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Plant Pathology

Efficacy of Bio-Fortified Compost in Controlling Anthracnose Disease of Chilli Caused by *Colletotrichum capsici* and Improvement the Crop Production

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Abstract | **Original Research Article**

A series of experiments were carried out in laboratory and field to find out the comparative performance of *Trichoderma* at different combinations to establish an eco-friendly management of anthracnose disease of chilli caused by *Colletotrichum capsici*. Twenty isolates of *Trichoderma harzianum* was screened following dual plate technique on PDA against *C. capsici* isolate CA-2. *In-vitro* studies clearly showed that *T. harzianum* isolate Pb-7 had the ability to inhibit the highest radial mycelial growth of the tested pathogen. Pre- and post-emergence mortality of plants due to anthracnose disease were significantly reduced after seed treated with spore suspension of *T. harzianum* isolate Pb-7 and field treated with bio-fortified composted poultry refuge. The lowest anthracnose disease incidence and severity was recorded in the treatment T_8 where seed treated with spore suspension of *T. harzianum* isolate Pb-7 and field treated with bio-fortified composted poultry refuge. The highest disease incidence and severity was recorded in control-2 (T_2) where soil inoculated with *C. capsici* followed by untreated control-1 where no treatments were given. The maximum reduction of the seedling mortality (76.92%), disease incidence (73.71%), severity (78.87%), plant height (63.4 cm) and the highest yield (5.00 tha^{-1}) were recorded in the treatment T_8 where seed were treated with spore suspension of *T. harzianum* isolate Pb-7 and field treated with bio-fortified composted poultry refuge.

Keywords: Anthracnose disease of chilli, *C. capsici*, *T. harzianum*, bio-fortified compost.

INTRODUCTION

Chilli (*Capsicum frutescens* L.) under the family of solanaceae is one of the most important spice crops not only in Bangladesh but also in many countries of the world. It has a significant economic value in Bangladesh. Moreover, fresh green chilli contains more vitamin C than citrus fruits and fresh red chilli has more vitamin A than carrot [1].

However, in tropical and subtropical areas, there are a plethora of problems for production of chilii has been identified. Among of these problems, anthracnose disease is important one [2]. Anthracnose disease is mainly infect on mature fruits, causing severe pre-and post- harvest fruit decay [3] and may cause 50% yield loss [4]. Under the suitable weather condition, this disease may reduce marketable yield up to 80% [5].

Traditionally, farmer uses different kinds of chemical fungicides for minimizing plant disease. But indiscriminate use of chemicals resulted environmental pollution and health hazards. As an alternate means of avoiding these problems, biological agents are being used for combating the disease as well as increasing crop production. The biological control of pathogen offers environmentally safe, durable and cost effective alternative to chemical compounds [6]. For instance, *Trichoderma* sp. is an effective biocontrol agent against different soil-borne pathogens. It was demonstrated that *T. harzianum* induced defense responses and systemic resistance in addition to control of plant pathogens. *Tricho*-compost, a *Trichoderma* based compost fertilizer, was developed by mixing a definite concentration of spore suspension of a *T. harzianum* strain with measured amounts of processed raw materials, such as poultry refuse, water hyacinth, vegetable waste, pea waste etc. *Trichoderma*-fortified compost was very effective in reducing different diseases of tomato and also significantly increased yield of tomato [7].

Among these practices used, seed treatment is the cheapest and safest method of direct plant disease

control. In many countries, regular practice of seed treatment is considered as a preventive protection against the building of inocula and has greatly effect on yield production as well as improve the quality in many crops including chilli. Considering the facts mentioned earlier, the proposed research program was undertaken to determine the effectiveness of *T. harzianum* at different formulation in controlling anthracnose disease of chilli and study the effect of *T. harzianum* on growth promotion and yield of chilli.

