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Bio-efficacy of Trichoderma-fortified compost in controlling onion diseases and improving yield of onion (*Allium cepa* L.)

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Abstract

In recent years, the genus *Trichoderma* has achieved a special position in the field of agriculture as a potent fungal bio-agent besides being a plant growth promoter and improving composting ability. They are enormously interactive in root, soil and foliar environments and economically viable for combating a wide range of plant diseases. In this study, an attempt was made to control seedling mortality, damping off diseases of onion at different growth stages caused by several fungal pathogens using *Trichoderma*-fortified compost mixed with different substrates including saw dust, cowdung, tea waste, water hyacinth and poultry refuges. Pathogens included *Alternaria porri, Fusarium oxysporum*, *Rhizoctonia solani,* and *Sclerotium rolfsii* in onion field under natural epiphytotic conditions. Additionally we also observed the effect of *Trichoderma*-fortified composts on growth promotion components and yield of onion. Among the 20 isolates screened in dual culture assay on PDA, *T. harzianum* isolate Pb-9 was found to show the highest radial growth inhibition of *Alternaria porri, Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotium rolfsii*. *Trichoderma*-fortified compost with poultry manure was found most effective in reducing seedling mortality, damping off, incidence and severity of foliar and soil-borne diseases in onion field. The lowest disease incidence and severity were observed with spraying spore suspension of *T. harzianum* Pb-9 on leaf in controlling purple leaf blight disease of onion caused by *A. porri*. All the treatments significantly increased the growth promoting components and yield where the highest increased was achieved with the treatment *Trichoderma*-fortified poultry manure compost.

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Introduction

Allium cepa L., the onion (also called bulb onion or common onion) is a monocot bulbous perennial (often biannual) crop. It is the most widely cultivated species of the genus *Allium*, which includes other important species such as garlic (*A. sativum*) and leeks (*A. ampeloprasum*). It is also used as very important vegetable worldwide in our daily diet. Onion has also been used as a folk remedy for its anti-infective properties and other beneficial effects such as in preventing or treating heart disease and atherosclerosis, diabetes, cancer, and possibly asthma (Griffiths *et al.*, 2002). Out of 15 vegetables listed by the Food and Agricultural Organization (FAO), onion ranks second in term of total annual world production. Onion is now grown almost all over Bangladesh and amongst all spices, it falls first in terms of production and second with respect to cultivated area. However, domestic production is insufficient to meet countries demand, so imports are necessary.

Among several factors, diseases are the most important factor associated with low productivity in onion due to susceptible to several foliar, bulb and root pathogens that reduce yield and quality (Cramer, 2000). Most of the diseases are caused by fungi that attack the crop during various growth stages of onion. Among the various diseases, purple leaf blotch (PLB) of onion caused by *Alternaria porri* (Aveling *et al.*, 1994, Suheri, and Price, 2000) is a major constrain in the production of both bulb and seed yield of onion worldwide especially in warm and humid environments like Bangladesh (Maude, 2006; Miller and Lacy, 1995, Ali *et al.*, 2016;). Early symptoms appear on the older leaves as white flecks and then under suitable environmental conditions the white flecks expand and produce sunken purple lesions that are often elliptical with a yellow to pale-brown border. Moreover, soil-borne disease problems of seeded and transplanted onions including damping-off, southern blight, pink root, Fusarium basal or plate rot etc. were also a major limiting factors in the production of onion (Maude, 2006). Soil-borne diseases such as damping off caused by *Fusarium* and *Rhizoctonia* species and southern blight caused by *Sclerotium rolfsii* can reduce plant survival, bulb size and quality and thereby affect crop productivity. Therefore, it is an urgent need to take efficient control strategies against these pathogens for upsurge the production of onion.

