

Management of Stem and Root Rot Disease of Lentil through Soil Amendment with Tricho-Compost

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ABSTRACT

The efficacy of *Trichoderma harzianum* based Tricho-inocula, Tricho-compost and seed treating chemical fungicide Provax was tested against foot and root rot diseases of lentil caused by *Sclerotium rolfsii* and *Fusarium oxysporum* in two different agro-ecological zones of 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 bran and poultry refuse colonized by *T. harzianum*. The results exhibited that the seedling mortality of lentil was significantly reduced by the Tricho-inocula, Tricho-compost and Provax. The yield of lentil was sharply increased over control due to the *T. harzianum* formulations and Provax in both locations. Among the treatment, Tricho-compost was found more efficient in the reduction of seedling mortality and acceleration of plant growth with increased grain yield of lentil under *S. rolfsii* and *F. oxysporum* inoculated pot culture as well as field experiments in two different agro-ecological zones of Bangladesh.

Keywords : Trichoderma Harzianum, Sclerotium Rolfsii, Fusarium Oxysporum, Lentil, Lens Culinaris

I. INTRODUCTION

Lentil (*Lens culinaris*) is the most familiar second important pulse crop in terms of area (154,000 ha) and production (116,000 t), but it the first consumed and cheap source of protein for human beings and animals in Bangladesh (BBS. 2011; Sattar *et al.*, 1996). The cultivated area and production of lentil is gradually decreasing from the last decades. The average yield of lentil per unit area in Bangladesh is low as compared to that of other lentil growing countries like Syria, Turkey, Canada, USA and Ethiopia (Hossain *et al.* 1999) due to various abiotic and biotic factors, especially the diseases that cause 30-40% yield loss in lentil (Begum 2003). Foot and root rot, one of the major diseases of lentil caused by the pathogens *S. rolfsii* Sacc. and *F. oxysporum* Schlecht in congenial conditions resulting up to 100% seedling mortality culminating drastic reduction in grain yield (Begum 2003). There is no effective resistant variety or fungicide for the successfully management of this disease in the field. Numerous studies have shown that biological control offers an

environmentally friendly alternative to protect plants from soil-borne pathogens (Harman 2011; Singh *et al.*, 2011). Various fungal species are used as biological agents that effectively control plant diseases, and about 90% of such biocontrol agents are different species of *Trichoderma* genus such as *T. harzianum*, *T. virens*, *T. viride* (Benítez *et al.*, 2004). The effect of *T. harzianum* as biocontrol agent against *S. rolfsii* and *F. oxysporum* was reported by many investigators (Goes *et al.*, 2002, Cherif and Benhamou, 1990, Shalini *et al.*, 2006). The suppression of plant disease by *Trichoderma* is based on hyper-parasitism, antibiosis, induced resistance in the host plant and competition for nutrients and space (Harman *et al.*, 2004). The *T. harzianum* is commercially used as preventive measure for several soil borne plant pathogenic fungi (Harman 2006, Shalini *et al.*, 2006). Mass production of *Trichoderma* biological control agents is less prevalent because of high-cost raw materials like Mendel's medium, molasses, corn steep liquor and other (Verma *et al.*, 2005). 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, groundnut shell, black ash, coir waste, spent straw from mushroom bed, talc, vermiculite (Rettinassababady and Ramadoss 2000), sewage sludge compost (Cotxarrera *et al.* 2002). Information on mass production of *T. harzianum* to control foot and root rot disease of lentil is inadequate in the country. Therefore, the present study was aimed to find out a suitable solid substrate for mass production of *T. harzianum* to formulate Tricho-compost and also its inocula for controlling seedling mortality of lentil caused by *S. rolfsii* Sacc. and *F. oxysporum* Schlecht.

II. METHODS AND MATERIAL

Two *Trichoderma harzianum* based products (Tricho-inocula, Tricho-composts), one chemical fungicide (Provax 200 WP), two lentil pathogens (*Sclerotium rolfsii* and *F. oxysporum*) were used in both pot and field experiments. The pot experiment was carried out at the Plant Pathology Division of Bangladesh Agricultural Research Institute (BARI), Gazipur. The field experiments were conducted in two agro-ecological zones namely BARI, Gazipur and Pulses Research Station (PRS), Madaripur during 2012-13 and 2013-14 cropping seasons.