MATERIALS AND METHODS

Collection, isolation and preservation of *C. capsici* isolates

Three isolates of *C. capsici* designated as CA-1, CA-2 and CA-3 were isolated from the infected rhizosphere region or plant part of chilli. The specimens which had typical symptoms of anthracnose were selected from the infected fields. The fungal isolates were isolated following typical method [8]. The fungal colonies were grown on PDA and morphologically identified by following standard key [9]. The pure culture of *C. capsici* isolates were preserved by using PDA slant at 10℃ in refrigerator for future use.

Inoculum preparation of test pathogen

Inoculum of the *C. capsici* isolates were prepared and stored following standard method [10].

Pathogenicity of test pathogen on Chilli seedling

The pathogenicity test of *C. capsici* isolates were done according to Rubayet *et al.* [11].

Inoculation with test pathogen

C. capsici conidial suspensions (5×10^5 conidia ml^{-1}) was prepared from 10 days old pure culture. Before using the inoculum, Tween-20 @ 0.1 ml L^{-1} was added to the conidial suspension for avoiding conidia aggregation and proper attachment [12].

Collection, isolation and preservation of *T. harzianum* isolates

A total of 20 isolates of *T. harzianum*, whereas that 10 isolates were isolated from the different crop fields of Pabna district of Bangladesh following the soil dilution plate technique [8]. And rest of 10 isolates were collected directly from the plant pathology laboratory, BSMRAU, Bangladesh. All the isolated *Trichoderma* spp. were identified as *T. harzianum* based on the different morphological characteristics like hyphal growth, spore formation and color [13]. The pure culture of *T. harzianum* was preserved following regular method [14-15] for future research purpose.

Screening of *T. harzianum* isolates against test pathogen

In-vitro screening was conducted to evaluate the antagonistic effect of selected 20 isolates of *T. harzianum* against *C. capsici* isolate CA-2 on Potato Dextrose Agar (PDA) medium by dual plate culture technique [16]. After 7 days of incubation the inhibition percentage of radial growth of *C. capsici* isolate CA-2 was calculated using following formula [17].

$$\% \text{ Inhibition of growth} = \frac{A - B}{A} \times 100$$

Where, A = Mycelial growth of pathogen in absence of *T. harzianum* (control)

B= Mycelial growth of pathogen in presence of *T. harzianum*

Preparation of wheat grain colonized *T. harzianum* isolate Pb-7

The highly antagonist isolate of *T. harzianum* Pb-7 was selected on the basis of screening test. Then, inoculum of the *T. harzianum* isolate Pb-7 was prepared with autoclaved moist wheat grains by following typical method [10]. After preparation, the inoculum was stored at 4°C for future use.

Preparation of spore suspension of *T. harzianum* isolate Pb-7

A spore suspension of the *T. harzianum* isolate Pb-7 was prepared according to the method of Das *et al.* [18]. The spore concentration was adjusted to 5×10^6 spore ml^{-1} using sterilized distilled water.

Preparation of bio-fortified compost

Bio-fortified compost was made using well decomposed poultry refuge. The compost pits were prepared 1.5 m^3 at size and refilled with well decomposed @ 40 Kg poultry refuge and @ 2.5 Kg of wheat grain colonized *T. harzianum* isolate Pb-7 inoculum. Finally, pits were covered with polythene sheet and kept undisturbed for 45 days before applying at the field.

Seed treatment with *T. harzianum* isolate Pb-7 and bio-fortified composted poultry refuge

The seeds of chilli variety 'Rangpur marich 20' were treated with *T. harzianum* isolate Pb-7 @ 3 g Kg^{-1} seed according to Arefin *et al.* [12]. On the contrary, poultry refuge extract was prepared according to the method described by Brinton [19] and treated the seeds following standard methods [18, 20-21].