Several investigators demonstrated different botanical extracts, bioagents and fungicides and they found effective against onion leaf diseases. Though it is established that pesticides are effective in controlling different onion diseases including purple blotch but it creates lots of problems such as deterioration of soil texture and structure; contaminate soil by toxic substances, harmful effect on beneficial microorganisms. As a result, soil productivity, plant resistance and agro-ecosystem as a whole is now in threaten. Therefore, for the protection of our soil environment from chemical fertilizer and pesticides, huge amount of rapid bio-agent enriched compost application on soil is indispensible. The biocontrol of plant pathogens such by antagonistic fungus *Trichoderma* is the best alternative, due to the advantages such as cost-effective, eco-friendly, enhanced penetration and composting remarkably (Saba *et al.*, 2012). The beauty of *Trichoderma* is that it can be used to combat almost every pathogenic fungus that people want to control. *Trichoderma* may be produced in liquid form to be sprayed on leaves for the treatment of foliar fungal diseases. *Trichoderma* spp. with different formulations can be applied as seed treatment, soil treatment, seedling dips and foliar spray against the fungal pathogens of vegetables (Bhattacharjee and Dey, 2014). Several workers investigated that application of *Trichoderma harzianum* in the field resulted lower disease incidence of purple blotch disease (Abo-Elyousr *et al.*, 2014). Recently in Bangladesh, *Trichoderma*-fortified compost was found most effective in controlling different diseases as well as increased yield of other vegetables significantly (Liton, 2014). The quality and quantity of compost applied to soil affects the growth of plants and its disease suppressive capability (Gomez, 1998; Noble and conventry, 2005; Hadar and Papadopoulou, 2012).

In Bangladesh, research on rapid preparation of compost using *Trichoderma* and value addition of compost with mixing *Trichoderma* and other agents before field application was not adequate. Considering the above mentioned facts, the aims of the current study were to find out the most effective isolates of *Trichoderma* as antagonist against seedling mortality/damping off and foliar diseases of onion, to select the best composting material compatible with *T. harzianum,* and to develop a management strategy for suppressing diseases of onion and increasing yield using effective *Trichoderma*-fortified compost.

Materials and methods

The experiment was carried out in the laboratory and research field of the Plant Pathology Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur during 2014-2015. An attempt was made to reduce the post-emergence seedling mortality/damping off, diseases of plants at different growth stages and bulb diseases of onion caused by several fungal pathogens in the field under natural epiphytotic conditions and also to increase the growth promotion factors and yield of onion through the application of *Trichoderma*-fortified compost. A series of preliminary experiments were conducted before the field trial to select an effective isolate of *Trichoderma* species.

Collection, Isolation and identification of Trichoderma harzianum

The isolates of *Trichoderma* species were incubated from soils of twenty different crop fields of Pabna districts of Bangladesh. Soil samples were collected from rhizophere soil of carrot, radish, tomato, potato, brinjal, Chilli, soybean, papaya during the period of May-July 2014. Fungi were isolated from individual samples following the soil dilution plate technique (Warcup, 1957). Soils were collected from at least three place of a particular crop field and mixed together to make an individual sample. Ten gm of soil from a sample was mixed with 90 ml of sterilized water in a sterile conical flask and the content was stirred with a magnetic stirrer for about 20 minutes. A series of soil dilution prepared in flask were agitated on a vortex for two minutes for thoroughly mixing. Then 5ml of each dilution was incorporated into a potato dextrose agar (PDA) amended by 100 ppm streptomycin sulfate. The soil suspension in PDA plate was spread evenly using a turntable. The Petri dishes were incubated for 5-7 days at room temperature (25±2 ^{o} C). Fungus was purified on PDA following hyphal tip culture technique (Tuite, 1969). A total of 20 fungal isolates were identified as *Trichoderma harzianum* on the basis of growth, colony and morphological characters following the standard key (Barnett and Hunter, 1998). The other isolated fungi were discarded. The isolates of the pure culture of *T. harzianum* were preserved by using PDA slants at 15^{o}C in refrigerator as stock culture for future use.

