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Effect of *Trichoderma* Fortified Compost on Disease Suppression, Growth and Yield of Chickpea

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Abstract- *Trichoderma species are commonly used as effective biological control agents against phytopathogens especially the soil-borne fungi while some isolates are able to ameliorate plant growth. In the present study, Trichoderma fortified compost with different substrates were evaluated to reduce the pre-emergence and post-emergence seedling mortality, diseases of stem and root of chickpea caused by several soil-borne fungal pathogens, including Fusarium oxysporum, Rhizoctonia solani and Sclerotium rolfsii at different growth stages in the field under natural epiphytotic conditions. Among the twenty isolates of T. harzianum, Co-7 showed the most effective antagonist against the test pathogens in dual culture method. In field experiment, subsequently it was used for inoculum preparation with colonized wheat grain and mixed with well-matured decomposed composting materials like, saw dust, cow dung, tea waste, water hyacinth and poultry manure. Trichoderma fortified compost with poultry manure was found significantly effective in reducing pre-emergence and post emergence seedling mortality, disease incidence and disease severity of chickpea in the field. Interestingly, all the treatments significantly increased but Trichoderma fortified compost with poultry manure was the best to boost seed yield and quality.*
Keywords— biocontrol, Cicer arietinum, seedling diseases, soil-borne fungi, yield.

I. INTRODUCTION

Chickpea (*Cicer arietinum*) is the world's fourth most important pulse crop after soybeans, beans and peas (FAO, 2012). Diseases are one of the main constrains for the low production of this crop (Godhani *et al*., 2010). Chickpea crop is attacked by wide range of pathogens including fungi, viruses, bacteria, nematodes and mycoplasma (Nene and Sheila, 1996). Some of the serious pathogens usually known as soil-borne pathogens in order of their importance including *Fusarium oxysporum* f. sp. *ciceri, Rhizoctonia solani*, and *Sclerotium rolfsii* are the most devastating in both seedling and mature stage. Under favourable conditions, outbreaks of these pathogens it cause yield and quality deterioration or, even there is a total crop failure and result in a huge economic losses.

Many attempts have been made to control *F. oxysporum* f. sp. *ciceri, R. solani,* and *S. rolfsii* including cultural or chemical methods (Jaacov, 2000) but neither cultural nor chemical measures alone were found to be effective against these pathogens. Controlling of these soil-borne diseases with fungicides is uneconomical and difficult to achieve because of the soil and seed-borne nature of the pathogen (Ahmad *et al*., 2010). Moreover, the application of fungicides causes groundwater pollution, killing of non-target beneficial flora and evolving fungicidal resistance variants of the pathogen. The antagonistic *Trichoderma*, a cosmopolitan soil and compost-borne saprophytic fungus can be used to suppress soil-borne pathogens that cause diseases such as damping-off, root rots, stem rots, and wilting in many vegetables. In addition to its effect as a natural enemy of plant pathogens, *Trichoderma* has also a positive impact on plant growth as it produces different kinds of secondary metabolites which are important for plant growth regulation (Vinale *et al.*, 2009) and improves the soil fertility by acting as decomposer. Composts or compost extracts used as an organic fertilizer have beneficial effects on plant growth and considered as a valuable soil amendment (Gharib *et al*., 2008). Recently *Trichoderma* fortified compost have been used in many countries and appeared very effective in controlling different soil-borne pathogen as well as increasing growth and yield of many crops (Kaewchai *et al*., 2009, Rahman, 2013). However, scanty published reports on disease suppression and improvement of growth and yield of this pulse crop are available in Bangladesh utilizing *Trichoderma* fortified compost. Therefore, this study was undertaken to select the most effective isolates of *Trichoderma* species against different soil-borne fungi of chickpea identified under lab condition and to assess the potential of *Trichoderma*-fortified compost in controlling fungal diseases and enhancing growth and yield of chickpea in the field.