Application rate of test pathogen, bio-agent and bio-fortified compost

The inoculum of test pathogen was incorporated with soil @ 90 gm^{-2} in all of these treatments except T_1 before 21 days of seedling transplanting. On the other hand, wheat grain colonized

T. harzianum isolate Pb-7 inoculum was applied @ 90 gm^{-2} in the target plots before 14 days of seedling transplanting. However, bio-fortified composted poultry refuge was mixed in the each selected plot @ 2.5 Kg $plot^{-1}$ (4.17 tha^{-1}) before 3 days of seedling transplanting.

Treatments of the Experiment

T_1 = Untreated healthy seeds without pathogen (Control-1)
T_2 = Seeds treated with *C. capsici* isolate CA-2 (Control-2)
T_3 = T_2 + seeds treated with spore suspension of *T. harzianum* isolate Pb-7
T_4 = T_2 + seeds treated with extract of bio-fortified composted poultry refuge
T_5 = T_2 + field treated with bio-fortified composted poultry refuge
T_6 = T_2 + field treated with composted poultry refuge
T_7 = T_2 + field treated with wheat grain colonized *T. harzianum* isolate Pb-7
T_8 = T_3 + T_5

Cultivation of chilli in the field

Land was prepared by using a tractor driven disc plough, rotavator and harrow. The experiment was designed in the Randomized Complete Block Design with 3 replications. The unit plot size of the experiment was 6 m^2 where row to row distance 30 cm. Distance between block to block was 1.0 m. Drains were made surrounding the each unit plots and the excavated soil was used for raising plots 15 cm high from soil surface. Healthy seedlings of chilli were transplanted where row to row 60 cm and plant to plant distance was 20 cm. Weeding, mulching and irrigation were done in the experimental field whenever necessary.

Data recording

Data were recorded on mortality percentage, number of healthy plants, number of infected plants and fruits, disease incidence and disease severity, plant height and number of branches and quantity of yield. Then, the disease incidence, disease severity (Disease severity of three replicates, rated 0-4, in which 0=No symptoms, 1=1-25%, 2=26-50%, 3= 51-75% and 4= 76-100% of the organ covered with lesions) and total yield were measured by the following formulas [22-23].

$$\text{Disease Incidence (DI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \text{ x } 100$$

$$\text{Percent Disease Index (PDI)} = \frac{\text{Summation of all ratings}}{\text{Total number of rating x Maximum disease grade (4)}} \text{ x } 100$$

$$\text{Total fruit yield } (\text{tha}^{-1}) = \frac{\text{Yield per plot (kg)}}{\text{Area of plot } (\text{m}^2) \text{ x } 1000 \text{ (Kg)}} \text{ x } 10000 \text{ m}^2$$

DATA ANALYSIS

Statistically data were analyzed by using the Statistix-10 program. The treatment means were compared following Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Collection, isolation and identification of anthracnose disease and causal organism

Characteristic symptoms of anthracnose diseases of chilli were noted from the infected plant samples. The fungus was isolated from infected plant parts and fruits of chilli following standard phyto-pathological procedures [24].

Symptoms of anthracnose disease on Chilli

The results of the study indicate that *Colletotrichum* was capable of causing disease on almost all parts of the chilli plants during any stage of plant growth. Plant symptoms were initially expressed as water-soaked, slightly sunken, dark dot like lesions on leaf blade. Within 2 to 3 days the lesions increased rapidly and most of the leaves were infected and the infected plant started to die from the top. The leaves and flowers of infected plants became soft and dropped from the plants. Within 7 to 10 days the disease became very severe and infected plants died Plate I (A-D). Fruit symptoms initially developed as water-soaked lesions that became soft, slightly sunken, and became tan. The lesions could cover most of the fruit surface and multiple lesions occurred. Black, minute spots might also be developed on seed of infected fruits. The asexual fruiting bodies called acervulus appeared as numerous black dots on the lesions.