In vitro screening of Trichoderma harzianum isolates against Alternaria porri, Fusarium oxysporum, Rhizoctonia solani and Sclerotium rolfsii

An *in vitro* study was undertaken to find out the antagonistic effect of 20 selected isolates of *T. harzianum* against *A. porri, F. oxysporum, R. solani* and *S. rolfsii* on PDA following dual plate culture technique (Dhingra and Sinclair , 1995). The selected virulent isolates of the pathogens were collected from the stock culture of the laboratory of the Department of Plant Pathology, BSMRAU. Discs of mycelium (5mm diameter) of each of the isolates of *T. harzianum* and *A. porri*, *F. oxysporum, R. solani* and *S. rolfsii* were cut from the edge of an actively growing colony with a cork borer (5 mm diameter). One mycelial disc of individual isolate of *T. harzianum* and one disk of test fungal pathogen was placed simultaneously on the edge of the each PDA Petri plate at opposite direction. The plates only with the discs *of A. porri*, *F. oxysporum, R. solani and S. rolfsii* in the centre were used as controlled plate. The plates were then incubated at room temperature until the mycelium of pathogen covered the whole plate. The growth of the colonies of each tested pathogens were measured after the complete growth of the control plates. Inhibition of the radial growth of *A. porri*, *F. oxysporum, R. solani* and *S. rolfsii* were calculated based on the following the formula as suggested by Sundar *et al.*, (1995).

$$\% \text{ Inhibition of growth} = \frac{X - Y}{X} \times 100$$

Where, X= Mycelial growth of the pathogen in absence of antagonist i.e. control and Y= Mycelial growth of the pathogen in presence of antagonist.

Isolates having strong suppressive effect on mycelial growth of *A. porri, F. oxysporum, R. solani* and *S. rolfsii* were selected for further study. In addition, we also scored the lysed mycelium of *A. porri, F. oxysporum, R. solani* and *S. rolfsii* by *T. harzianum* using the modified Bell's scale (Bell *et al.*, 1982), where R1= 100% overgrowth, R2= 75% overgrowth, R3= 50% overgrowth, R4= block at the point contact, R5= pathogen overgrowth antagonistic. The plates were arranged in Completely Randomized Design (CRD) with three replications.

Preparation of T. harzianum inoculum

Based on the in vitro screening test, the inoculum of the highly antagonist isolate of *Trichoderma harzianum* Pb-9 against the highly virulent isolates of *A. porri*, *F. oxysporum*, *R. solani* and *S. rolfsii* were prepared on wheat grains soaked in water for 12 hours in 1000 ml Erlenmeyer flask prescribed by Chang and Tin-En (2008). Wheat grains (500g) were drained off and poured into 1000 ml Erlenmeyer flask. Twelve to fifteen mycelia discs (5 mm) of T. harzianum isolate Pb-9 grown on PDA were added to the flasks containing autoclaved wheat grain and incubated at 250 C for 21 days. They were shaken by hand at 2-3 days interval for even colonization. The colonized wheat grain *T. harzianum* was air dried for 1 week and stored at 100C for using as inocula of colonized wheat grain *T. harzianum* isolate Pb-9.

Preparation of T. harzianum-fortified compost with different substrates

Before setting the experiment in the field, *T. harzianum*-fortified compost in different substrates were prepared using saw dust, cow dung, tea waste, water hyacinth and poultry refuges separately in a 1.0 m x 1.0m x 1.5m different composting pit and covered with polythene sheet.

A total of five compost pits were prepared where each compost pit contains 40 kg saw dust, cow dung, tea waste, water hyacinth and poultry refuges, respectively and after 45 days of decomposition, 2.5 kg of wheat grain colonized *T. harzianum* inocula prepared previously was mixed in each five different compost pits. Compost pits were allowed for 90 days for decomposition and degradation following the procedure of the preparation of standard quality compost.

Trichoderma-fortified compost of different substrates applied in the field

A field study was conducted to assess the most effective composting substrate to mix with *T. harzianum* isolate Pb-9 for controlling foliar and soil borne diseases as well as its impact on growth promotion and increasing yield of onion. Prepared *Trichoderma*–fortified compost in different substrates as described earlier were used in the field as the treatments. Well decomposed *Trichoderma*-fortified compost@ 5 kg/ plot (8.33 t ha^{-1}) was applied during the land preparation on the basis of the treatment layout. Three sprays of *T. harzianum* spore suspension were given at 15 days interval starting after one month of planting in all the treatments except in the untreated control treatment. No chemical fertilizer was used in any of the treatment.

Plantation of seedlings

Twenty five days aged apparently healthy onion seedlings variety 'Taherpuri" collected from local market were planted at 30-35 cm of row to row distance and 10-12 cm of plant to plant distance. A total of 168 seedlings were planted in each plot and also maintained intercultural operations.