II. MATERIALS AND METHODS

2.1 Collection, isolation and preservation of *Trichoderma* spp. isolates

A total of 20 isolates of *Trichoderma* spp. were collected from soils of different crop fields of Chandina Upazilla of Comilla districts of Bangladesh. Soils samples were collected from rhizophere soil of carrot, radish, tomato, potato, brinjal and chilli. Fungi were isolated from individual samples following the soil dilution plate technique (Mian, I995). Briefly, a total of 10 gm of soil from a sample was mixed with 90 ml of sterilized water in a sterile conical flask while suspension was in motion. The initial soil sample was diluted through serial dilutions in order to achieve a small number of colonies on each plate. Then 5 ml of each dilution was incorporated into a plate with PDA (potato, 250 g; dextrose, 20 g; agar, 20 g; distilled water upto 1L) amended by 100 ppm streptomycin sulfate. The Petri dishes were incubated for 3-5 days at room temperature (25±2°C). Fungus was purified on PDA following hyphal tip culture technique (Tuite, 1969). A total of 20 fungal isolates were identified as *T. harzianum* on the basis of growth, colony and morphological characters following the standard key (Barnett and Hunter, l998). The other isolated fungi were discarded.

2.2 Testing the antagonistic activity of *T. harzianum* isolates *in vitro*

Twenty isolates of *T. harzianum* were tested against *F. oxysporum, R. solani* and *S. rolfsii* on potato dextrose agar (PDA) medium f dual culture technique by placing 5 mm diameter young mycelium disc of pathogen at one end and that of the biocontrol agent at the other end of a 9 cm diameter Petri dish (Dhingra and Sinclair, 1985). The selected virulent isolates of test pathogens were collected from the stock culture of the laboratory of the Department of Plant Pathology, BSMRAU. All plates were incubated in the dark at 25°C until the mycelium of *F. oxysporum, R. solani* and *S. rolfsii* covered the whole area of the control plate. Inhibition percentage of the radial growth of *F. oxysporum, R. solani* and *S. rolfsii* were calculated following the formula as suggested by Sundar *et al.* (1995):

$$\text{\% Inhibition of growth} = \frac{X - Y}{X} \times 100 \quad \text{(equation 1)}$$

Where, X = mycelial growth of pathogen in absence of *T. harzianum* (control), Y= mycelial growth of pathogen in presence of *T. harzianum*).

2.3 Preparation of *Trichoderma* fortified compost with different substrates

Before setting the experiment in the field, a total of five compost pits (1.0 m x 1.0 m x 1.5 m) were prepared separately where each compost pit contained 40 kg each of saw dust, tea waste, poultry manure, water hyacinth and cow dung. After 45 days of decomposition wheat grain colonized *Trichoderma* inoculum @2.5 kg was mixed in each compost pit. Then it was left for 90 days for decomposition and degradation following the procedure of the preparation of standard quality compost as described by James (2008). On the basis of screening test against the studied virulent isolates of *F. oxysporum, R. solani* and *S. rolfsii*, the highly antagonist isolate of *T. harzianum* Co-7 was selected for the field experiment

2.4 Application *Trichoderma* fortified compost with different substrates for controlling major diseases of chickpea in field condition

A field experiment was conducted to evaluate the most effective composting substrate mixed with *T. harzianum* isolate Co-7 for controlling major soil borne diseases of chickpea as well as its impact on growth promotion and increasing the yield of chickpea. Prepared *Trichoderma* fortified compost @ 5 kg plot^{-1} (8.33 t ha^{-1}) with individual substrates as described earlier were used in the field as treatments. Seeds of chickpea (*Cicer arietanum*) variety BARI Chola-5 collected from Bangladesh Agricultural Research Institute, Joydebpur, Gazipur were grown for this purpose in a non-sterile field soil. This experiment included following seven treatments:

- T_1=sowing of seeds only in field soil (Control 1)
- T_2= Wheat bran colonized *Trichoderma* without compost (Control 2)
- T_3=Colonized *Trichoderma* with saw dust
- T_4=Colonized *Trichoderma* with cow dung
- T_5=Colonized *Trichoderma* with tea waste
- T_6=Colonized *Trichoderma* with water hyacinth
- T_7=Colonized *Trichoderma* with poultry manure

