpea was sharply reduced in Gazipur as well as Madaripur locations due to the soil amendment with Tricho-composts and seed treatment with Tricho-inocula and Provax 200 WP as well (Table 4). The range of seedling mortality was higher in Gazipur (4.46-13.23%) than that in Madaripur (6.14-10.29%) whereas untreated control plots showed much higher mortality (25.23% and 25.80%). The grain yield of chickpea was significantly increased over untreated control plot in both locations though the treatment effects did not differ statistically among themselves. The percent increase in chickpea grain yield was higher in Gazipur (35.68-45.09%) and lower in Madaripur (21.25-24.99%).

The results of the second year field experiment showed distinct variation among the tested treatments with respect to seedling mortality and grain yield of chickpea both in Gazipur and Madaripur locations (Table 5). In Gazipur location maximum seedling mortality (28.67%) was recorded in the untreated control plot followed by Tricho-inocula, Provax 200 WP, and Tricho-compost treated plots. Tricho-composts gave lower seedling mortality (6.33-6.67%) as compared to Provax 200 WP (9.67%) and Tricho-inocula (15.00-16.67%). The trend of seedling mortality in Madaripur location was almost similar to that of Gazipur location.

The grain yield of chickpea was significantly increased in both Gazipur and Madaripur locations due to *T. harzianum* and Provax 200 WP (Table 5). In Gazipur location the grain yield was ranged from 2089 kg ha^{-1} to 2711 kg ha^{-1} among the treated plots and it was lower (1689 kg ha^{-1}) in the untreated control plot. Similarly, better grain yields (1625-1755 kg ha^{-1}) were obtained from the treated plots of Madaripur location where minimum grain yield (1345 kg ha^{-1}) was recorded from the untreated control plot. Both Gazipur and Madaripur locations showed a sharp increase in chickpea grain yields ranging from 19.15-37.69% and 17.23-23.36% respectively, due to various treatments (Table 5).

Table 4. Effect of *T. harzianum* on seedling mortality and grain yield of chickpea under *S. rolfsii* and *F. oxysporum* f. sp. *ciceri* inoculated field soils during 2011-2012.

Treatments	Seedling mortality in two locations (%)		Chickpea yield in two locations (kg/ha)		Yield increased over control (%)	
	BARI, Gazipur	PRS, Madaripur	BARI, Gazipur	PRS, Madaripur	BARI, Gazipur	PRS, Madaripur
Seed treatment						
Provax 200 WP	11.26	6.14	2667 a	1624 ab	40.65	22.17
Tricho-inocola-1	13.11	9.35	2460 a	1605 b	35.65	21.25
Tricho-inocola-2	13.23	10.29	2500 a	1609 b	36.68	21.44
Soil amendment						
Tricho-compost-1	5.00	8.22	2583 a	1663 ab	38.72	23.99
Tricho-compost-2	4.46	6.73	2883 a	1685 a	45.09	24.99
Untreated control	25.23	25.80	1583 b	1264 c	-	-

Values in a column having same letter did not differ significantly (P=0.05) by LSD.

Table 5. Effect of *T. harzianum* on growth and grain yield of chickpea under *S. rolfsii* and *F. oxysporum* f. sp. *ciceri* inoculated field soils during 2012-2013.

Treatments	Seedling mortality in two locations (%)		Chickpea yield in two locations (kg/ha)		Yield increased over control (%)	
	BARI, Gazipur	PRS, Madaripur	BARI, Gazipur	PRS, Madaripur	BARI, Gazipur	PRS, Madaripur
Seed treatment						
Provax 200 WP	9.67	3.14	2356 ab	1684 ab	28.31	20.13
Tricho-inocola-1	15.00	3.35	2089 ab	1625 b	19.15	17.23
Tricho-inocola-2	16.67	3.29	2200 ab	1655 ab	23.22	18.73
Soil amendment						
Tricho-compost-1	6.33	3.22	2356 ab	1683 ab	28.31	20.08
Tricho-compost-2	6.67	2.73	2711 a	1755 a	37.69	23.36
Untreated control	28.67	13.80	1689 b	1345 c	-	-

Values in a column having same letter did not differ significantly (P=0.05) by LSD.

Therefore, considering the reduction of seedling mortality and enhancing of grain application of *T. harzianum* under pot culture conditions (Table 1). Tricho-composts, Tricho-application of *T. harzianum* under pot culture conditions (Table 1). Tricho-composts, Tricho-yield of chickpea, the effect of Tricho-composts were seemed to be superior over Tricho-inocula and Provax 200 WP.

DISCUSSION

The saprophytic fungus *Trichoderma* was found in almost all agricultural soil that had been investigated as potential biocontrol agents because of their ability to reduce the incidence and severity of disease caused by plant pathogenic fungi, particularly many soil borne pathogens (Freeman et al., 2004, Ashrafizadeh et al., 2005, Dubey et al., 2007), although some have been occasionally recorded as plant pathogens (Menzies, 1993). The use of *Trichoderma* as a biological agent of plant diseases had long been known (Tran 1998) but its potentiality in Bangladesh agriculture was yet to be explored. Therefore, soil application of Tricho-composts as well as seed treatment with *Trichoderma* inocula were evaluated against seedling diseases of chickpea in the pot house as well as in the field of two different agro ecological locations at Gazipur and Madaripur. Results came out from the studies proved that *Trichoderma* spp. cultured on compost materials could be used to control soil borne pathogens *S. rolfsii* and *F. oxysporum* f. sp. *ciceri* causing foot and root rot and wilt diseases of chickpea.

Several media and protocols for mass scale production of *T. harzianum* spore on costly conventional synthetic media were aimed especially to produce conidia, mycelium and chlamydospore. Therefore cost effective substrates such as wheat bran, khesari bran and rice bran alone or in combination with mustard oilcake were used for mass production of *T. harzianum* useful for large scale soil amendment. The results clearly indicated that Tricho-compost having biological control agent *T. harzianum* provided effective protection measure against seedling diseases of chickpea and also caused plant growth promotion with higher grain yield of chickpea. Different workers reported that the antagonistic activity of different *Trichoderma* isolates against various phtyopathogenic fungi such as *R. solani, F. oxysporum* and *S. rolfsii* (Deshmukh & Raut, 1992, Xu et al., 1993, Askew & Laing, 1994). It was reported that locally available organic media viz., coir pith, cow dung, and neem cake were the excellent sources of nutrition for antagonistic fungi like *T. harzianum* and *T. viride* (Rini & Sulochana, 2007). Besides, cow dung and neem cake mixture was reported as a recommended practice for field multiplication of *Trichoderma* (Jose et al., 2002). On the other hand several reports showed that soil and foliar application of *T. harzianum* reduced the population of soil-borne phytopathogens, especially *S. rolfsii*, *F. oxysporum*, *Rhizoctonia solani* and *S. sclerotiorum* (Sivan et al., 1984, Hoitink & Boehm, 1999). Thus it was revealed from the investigation that soil amendment with Tricho-compost was most effective option for reducing seedling mortality and increasing plant growth as well as for higher yield of chickpea. The other options were seed treatment with chemical fungicide Provax 200 WP or *T. harzianum* inocula for reducing seedling mortality and higher yield of chickpea.

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