Data Collection

Onion plants were observed regularly immediately after transplantation to record the incidence of post emergence seedling mortality/damping off, different diseases at different stages of growth. The diseases of the infected onion plants were identified based on characteristic symptoms of the diseases.

The causal agents of the recorded diseases were identified on isolation of the pathogen from the infected leaves and bulbs. The disease incidence was recorded continuously at 3 days interval from transplanting to final harvest. Observations were made by selecting six plants randomly from each plot. Diseases of the crop were measured by using the following formula and expressed as percentage:

% Disease incidence=

$$\frac{\text{Number of infected plants}}{\text{Total number of plant observed}} X100$$

Development of the leaf blight disease of onion seedlings were scored for disease severity by following 0-5 scale as given by Islam *et al.,* 1999: 0. No disease symptom 1. A few spots towards tip covering l0% leaf area 2. Several dark purplish brown patch covering up to 20% leaf area 3. Several patches with paler outer zone covering up to 40% leaf area 4. Leaf streaks covering up to 75% leaf area or breaking of the leaves from center 5. Complete drying of the leaves or breaking of the leaves from center.

Per cent Disease Index (disease severity) was calculated by using the formula as stated by Baker (1970).

Percent disease index (PDI)=

$$\frac{\text{Summation of all rating}}{\text{Number of plant observed} \times \text{Maximum rating in the scale}} \times 100$$

Growth promoting factors including root length, root diameter, plant height, number of leaves, number of branches, individual bulb weight, bulb diameter were recorded randomly taken five plants from each replication of all the treatments after certain maturity. Bulbs were harvested on March 2015 when necks of the onions were fallen.

Experimental design and statistical analysis

The experiment was laid out in the Randomized Complete Block Design (RCBD) with four replications. After necessary transformation data recorded on various disease components, growth promoting factors and yield were analyzed statistically using the MSTAT-C computer program. The means were compared following Duncan's Multiple Range Test (DMRT) using the statistical computer package program, MSTAT-C.

Results

Screening of Trichoderma harzianum against virulent isolates of test pathogens

To observe the antagonistic effect of *Trichoderma harzianum* a total of 20 selected isolates were tested against *A. porri, F. oxysporum, R. solani* and *S. rolfsii* on Potato Dextrose Agar (PDA) media by dual culture technique. All isolates of *T. harzianum* showed more than 50% inhibition of radial growth of the tested pathogens as compared to that of untreated control (Table 1 and Fig. 1.). Among them, *T. harzianum* Pb-9 displayed the highest inhibition of the radial growth against all the tested pathogens. The lowest 55.23% growth inhibition was observed against *F. oxysporum* and *R. Solani* with the isolate *T. harzianum* Pb-17 followed by 63.34% radial growth inhibition against *S. rolfsii* and 66.45% radial growth inhibition against *A. porri.*

Table 1. In vitro inhibition of radial growth of *Alternaria porri*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii* by different isolates of *Trichoderma harzianum* in dual culture technique.

Isolates	% Inhibition				Bell's scale*			
	A. porri	*F. oxysporum*	*R. solani*	*S. Rolfsii*	*A. porri*	*F. oxysporum*	*R. solani*	*S. Rolfsii*
T. harzianum Pb-1	68.56	65.54	70.00	65.55	R 2	R 2	R 2	R 2
T. harzianum Pb-2	67.99	63.23	62.55	67.38	R 2	R 2	R 2	R 2
T. harzianum Pb-3	71.11	72.55	55.56	77.76	R 2	R 2	R 2	R 1
T. harzianum Pb-4	68.89	68.94	75.57	76.66	R 2	R 2	R 2	R 1
T. harzianum Pb-5	71.54	71.67	71.23	78..56	R 2	R 2	R 2	R 1
T. harzianum Pb-6	72.05	68.98	64.56	63.52	R 2	R 2	R 2	R 2
T. harzianum Pb-7	69.26	76.56	66.58	58.28	R 2	R 1	R 2	R 2
T. harzianum Pb-8	73.13	73.57	68.99	75.55	R 2	R 2	R 2	R 2
T. harzianum Pb-9	76.50	82.54	79.66	81.12	R 1	R 1	R 1	R 1