2.5 Monitoring of disease development

Chickpea plants were observed regularly after sowing to record the incidence of pre-emergence and post-emergence seedling mortality, infection both on plant organs and pods at different growth stages. The causal agents of the recorded diseases were identified on isolation of the pathogen from the infected roots, stems and pod. The disease incidence was recorded continuously at 3 days interval from transplanting to final harvest. Observations were made by selecting five plants randomly from each plot. Disease severity of wilt, wet root rot and collar rot caused by *F. oxysporum, R. solani* and *S. rolfsii*, respectively was rated as 0-4 scale in which 0= no symptoms, 1=1-25%, 2=26-50%, 3=51-75% and 4=76-100% of chickpea organ covered with lesions (Morid *et al*., 2012). Diseases of the crop were expressed as percentage using the following formulae:

- Disease incidence (%) $= \frac{\text{Number of infected plants}}{\text{Total number of plant observed}} \times 100$ (Equation 2)
- Percent disease index (PDI) $= \frac{\text{Summation of all rating}}{\text{Number of plant observed} \times \text{Maximum rating}} \times 100$ (Equation 3)

2.6 Observation of growth promoting factors at maturity and yield

Growth promoting factors including plant height, no. of branches plant^{-1} and numbers of pods plant^{-1} were recorded randomly five plants from each replicated plots of all the treatments attain after certain maturity. Pods plant^{-1} was harvested and the total dry weight of seed was also measured.

2.7 Design of experiment and data analyses

The field experiment was laid out in the Randomized Complete Block Design (RCBD) with four replications. Data recorded on various diseases and yield components were analyzed statistically using the STATISTIX 10 computer program after proper transformation whenever necessary and the means were compared following DMRT.

III. RESULTS

3.1 Screening of *T. harzianum* isolates against *F. oxysporum* f. sp. *ciceri, R. solani,* and *S. rolfsii*

All the 20 isolates of *T. harzianum* showed more than 60% inhibition of radial growth of the tested pathogens as compared to untreated control except the isolate Co-6 where the growth inhibition against *R. solani* was 58.8%. Among the screened isolates of *T. harzianum* only four isolates namely Co-5, Co-7, Co-12 and Co-20 showed above 70% growth inhibition against all the tested pathogens (Table 1). The same isolate of *T. harzianum* had highly varied antagonistic ability and it ranged from 60 to 80% depending on the test pathogen. However, the tested isolates Co-7 showed the highest inhibition of the radial growth among all the tested pathogens (Table 1 and Fig. 1).

3.2 Assessment of *Trichoderma* fortified composts applied in the chickpea field in controlling soil-borne diseases

3.2.1 Effect on seedling mortality

Immediately after sowing of chickpea seed, pre-emergence and post-emergence seedling mortality caused by *F. oxysporum, R. solani* and *S. rolfsii* were recorded up to five weeks of the plant growth. The highest reduction of total seedling mortality over control (69.31%) was recorded in plants receiving a combined treatment with the *Trichoderma* fortified compost mixed with poultry manure as substrate (T_7). However, the similar result with T_7 was also observed in treatment T_4 and T_5 where the field soil received cow dung and tea waste with *T. harzianum* isolate, respectively (Table 2). Other treatments (T_6 and T_3) showed statistically similar effect on seedling mortality in comparison to the untreated control where only chemical fertilizers were mixed without *Trichoderma* fortified compost.

3.2.1 Effect on disease development

All the *Trichoderma* fortified composts reduced superiorly diseases of chickpea in comparison to untreated control. Among the diseases, wet root rot caused by *R. solani* was the most prevalent followed by collar rot caused by *Sclerotium rolfsii* and Fusarium wilt caused by *F. oxysporum* (Table 3). Significantly the highest reductions of the marked three diseases over control were achieved with *Trichoderma* fortified compost with poultry manure (T_7) followed by the treatment containing mixtures of *Trichoderma* and cow dung (Table 3). Additionally, the highest reduction of PDI over control was also observed in the treatment T_7 (64.9% of *F. oxysporum*, 67.8% of *S. rolfsii* & 65.7% of *R. solani*) in case of all the diseases but those of treatments T_4 and T_5 were numerically almost similar followed by T_6 in case of collar rot and wilt (Table 4). However, in case of the reduction of PDI caused *R. solani* treatment T_7 was highest (65.7%) but T_4 and T_5 were almost numerically identical to T_7.