Seventy two (72) isolates of *T. harzianum* were obtained from different locations of Bangladesh and their efficacy was tested against different soil borne pathogens including *S. rolfsii* and *F. oxysporum* in the laboratory. Some isolates of *T. harzianum* including TM11 were found more vigorous to suppress the soil borne pathogens. A pure culture of *T. harzianum* (TM11) was grown in potato dextrose agar (PDA) medium which was used to formulate in the substrates.

Tricho-compost preparation: Isolated *T. harzianum* (TM11) was initially multiplied on substrate containing a mixture of rice bran, wheat bran and mustard oilcake to formulate a *T. harzianum* based inoculant. The *T. harzianum* inoculant was used for mass multiplication in two different mixtures of cow dung based compost. Tricho-compost-1 was made with cow dung and rice bran and Tricho-compost-2 multiplied in cow dung, rice bran and poultry refuse. The *T. harzianum* inoculant was

added in between two layers of compost materials and kept for 45-50 days maintaining the moisture content approximately 60-70% for its rapid multiplication in the composting materials.

Pathogenic fungal inocula preparation: The pure cultures of the pathogenic fungi *S. rolfsii* and *F. oxysporum* were isolated from infected lentil seedlings grown on potato dextrose agar (PDA) medium. The inoculum of these soil borne pathogens were multiplied separately in a mixture of wheat bran, khesari bran and mustard oilcake (MOC).

Seed treatment: *T. harzianum* was cultured in potato dextrose agar (PDA) and potato dextrose broth (PDB) media and the spores were separately harvested from 10 days old culture. The seeds of lentil (BARI Masur-5) were treated with the spores suspension of *T. harzianum* at the concentration of $1 \times 10^8 \text{ ml}^{-1}$. Similarly, another set of seeds were also treated with Provax 200 WP at 2.5 g kg^{-1} seeds at the time of sowing.

Pot experiment: The pot experiment was carried out in the pot house of Plant Pathology Division, BARI, Gazipur during cropping season of 2011-2012. There were six treatments viz. (i) seed treatment with Provax 200 WP (Carboxin + Thiram 37.5% WS), (ii) seed treatment with *Trichoderma* spore suspension-1 at $1 \times 10^9 \text{ ml}^{-1}$ from PDA (Tricho-inocula 1), (iii) seed treatment with *Trichoderma* spore suspension-2 at $1 \times 10^9 \text{ ml}^{-1}$ from PDB (Tricho-inocula 2), (iv) soil amended with Tricho-compost-1, (v) soil amended with Tricho-compost-2 and (vi) untreated control. The pot experiment was conducted in completely randomized design (CRD) with 5 replications. The sterilized soil in the pot was inoculated with the *S. rolfsii* and *F. oxysporum* colonized substrates at 20 g kg^{-1} soil. Inoculated soil was incubated for 10 days to grow the pathogens and then the soil was amended with Tricho-compost at 100 g kg^{-1} soil and kept for 7 days with proper soil moisture. One hundred seeds of lentil BARI Masur-5 were sown in each pot (size 20 cm) 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 2012-13 and 2013-14 cropping years. The treatment combinations

were similar as used in the pot experiment. 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* colonized substrate at 100 g m⁻² of soil and the introduced pathogen was allowed to colonize the soil for 10 days before seed sowing. The field soil was thereafter treated with Tricho-compost at 3t ha⁻¹ and left for 7 days. The seeds of lentil BARI Masur-5 were sown at 35 kg ha⁻¹ in the experimental plots maintaining row to row distance of 20 cm. Proper intercultural operations were done for better growth of lentil in the field. No plant protecting chemicals (insecticides or fungicides) were applied in the field.

Incidence of foot and root rot disease: The experimental plots were inspected routinely to record the foot and root rot infected plants. The complex symptoms-bearing plants were brought to the laboratory and the fungi were isolated on PDA. The isolated fungi were identified as *F. oxysporum* and *S. rolfsii* according to reference mycology books and manuals (Barnett and Hunter, 1972, Booth, 1971). The pure cultures of the fungi were preserved in PDA slants at 4°C.

Data collection and analysis: Data on different parameters viz., germination, post-emergence seedling mortality, shoot length, root length, shoot weight, root weight, yield of lentil were taken. Data were analyzed in MSTATC program and the treatment means were separated in least significant difference (LSD) test at p = 0.05 level.