Isolates	% Inhibition				Bell's scale *			
	A. porri	*F. oxysporum*	*R. solani*	*S. Rolfsii*	*A. porri*	*F. oxysporum*	*R. solani*	*S. Rolfsii*
T. harzianum Pb-10	74.31	77.57	70.00	62.24	R 2	R 1	R 2	R 2
T. harzianum Pb-11	73.33	66.43	66.65	65.58	R 2	R 2	R 2	R 2
T. harzianum Pb-12	72.33	56.44	73.34	73.34	R 2	R 2	R 2	R 2
T. harzianum Pb-13	74.66	68.98	74.55	78.48	R 2	R 2	R 2	R 1
T. harzianum Pb-14	67.56	71.90	62.21	80.00	R 2	R 2	R 2	R 1
T. harzianum Pb-15	72.22	73.95	57.27	75.55	R 2	R 2	R 2	R 2
T. harzianum Pb-16	71.43	78.58	63.38	66.62	R 2	R 1	R 2	R 2
T. harzianum Pb-17	66.45	55.23	55.23	63.34	R 2	R 2	R 2	R 2
T. harzianum Pb-18	72.52	75.56	74.54	71.11	R 2	R 2	R 2	R 2
T. harzianum Pb-19	69.58	75.64	77.77	73.50	R 2	R 2	R 1	R 2
T. harzianum Pb-20	73.24	67.77	71.76	78.88	R 2	R 2	R 2	R 1

* R_1=>75-100% growth inhibition of the pathogens, R_2= >50% - <75% growth inhibition of the pathogens, R_3 = > 25% - <50% growth inhibition of the pathogens, R_4= > 10% - <25% growth inhibition of the pathogens, R_5= pathogen overgrowth antagonist.

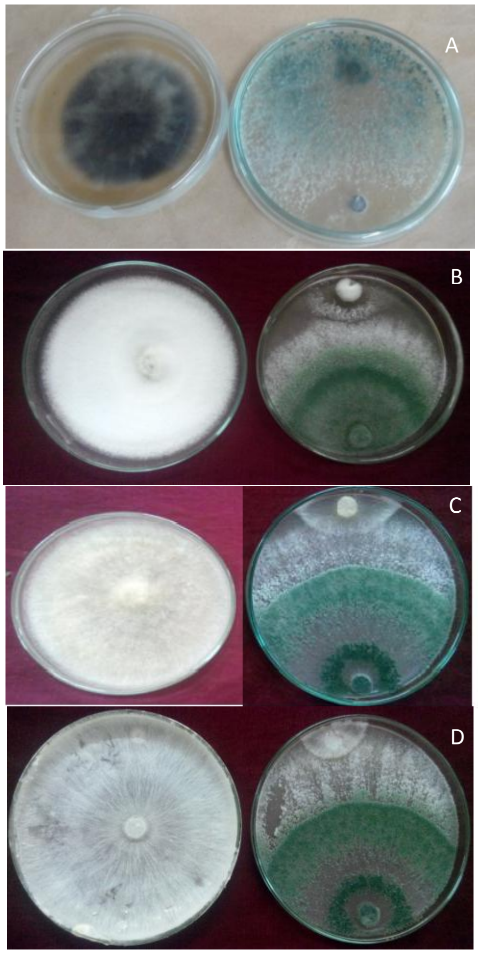

Fig. 1. Growth inhibition of different pathogens by *T. harzianum* isolate Pb-9 in dual culture PDA plate (right) and untreated control plate (left) along with dual culture. [Plate (right): *T. harzianum* isolate Pb-9 with A) *A. porri;* B) *F. Oxysporum;* C) *R. solani;* and D) *S. rolfsii*].