3.3 Performance of *Trichoderma* fortified compost on growth promoting components and yield of chickpea in the field

Trichoderma fortified compost increased the growth promoting components including the number of branch plant^{-1} and plant height compared to those of untreated control (T_1). In case of pods plant^{-1} treatments T_2, T_3, T_5, T_6 were statistically identical but significantly lower to those of T_7 treatment where *Trichoderma* were mixed with poultry manure (Table 5). The treatments containing mixtures of colonized *Trichoderma* with cow dung and *Trichoderma* with poultry refuses were found statistically similar in increasing all growth promoting characters. Similarly significantly the highest seed yield (1.54 t ha^{-1}) was recorded in the plot with *Trichoderma* compost using poultry manure (T_7) followed by treatment T_4 using cow dung as compost. On the other hand, identical seed yield and seed weight were observed in T_5 and T_6 treated plot but they were significantly lower than that of T_7 in seed yield (Table 5).

IV. DISCUSSION

Soil-borne pathogens such as *F. oxysporum* f. sp. *ciceri*, *R. solani* and *S. rolfsii* are primarily known as devastating pathogens to cause seedling diseases in chick pea cultivation. These pathogens are mostly difficult to control as they often reside alive for many years as sclerotia in soil or as mycelia in soil under several environmental conditions and subsequently attack the crops resulting poor yield. Now a-days, *Trichoderma* is widely considered as a potential cost-effective means against several pathogens attacking vegetables, fruits, field and industrial crops (Tran, 2010). In our study, we got many isolates of *Trichoderma harzianum* from rhizophere soil of different vegetables in Bangladesh. *In vitro* assay clearly showed that antagonists *T. harzianum* halted the radial mycelium growth of highly virulent isolate *F. oxysporum*, *R. solani* and *S. rolfsii*, with varying levels of antagonism. Similar antagonistic effect of *T. harzianum* against these soil-borne pathogens infecting many other crops was also observed by several other investigators (Sundar *et al*., 1995, Bhuiyan *et al*., 2007, Nitu et al., 2016). The variation among the different isolates of *T. harzianum* may be come about due to their genetic makeup for the antagonistic activity (Shanmugam *et al.*, 2008, Kumar *et al.*, 2011), production of virulence factor such as metabolites (Shentu *et al.*, 2014), trichodene (Malmierca *et al*., 2015) etc. Moreover, a variety of extracellular lytic enzymes such as high chitinase and β-(1,3)-glucanase have been reported to be produced by *T. harzianum* (Kumar *et al.,* 2012), and there may be relationship between the production of these enzymes and the ability to inhibit the pathogen (Elad et al., 1982, Sivan and Chet, 1989). Results in this study also revealed that the radial mycelial growth of the pathogens was interfered by *T. harzianum* within the contact area or interface zone.