III. RESULT AND DISCUSSION

Pot Experiment

Emergence and growth of lentil seedlings were significantly increased by the application of *T. harzianum* based inoculants under pot culture conditions (Table 1). Tricho-composts, Tricho-inocula and Provax 200 WP were equally effective for enhancing seedling emergence of lentil up to 99.67% as compared to untreated control (76.33%). Pre-emergence mortality of lentil seedlings was as high as 23.67% in untreated control pot which was much lower (0.33-11%) in the treated plots. Similarly, post-emergence seedling mortality was considerably lower among the

formulations of *T. harzianum* (7.33-9%) and Provax 200 WP (5.67%) while it was higher in untreated control pot (34%). Besides, the length and weight of shoot and root of lentil seedlings were significantly higher when soil was amended with Tricho-compost followed by seed treatment with Provax and Tricho-inocula. The lowest shoot and root growth were recorded from untreated control pot.

Field Experiment

Seedling mortality

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 2). At Gazipur the range of seedling mortality was 6.33-13.33% in first year and 5.00-11.67% in the second year due to the soil amendment with Tricho-composts and seed treatment with Tricho-inocula and Provax 200 WP whereas untreated control plots showed much higher mortality of 30.67% and 23.33% respectively in 2012-13 and 2013-14.. The seedling mortality was reduced over control up to 79.36% in first year and 78.56% in second year trial at Gazipur. In Madaripur location maximum seedling mortality of 20.17% and 28.00% were recorded from the untreated control plot followed by Tricho-inocula and Provax 200 WP treated plots during the consecutive years while Tricho-composts gave lower seedling mortality of 11.46-12.41% and 9.67-10.33%, respectively during same years. The reduction of seedling mortality was up to 43.18% in first year and 65.46% in second year due to the treatments (Table 2).

Seedling growth

Vegetative growth of lentil seedlings comprising shoot heights and shoot weights were significantly accelerated in both the locations i.e. Gazipur and Madaripur districts due to the soil amendment with *T. harzianum* based Tricho-compost, seed treatment with Provax 200 WP and *T. harzianum* inocula as compared to untreated control. The Tricho-compost-2 was the best treatment in increasing lentil shoot heights at Gazipur (24.60 cm and 36.73 cm) and also at Madaripur (25.23 cm and 29.60 cm) in both years (Table 3). It was followed by Tricho-compost-1, Provax 200 WP and Tricho-inocula. The

untreated plots showed lower shoot height in Gazipur (14.60 cm and 16.40 cm) and Madaripur location (21.30 cm and 17.33 cm) during the consecutive years. At Gazipur, in 2012-13, the highest lentil shoot weight was produced by Tricho-compost-1 with 6.92 g plant⁻¹ while, in 2013-14, the highest shoot was obtained from Tricho-compost-2 treated plants with 7.07 g plant⁻¹. In both years, the lowest lentil shoot weights were from the untreated control plots with 3.72 and 4.43 g plant⁻¹, respectively in 2012-13 and 2013-14.

Root length and root weight of lentil plants were significantly increased in both Gazipur and Madaripur locations due to the soil amendment with different formulations of *T. harzianum* based composts and seed treatments with *T. harzianum* based inoculants, and Provax 200 WP compared to untreated control plots during 2012-13 and 2013-14 cropping seasons (Table 4). Among the treatments, soil amendments with Tricho-composts were found to be slightly more efficient than others in both locations in the enhancement of lentil root length and root weights. The lowest root lengths and root weights of lentil were observed in untreated control in both locations during the two cropping seasons.

Grain yield of lentil

At Gazipur, the grain yield of lentil was significantly increased in both by the application of *T. harzianum* based products and Provax 200 WP (Table 5). The grain yield of lentil in Gazipur location ranged from 1307 to 1622 kg ha⁻¹ in 2012-13 and 1282 to 1936 kg ha⁻¹ in 2013-14 for the treated plots. The lowest grain yields were obtained from the untreated control for both cropping seasons with 1169 kg ha⁻¹ and 962 kg ha⁻¹.

At Madaripur, the application of *Trichoderma* based product and Provax also significantly increased lentil grain yields. For the treated plots, grain yields ranged from 962-1134 kg ha⁻¹ in 2012-13 and 1426-1700 kg ha⁻¹ in 2013-14. The untreated control plots produced the lowest grain yields of 875 kg ha⁻¹ and 1023 kg ha⁻¹ during the same cropping seasons. The yield increases in Gazipur and Madaripur locations were respectively up to 27.93% and 22.84% for 2012-13 cropping season, and 50.31% and 39.82% for the 2013-14 cropping season (Table 5). Since Tricho-compost treatments produced the highest grain yield, it could be stated that their effects were superior over Tricho-inocula and Provax-200WP in respect of seedling mortality reduction and enhancing grain yield of lentil in Gazipur and Madaripur districts during two consecutive years.