Among the tested isolates, *T. harzianum* Pb-9 exhibited growth inhibition at Bell's scale R1 (>75-100% overgrowth on the mycelial growth) against all the tested pathogens (Table 1 and Fig. 1.). Additionally, majority of the *T. harzianum* isolates showed R2 antagonism (>50% - <75% overgrowth of *Trichoderma*) against all the tested pathogens at Bell's Scale. However, four *T. harzianum* isolates namely Pb-7, Pb-9, Pb-10 and Pb-16 showed growth inhibition at Bell's scale R1 against *F. oxysporum* and two isolates *T. harzianum* Pb-9 and *T. harzianum* Pb-19 showed growth inhibition at Bell's scale R1 against *R. solani* and seven isolates of *T. harzianum* namely Pb-3, Pb-4, Pb-5, Pb-9, Pb-13, Pb-14, and Pb-19 showed growth inhibition at Bell's scale R1 against *S. rolfsii*. Based on the screening test, the highly antagonist *Trichoderma* isolate Pb-9 was selected to prepare the *Trichoderma* -fortified compost and preserved in the PDA slant at 10° C for further use.

Consequence on seedling mortality

Immediately after transplantation of onion seedlings, seedling mortality/damping off caused by *F. oxysporum, R. solani*, and *S. rolfsii* were recorded until two weeks of the field growth. The highest reduction of total seedling mortality (45.51%) over control was observed in the *Trichoderma*-fortified compost mixed with poultry manure used as substrate. In contrast, *Trichoderma*-fortified composts where poultry manure, water hyacinth and tea waste were used as substrates in the treatments, respectively were identical in reducing seedling mortality/ damping off and appeared superior to other compost (Table 2).

Table 2. Effect of *Trichoderma*-fortified compost on seedling mortality of onion in the field.

Treatments	% Seedling mortality/ damping off*	% Reduction of mortality over control
Untreated control	11.45 e	....
Only *Trichoderma* inoculum	10.56 de	7.77
Colonized *Trichoderma* with saw dust	9.97cde	12.93
Colonized *Trichoderma* with cow dung	8.48bcd	25.94
Colonized *Trichoderma* with tea waste	7.44abc	35.02
Colonized *Trichoderma* with water hyacinth	8.63ab	24.63
Colonized *Trichoderma* with poultry manure	6.25 a	45.41

*Means in a column followed by the same letters does not differ significantly (p= 0.05) according to Duncan's multiple range test.

Control of disease development in the onion field

The development of diseases at different stages of plant growth was recorded continuously at 3 days interval from transplanting to final harvest. The two major diseases observed in the field were leaf blight/ purple blotch caused by *Alternaria porri* and southern blight caused by *Sclerotium rolfsii*. Among the treatments, *Trichoderma*-fortified compost mixed with poultry manure substrate had the lowest disease incidence (10.34%) while the highest disease incidence due to the prevalence of the foliar and soil-borne pathogens in the natural onion field was recorded in the untreated control plot (37.03%) where no *Trichoderma*-fortified compost was applied (Table 3). However, the level of disease incidence did not differ statistically between the colonized *Trichoderma* with poultry manure and with tea waste against *A. porri* while it was similar among the *Trichoderma*-fortified with poultry manure, tea waste, cow dung, and water hyacinth as substrates against *Sclerotium rolfsii*. In addition, all the *Trichoderma*-fortified composts were also effective to reduce the incidence and severity of both the diseases compared to that of untreated control (Table 3).

Table 3. Effect of *Trichoderma*-fortified compost on disease incidence and severity (PDI) of onion diseases in the field condition.

Treatments	Purple leaf blotch			Southern blight		
	% Disease Incidence*	% Reduction over control	PDI	%Disease Incidence*	% Reduction over control	PDI
Untreated control	37.03 a	....	34 a	24.20 a	....	27.50 a
Only *Trichoderma* inoculum	29.45 b	20.46	29ab	23.08 a	4.65	23.75ab
Colonized *Trichoderma* with saw dust	26.41 b	28.69	20bc	22.40 a	7.43	21.25ab
Colonized *Trichoderma* with cow dung	24.63 b	50.37	12cde	14.98 b	38.12	16.25bc
Colonized *Trichoderma* with tea waste	16.95 c	54.23	10 de	14.35 b	40.70	13.75bc
Colonized *Trichoderma* with water hyacinth	25.74 b	30.50	18 cd	22.35 a	7.64	18.75abc
Colonized *Trichoderma* with poultry manure	10.34 c	72.08	8 e	8.58 c	64.53	8.75 c

*Means within same column followed by common letter(s) are not significantly different (P=0.05) by DMRT.