Under field conditions, we have observed seedling mortality caused by *F. oxysporum*, *S. rolfsii*, and *R. solani*. Some reports accounted these soil-borne pathogens causing diseases in different vegetable crops including chick pea (Prashad *et al*. 2014, Akhter *et al.*, 2015). As these warm-dependent pathogens are mostly common in Bangladesh soil, soil amendments using composted agricultural wastes fortified with biocontrol agents could be acceptable approaches in this regard. A number of investigations have also showed that the organic amendments of soil with various origins were potential biological control agents in suppressing soil-borne plant pathogens (Litterick *et al*. 2004). Result of this study is also consistent with the consequence of a variety of soil amendments (Noble and Coventry 2010, Akter *et al.*, 2016). The highest disease occurrence in the untreated control plot (T_1) in case of all diseases followed by treatment T_2 where wheat bran colonized *Trichoderma* without compost was used indicated that the soil-borne pathogens were the most prevalent in the natural field condition even without artificial inoculation of the pathogens. Consequently, organic amendments with *Trichoderma* fortified with poultry manure yielded the lowest disease incidence as well as disease severity in this study. Moreover, crop treated with *T. harzianum* grown better and had higher yields to compare with the one without application. Different mechanisms have been suggested as being responsible for the action of individual bio-agents and composts. Disease suppressive effect of *Trichoderma* compost might be due to increase in microbial biomass of *Trichoderma* with the ideal food base compost, it aids in their introduction and establishment into the soil for sustained biocontrol activities of soil micro biota as stated by Hoitink and Boehm (1999). An antagonist parasitizes the pathogens, and poultry manure might be improved soil nutrients status and enhanced the efficacy of antagonist. The superior inhibitory effect of poultry manure is suggested to be related with the release of antifungal compounds from it. Poultry compost contains NH4-N and NO3-N while Brady (1974) claimed that nitrate-nitrogen can be readily taken by plants but where carbon-based organic residue are available, resident soil micro organisms tend to exploit NH4+ more quickly than plants. Considerable evidence has piled up to support the idea that ammonia liberated following application of high nitrogen amendments contributes to kill soil-borne pathogens (Shiau *et al*., 1999). Additionally, the bio-control activities of *T. harzianum* against *F. oxysporum* f. sp. *ciceri*, *R. solani* modulate the induced plant resistance and enhance the plant growth (Sivan, 1989, Malik *et al*, 2005, Saxena *et al*., 2015). These results support the present findings of controlling soil-borne diseases as well as increasing growth promoting parameters and seed yield of chick pea by *Trichoderma* fortified composting.

V. CONCLUSION

Results from this study showed that poultry manure was appeared to be excellent and promising substrates for the preparation of *Trichoderma* fortified compost in controlling soil-borne diseases of chickpea. *Trichoderma* fortified compost with different substrates had also better effect in increasing different growth parameters as well as yield of chickpea than the untreated control where *Trichoderma* was not mixed. Farmers can adopt eco-friendly control measures of different soil borne diseases of vegetables by using *Trichoderma* fortified compost with the lower cost in comparison to chemical pesticides which is a key to profitable organic farming. Overall, results obtained from this study clearly indicated that *Trichoderma* fortified compost was the most effective not only control the pathogenic diseases but also achieved directly through storage of compost carbon, and indirectly through enhanced plant growth and biological control which in turn contributes to increased soil carbon levels. However, the experiment should be repeated to standardize the ratio and composition of the compost substrate to prepare the most effective *Trichoderma* fortified compost.