Table 1. Role of *T. harzianum* on mortality and growth of lentil seedlings against *Sclerotium rolfsii* and *Fusarium oxysporum* under pot culture conditions

Treatments	Germination (%)	Seedling mortality (%)		Shoot length (cm)	Shoot weight (g/plant)	Root length (cm)	Root weight (mg/plant)
		Pre-emergence	Post-emergence				
Seed treatment with Provax	92.67	7.33	5.67	9.26 c	3.33 c	4.80 bc	25 ab
Seed treatment with Tricho-inocula-1	89.00	11.00	9.00	10.17 b	4.03 b	5.47 b	330 a
Seed treatment with Tricho-inocula-2	99.67	0.33	7.33	10.03 b	4.04 b	5.27 b	320 a
Soil amendment with Tricho-compost-1	90.00	10.00	8.67	11.73 a	4.30 ab	6.43 a	330 a
Soil amendment with Tricho-compost-2	97.33	2.67	8.33	12.20 a	4.53 a	6.50 a	330 a
Untreated Control	76.33	23.67	34.00	7.00 d	2.15 d	3.93c	210 b

Figures followed by the same letter are not statistically different according to the LSD test, at p = 0.05 level.

Table 2. Potential of different *T. harzianum* based products in controlling seedling mortality of lentil caused by *Sclerotium rolfsii* and *Fusarium oxysporum*

Treatments	Seedling mortality in two locations (%)				Reduction of seedling mortality over control (%)			
	BARI, Gazipur		PRS, Madaripur		BARI, Gazipur		PRS, Madaripur	
	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14
Seed treatment with Provax	6.67	5.67	12.67	11.67	78.25	75.69	37.18	58.32
Seed treatment with Tricho-inocula-1	13.33	11.67	13.74	12.33	55.43	49.98	31.88	55.96
Seed treatment with Tricho-inocula-2	9.67	10.33	13.02	12.33	68.47	55.72	35.45	55.96
Soil amendment with Tricho-compost-1	7.00	5.00	12.41	10.33	77.18	78.56	38.47	63.11
Soil amendment with Tricho-compost-2	6.33	5.33	11.46	9.67	79.36	77.15	43.18	65.46
Untreated control	30.67	23.33	20.17	28.00	-	-	-	-

PRS= Pulses Research Station

Table 3. Effect of *T. harzianum* on shoot growth of lentil in *Sclerotium rolfsii* and *Fusarium oxysporum* inoculated field soil conditions during two consecutive years

Treatments	Lentil shoot height (cm)				Lentil shoot weight (g/plant)			
	BARI, Gazipur		PRS, Madaripur		BARI, Gazipur		PRS, Madaripur	
	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14
Seed treatment with Provax	25.00 a	22.93b	24.12 b	23.87 b	5.78 a	5.43 b	6.89 b	5.88 b
Seed treatment with Tricho-inocula-1	23.63 a	23.10b	21.93 d	22.67 b	6.20 a	5.93 b	6.68 b	5.87 b
Seed treatment with Tricho-inocula-2	22.12 a	24.60b	23.49 c	23.20 b	5.93 a	6.03 b	6.68b	5.92 b
Soil amendment with Tricho-compost-1	23.55 a	32.67a	24.45 b	28.40 a	6.92 a	7.00 a	7.83 a	7.13 a
Soil amendment with Tricho-compost-2	24.60 a	36.73a	25.23 a	29.60 a	6.70 a	7.07 a	8.15 a	7.34 a
Untreated control	14.60 b	16.40c	21.30 e	17.33 c	3.72 b	4.43 c	5.07 c	4.14 c

PRS= Pulses Research Station

Table 4. Effect of *T. harzianum* on root growth of lentil in *Sclerotium rolfsii* and *Fusarium oxysporum* inoculated field soil conditions during two consecutive years

Treatments	Lentil root length in two locations (cm)				Lentil root weight in two locations (mg/plant)			
	BARI, Gazipur		PRS, Madaripur		BARI, Gazipur		PRS, Madaripur	
	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14
Seed treatment with Provax	9.15 a	7.40 bc	6.08 bc	7.63 b	290 bc	390 ab	780 a	650 b
Seed treatment with Tricho-inocula-1	10.13 a	6.37 c	5.62 d	8.87 b	290 bc	330 ab	630 b	630 b
Seed treatment with Tricho-inocula-2	9.32 a	7.33 bc	5.95 c	7.93 b	270 cd	370 ab	670 b	580 b
Soil amendment with Tricho-compost-1	9.97 a	8.53 ab	6.30 ab	9.80 a	360 a	450 a	780 a	760 a