Effect of Trichoderma-fortified compost on growth promotion components and improving yield of onion

All the *Trichoderma*-fortified compost treatments significantly increased the growth promotion components compared to untreated control plot. Statistically similar plant heights were recorded in all the treatments except the untreated control but *Trichoderma* with poultry manure compost provided numerically higher plant height (Table 4).

The growth promotion components including number of leaflet plant^{-1} and root length were also increased significantly in the treatment where *Trichoderma* were mixed with poultry manure. *Trichoderma* colonized with poultry manure numerically provided the highest production of bulb yield 8.67 ton ha^{-1} followed by colonized *Trichoderma* with tea waste (7.92 ton ha^{-1}), cow dung (7.08 ton ha^{-1}), and water hyacinth treated plots (6.75 ton ha^{-1}), respectively but statistically there were no differences among these *Trichoderma*-fortified treatments (Table 4). However, all the *Trichoderma*-fortified compost treatments significantly increased the bulb yield and yield related components in comparison to untreated control.

Table 4. Effect of *Trichoderma*-fortified compost on growth promotion and yield components of onion in field condition.

Treatments	Plant height (cm)	No of leaflet /plant	Root length (cm)	No of bulb /plot	Individual bulb wt.(g)	Bulb diameter (mm)	Bulb length (mm)	Total yield (ton ha^{-1})
Untreated control	16.94 b	4.75 d	3.08 c	121.50 e	31.50 b	38.55 c	34.05 c	4.50 c
Trichoderma inoculum without any pathogen	19.64 ab	5.5 cd	4.62 bc	125.30 d	32.20 ab	39.35 c	35.63 bc	5.25 bc
Colonized *Trichoderma* with Saw dust	20.02 ab	6.0 cd	5.39 abc	128.00 d	29.80 d	39.98 bc	37.53 ab	5.58 bc
Colonized *Trichoderma* with Cow dung	21.94 a	7.0 bc	6.54 ab	134.30 c	31.17 bc	41.95 ab	38.33 ab	7.08 ab
Colonized *Trichoderma* with Tea waste	22.33 a	8.25 ab	6.93 ab	141.00 b	32.90 a	42.22 ab	38.53 ab	7.92 a
Colonized *Trichoderma* with water hyacinth	20.40 ab	6.25 cd	6.16ab	133.00 c	30.15 cd	40.72 abc	37.85 ab	6.75 abc
Colonized *Trichoderma* with Poultry manure	23.48 a	9.00 a	7.70 a	150.30 a	33.25 a	42.47a	40.65 a	8.70 a

*Means within same column followed by common letter(s) are not significantly different (P=0.05) by DMRT.

Discussion

Seedling mortality/damping off caused by *F. oxysporum*, *R. solani*, and *S. rolfsii* and purple blotch of onion infected by *Alternaria porri* are primarily recognized as one of the most serious threats in onion production all over Bangladesh. A series of experiment were carried out in laboratory and filed to find out the comparative performance of few organic wastes compatible with *T. harzianum,* and to establish a management strategy in reducing onion diseases and in improving yield using effective *Trichoderma*-fortified compost.

In the present experiment, twenty isolates of *Trichoderma harzianum* were screened against *A. porri*, *F. oxysporum*, *R. solani* and *S. rolfsii* following dual plate culture technique.

In vitro studies clearly showed that *T. harzianum* isolate Pb-9 had the ability to inhabit the highest radial mycelial growth of all the test pathogens although different isolates exhibited varying levels of antagonism against them. Many researchers reported the significant effect of *T. harzianum* against *R. solani* and *S. rolfsii* on PDA plates. In Bangladesh, different species of *Trichoderma* have been showed to be effective antagonist against *Fusarium oxysporum* and *Sclerotium rolfsii* (Hossain, 2000; Fayad, 2013; Kashem *et al.,* 2016). The mode of inhibition in mycelial growth of all the tested pathogen could be through various mechanisms such as mycoparasitism, antibiosis, lysis, competitive, metabolites secretions, competition and modulation of induced resistance (Schirmbock *et al.*, 1994, Fotoohiyan *et al.,* 2015).