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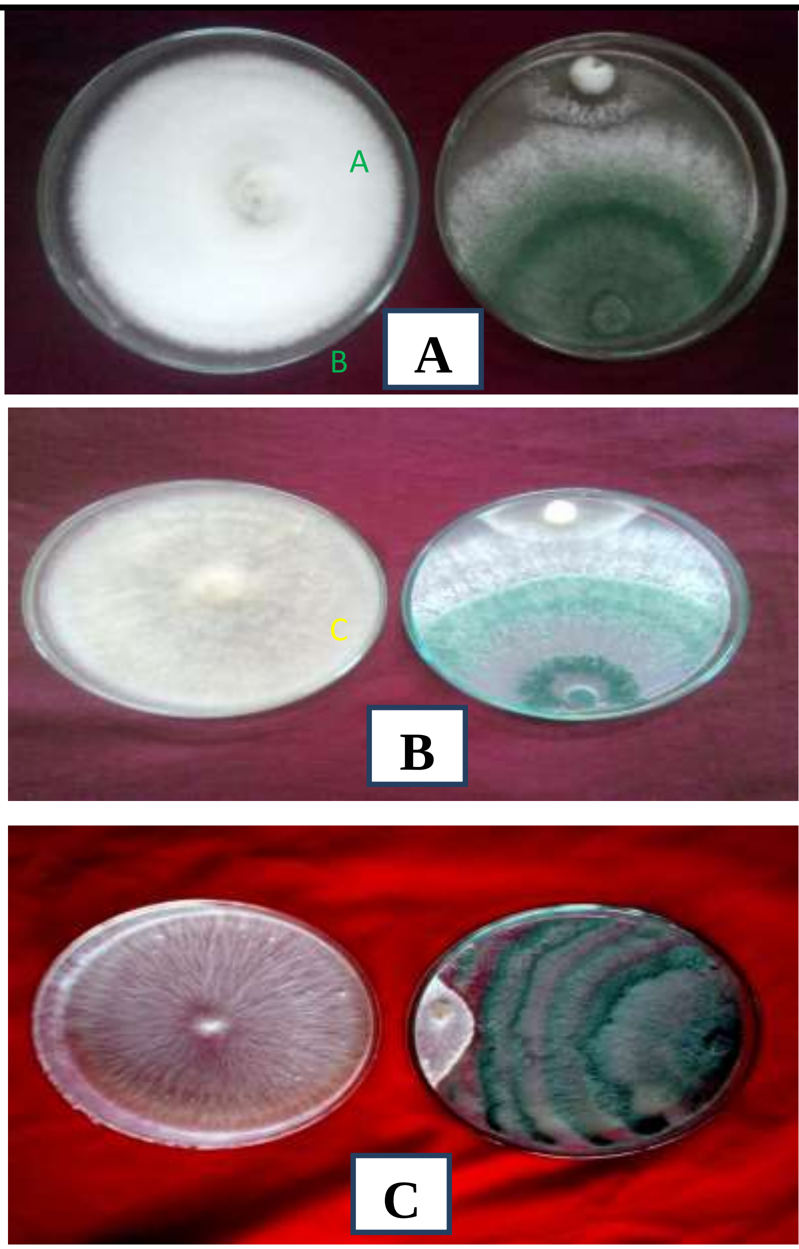

Fig. 1: Antagonism of T. harzianum isolate Co-7 in dual culture PDA plate against A. Trichoderma and Fusarium oxysporum, B. Trichoderma and Rhizoctonia solani, C. Trichoderma and Sclerotium rolfsii [right]; and control plates [left] along with dual culture.

Table 1. Screening of Trichodermaharzianum isolates against Fusarium oxysporum, Rhizoctonia solani and Sclerotium rolfsii by dual culture technique on PDA plates.

Isolates of *T. harzianum*	% inhibition of radial growth over control		
	F. oxysporum	*R. solani*	*S. rolfsii*
Co-1	70.00	67.7	77.7
Co-2	61.1	66.6	73.3
Co-3	71.1	64.4	68.8
Co-4	60.0	72.2	65.5
Co-5	76.6	73.3	77.7
Co-6	62.2	58.8	67.7
Co-7	82.2	78.8	83.3
Co-8	72.2	74.4	63.3

Co-9	64.4	62.2	66.6
Co-10	68.8	65.5	80.0
Co-11	62.2	81.1	71.1
Co-12	78.8	77.7	75.5
Co-13	71.1	80	67.7
Co-14	66.6	62.2	64.4
Co-15	76.6	68.8	72.2
Co-16	66.6	70.0	78.8
Co-17	73.3	65.5	61.1
Co-18	74.4	64.4	76.6
Co-19	72.2	63.3	66.6
Co-20	71.1	78.8	73.3

Table 2. Effect of Trichoderma fortified compost in controlling seedling mortality of chickpea in open field.

Treatments	% Mortality*			% Reduction of total mortality
	Pre-emergence	Post-emergence	Total	
T1= Untreated control	10.5 a	27.4 a	37.8 a	-
T_2= Wheat bran Colonized *Trichoderma* without compost	5.5 b	13.2 b	18.7 b	50.52
T_3= Colonized *Trichoderma* with saw dust	5.2 bc	12.0 b	17.3 b	54.23
T_4= Colonized *Trichoderma* with cow dung	4.1 d	8.9 d	13.0 d	65.6
T_5= Colonized *Trichoderma* with tea waste	4.5 bc	9.1 cd	13.6 cd	64.02
T_6= Colonized *Trichoderma* with water hyacinth	5.0 bc	11.6 bc	16.1 bc	57.4
T_7= Colonized *Trichoderma* with poultry manure	3.2 d	8.4 d	11.6 d	69.31

**Means in a column followed by the same letters does not differ significantly (p= 0.05) according to DMRT test.*

Table 3. Effect of Trichoderma fortified compost on incidence of chickpea diseases in the field.