Soil amendment with Tricho-compost-2	10.17 a	8.87 a	6.36 a	10.47 a	330 ab	420 ab	680 b	780 a
Untreated control	5.32 b	5.00 d	5.50 d	5.37 c	220 d	270 b	460 c	450 c

PRS= Pulses Research Station

Table 5. Effect of *T. harzianum* on grain yield of lentil in *Sclerotium rolfsii* and *Fusarium oxysporum* inoculated field soil conditions during two consecutive years

Treatments	Lentil yield in two locations (kg/ha)				Yield increased over control (%)			
	BARI, Gazipur		PRS, Madaripur		BARI, Gazipur		PRS, Madaripur	
	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14
Seed treatment with Provax	1560 a	1615 b	1100 a	1519 b	25.06	40.43	20.45	32.67
Seed treatment with Tricho-inocula-1	1329 ab	1506 b	962 c	1426 b	12.04	36.12	09.04	28.26
Seed treatment with Tricho-inocula-2	1307 ab	1282 c	1030 b	1453 b	10.56	24.96	15.05	29.59
Soil amendment with Tricho-compost-1	1573 a	1827 a	1056 b	1668 a	25.68	47.35	17.14	38.67
Soil amendment with Tricho-compost-2	1622 a	1936 a	1134 a	1700 a	27.93	50.31	22.84	39.82
Untreated control	1169 b	962 d	875 d	1023 c	-	-	-	-

PRS= Pulses Research Station

Discussion

The saprophytic fungus *Trichoderma* was found as potential biocontrol agent in almost all agricultural soils. This fungus had been studied 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 has not yet been explored. Therefore, soil application of Tricho-composts as well as seed treatment with *Trichoderma* inocula were evaluated against seedling diseases of lentil in the pot house as well as in the field of two different agro ecological zones viz. Gazipur and Madaripur. Results revealed that *T. harzianum* cultured on compost materials effectively controlled the soil borne pathogens *S. rolfsii* and *F. oxysporum* causing foot and root rot diseases of lentil.

Several costly media and protocols for mass production of *T. harzianum* spore were available to produce conidia, mycelium and chlamyospore. Therefore, cost effective compost materials such as rice bran, cow dung and poultry refuse were used for mass production of *T. harzianum*. 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 and Sulochana 2007). Post-emergence seedling mortality of lentil due to foot and root rot (*F. oxysporum* and *S. rolfsii*) was found to be reduced by soil amendments with Tricho-compost and also seed treatment with Tricho-inocula and Provax 200 WP. The mycoparasitism of *T. harzianum* on *F. oxysporum* f. sp. *phaseoli* causing wilt disease in beans was found that the pathogen was completely engulfed by the hyphae of *Trichoderma* and treated seeds reduced the post-emergence death of seedling (Younis, 2005). Several researchers also reported that the antagonistic activity of different *Trichoderma* isolates against various phytopathogenic fungi such as *R. solani*, *F. oxysporum* and *S. rolfsii* (Deshmukh and Raut 1992, Xu *et al.* 1993, Askew and Laing 1994). The results clearly indicated

that Tricho-compost possessing *T. harzianum* enhanced plant growth and gave higher grain yield of lentil. Several researchers reported that application of *Trichoderma* as a seed-treating agent resulted in higher shoot length, root length, and shoot weight of vegetable seedlings (Hossain and Shamsuzzaman 2003, Hossain and Naznin 2005, Shaban and El-Bramawy 2011). Hannan *et al.* (2012) reported that seed treatment with BINA bio-fertilizer and BAU-bio-fungicide as well as application of cow dung in the soil enhanced shoot and root growth with increased biomass production of lentil. This finding is also supported by other researchers (Devi *et al.* 2003, Hossain *et al.* 1999, Sultana and Hossain. 1999). On the other hand several reports showed that soil and foliar application of *T. harzianum* reduced the population of soil-borne phytopathogens, especially *S. rolfisii*, *F. oxysporum*, *Rhizoctonia solani* and *S. sclerotiorum* (Sivan *et al.* 1984, Sivasithamparam and Ghisalberti 1998, Hoitink and Boehm 1999). Thus it was revealed from the investigation that soil amendment with Tricho-compost was an effective option for reducing seedling mortality and increasing plant growth as well as grain yield of lentil in *S. rolfisii* and *Fusarium oxysporum* sick soil.

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