Trichoderma spp. are early colonizers of substrates and reduce the activity of other fungi simply by substrate occupation and exhaustion (Martin and Loper, 1999) and this would be in accord with the present observations. The variation among the different isolates of *T. harzianum may* be occurred due to their diverse genetic makeup (Hermosa *et al.*, 2000) for the antagonistic activity, virulence factor, degree of virulence, trichodene, etc. Other authors such as Kumar *et al.*, 2012 also observed that *T. harzianum* showed antagonistic activity by the production of significant chitinase and β-1,3-glucanase in growth medium. Our results revealed that the radial mycelial growth of the pathogens was limited within the contact area or interface zone, resulting in lysis and disrupting the mycelium of plant pathogenic fungi. Therefore, highest reduction of radial growth of the test pathogen obtained by *T. harzianum* pb-9 isolate in our study could be happened due to the more secretion of cell wall degrading enzymes.

Compost and compost extracts considered as bio-fertilizers have been found to enhance plant growth and to suppress pathogens (Gharib *et al.*, 2008; Naidu *et al.*, 2010). Our results in field experiment visibly exhibited that poultry manure mixed with *T. harzianum* isolate Pb-9 was able to reduce the seedling mortality, damping off and both the incidence and severity of foliar and soil-borne diseases of onion while *Trichoderma mixed* with all other substrates also significantly reduced diseases as compared to untreated control. Interestingly, our observations showed that application of spore suspension of *Trichoderma* considerably reduced the appearance of purple blotch in the field. Several authors demonstrated that some native isolates of *Trichoderma* spp. were found to exhibit successful antagonism against not only of soil-borne pathogens (Amin and Razdan, 2010) but also of foliage pathogens (Elad, 1994). The amendments of soil with *Trichoderma* isolates effectively enhanced decay of the often heterogeneous substrates resulting expanded penetration into the host tissues.

Additionally organic amendment produces volatile and nonvolatile sub-stances during their decomposition and aids in the introduction and establishment of antagonist into the soil for sustained bio-control activities of soil microbiota (Hoitink and Boehm, 1999). The results obtained from this study support the idea showed suppression of the colonization of roots by the pathogens because *Trichoderma* spp. is predominant in niche and efficiently nutrient competition. Some authors such as Uddin *et al.* (2011) reported that seed treatment with *T. harzianum* along with soil amendment of poultry refuse and vermicompost offered better performance against damping off disease caused by *S. rolfsii, F. oxysporum, Pythium* spp.*, R. solani* and *Phytophthora* spp. of potato and chilli. These results also support our observations examined the effects of *T. harzianum* along with composting substrates in promoting seedling establishment, enhancement of plant growth and reduction of plant diseases. *Trichoderma*-fortified compost with poultry manure was found to be significantly superior compared to other treatments in respect of disease incidence and percent disease index (PDI).

Under field conditions, an integrated management strategy that combined the use of *T. harzianum* with poultry substrates was appeared to be significantly more superior in improving growth promotion components and yield in onion when compared to dual and individual application of them although all the treatments significantly increased all the growth promoting components and yield in comparison to the untreated control. In the management of soil-borne diseases of several crops, an integrated approach involving microbial antagonist and different composting substrates were found highly effective and resulted in enhanced crop yield (Gharib *et al.* 2008, Nahar *et al.*, 2013; Olabiyi *et al.*, 2013). In this integration, an antagonist parasitizes the pathogens, and composting substrates improves soil nutrients status and properties and enhances the efficacy of antagonist (Chattopadhyay *et al.,* 1996) which are in partial agreement with findings of the present study.

Moreover, composting substrates such as poultry manure could be influence the more activity of *T. harzianum,* being developed as promising biological fungicides, and their weaponry function also through producing secondary metabolites with potential applications in the field of agriculture organic.

Results of the aforesaid study revealed that *Trichoderma* compost prepared by using different composing substrates has high potential for success, especially for poultry refuses in controlling foliar and soil-borne diseases of onion as well as in increasing the bulb yield. However, the study should be continued to standardize the ratio and composition of the substrate to prepare the most effective *Trichoderma*-fortified compost. The farmers are therefore advised to use integration management strategies in order to effectively control the disease and get better crop performance. Otherwise it may necessitate the development of tolerant biotypes of the biological control agents to be utilized in the integrated approach.

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