Treatments	Fusarium wilt (*F. oxysporum*)		Collar rot (*S. rolfsii*)		Wet root rot (*R. solani*)	
	Disease incidence	% reduction over control	Disease incidence	% reduction over control	Disease incidence	% reduction over control
T1= Untreated control	36.4 a	-	35.9 a	-	25.7 a	-
T_2= Wheat bran Colonized *Trichoderma*without compost	26.6 b	26.9	25.2 b	29.8	20.9 b	18.7
T_3= Colonized *Trichoderma* with saw dust	22.7 c	37.6	21.8 c	39.3	16.2 c	36.9
T_4= Colonized *Trichoderma* with cow dung	17.3 e	52.5	16.8 e	53.2	12.9 e	49.8
T_5= Colonized *Trichoderma* with tea waste	19.5 de	46.4	19.5 de	45.7	14.6 de	43.2
T_6= Colonized *Trichoderma* with water hyacinth	22.5 c	38.2	21.4 c	40.4	15.2 cd	40.9
T_7= Colonized *Trichoderma* with poultry manure	13.2 f	63.7	12.9 f	64.1	12.5 f	51.4

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

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Table 4. Effect of Trichoderma fortified compost on severity of chickpea diseases in field.

Treatments	Fusarium wilt (*F. oxysporum*)		Collar rot (*S. rolfsii*)		Wet root rot (*R. solani*)	
	PDI	% reduction over control	PDI	% reduction over control	PDI	% reduction over control
T_1= Untreated control	38.5 a	-	32.3 a	-	36.4 a	-
T_2= Wheat bran Colonized *Trichoderma*without compost	30.3 b	21.3	28.1 b	13.0	27.1 b	25.5
T_3= Colonized *Trichoderma* with saw dust	25.0 c	35.1	22.9 c	29.1	23.9 b	34.3
T_4= Colonized *Trichoderma* with cow dung	15.7 ef	59.2	12.5 de	61.3	14.6 c	59.9
T_5= Colonized *Trichoderma* with tea waste	18.8 de	51.2	15.7 d	51.4	16.7 c	54.1
T_6= Colonized *Trichoderma* with water hyacinth	22.9 cd	40.5	20.8 c	35.6	22.9 b	37.1
T_7= Colonized *Trichoderma* with poultry manure	13.5 f	64.9	10.4 e	67.8	12.5 c	65.7

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

Table 5. Effect of Trichoderma fortified compost on growth promoting and yield components of chickpea

Treatments	No. of branch $plant^{-1}$	No. of pods $plant^{-1}$	Plant height (cm)	1000 seed wt. (g)	Seed yield (t ha^{-1})
T_1= Untreated control	3.0 e	32.8 c	42.1 d	140.2 d	1.02 f
T_2= Wheat bran Colonized *Trichoderma* without compost	3.2 de	35.5 c	46.1 c	140.9 c	1.13 e
T_3= Colonized *Trichoderma* with saw dust	3.5 cd	37.0 c	47.1 c	150.1 c	1.19 de
T_4= Colonized *Trichoderma* with cow dung	4.2 ab	43.0 ab	52.1 a	160.5 a	1.42 b
T_5= Colonized *Trichoderma* with tea waste	4.1 b	38.0 bc	51.5 ab	150.7 b	1.30 c
T_6= Colonized *Trichoderma* with water hyacinth	3.6 c	37.2 bc	47.5 bc	150.3 bc	1.27 cd
T_7= Colonized *Trichoderma* with poultry manure	4.5 a	46.8 a	55.2 a	160.9 a	1.54 a

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