Plate-I: Symptoms of infected chilli plants due to *C. capsici*
A & C. Anthracnose symptoms on infected stem
B & D. Anthracnose symptoms on infected leaves

Identification of causal organism

The characteristic whitish gray colony was appeared on PDA after culturing the pathogen. The asexual fruiting bodies/structures called acervuli were present with abundant setae. The setae were mostly pointed, rarely blunt, elongate, strait or slightly curved, aseptate, smooth and dark brown. Conidiophores were short, bearing with falcate shape conidia. Conidia were hyaline with one or both end curved and pointed as well as sudden or gradual tapering towards the both ends (Plate II). These morphological features of the causal organism including setae, conidiophores and conidia were similar with the findings of [25-26]. The symptoms, pathogenicity test, setae characteristics, conidiophores and conidial structures proved that the pathogen was *C. capsici*.

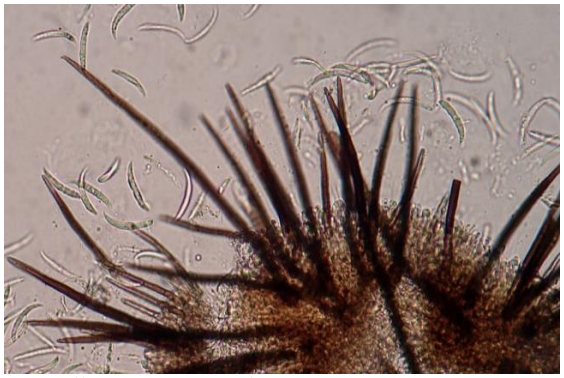

Plate-II: Different structures of *C. capsici* observed under microscope

Pathogenicity of the test pathogen

The pathogenicity test was done in the pot culture within the three selected isolates of *C. capsici* for identifying the most virulent isolate of test pathogen. All the isolates of the tested pathogen were virulent but variable in causing total seedling mortality of chilli ranged from 66.54- 90.64 %. The *C. capsici* isolate CA-2 was appeared to be the most virulent isolate causing the highest 90.64% total seedling mortality followed by isolate CA-1 (Table 1). Significantly the lowest 66.54% total seedling mortality was observed with the isolate CA-3. The pre- and post-emergence mortality of different vegetable crops including chilli caused by the tested pathogen are also reported by several investigators [27-28].

Table-1: Pathogenicity test of *C. capsici* isolates against chilli

C. capsici isolates	% Mortality		Total mortality
	Pre-emergence	Post-emergence	
CA-1	**42.50**	**29.02**	**71.52 b**
CA-2	**51.50**	**39.14**	**90.64 a**
CA-3	**31.25**	**35.29**	**66.54 c**
Untreated control	**0.00**	**0.00**	**0.00**

Screening of *T. harzianum* isolates against test pathogen

A total of 20 selected isolates of *T. harzianum* were tested against *C. capsici* isolate CA-2 on Potato Dextrose Agar (PDA) by dual culture technique to observe the antagonistic effect. All the tested 20 isolates of *T. harzianum* showed more than 50% inhibition of radial growth of the tested pathogen *C. capsici* as compared with the control. Among the 20 isolates of *T. harzianum*, Pb-7, Pb-8, Pb-12 and Pb-17 isolates showed growth inhibition at maximum level against *C. capsici* (Fig. 1 and Plate III). The highest 82.96% inhibition of the radial growth of *C. capsici* was observed with the isolates *T. harzianum* Pb-7 followed by Pb-17, Pb-12, respectively. The lowest 54.07% inhibition of the radial growth of *C. capsici* was observed with the isolates *T. harzianum* Pb-10 followed by Pb-15, Pb-14, respectively. The study of the screening of *T. harzianum* isolates against the seed-borne pathogen *C. capsici* by dual culture technique were also observed by several investigators [15, 27-29]. Based on the screening the highly antagonist *T. harzianum* isolate Pb-7 was selected to prepare the *T. harzianum* spore suspension and preserved for further use in the PDA slant at 10^{o}C.

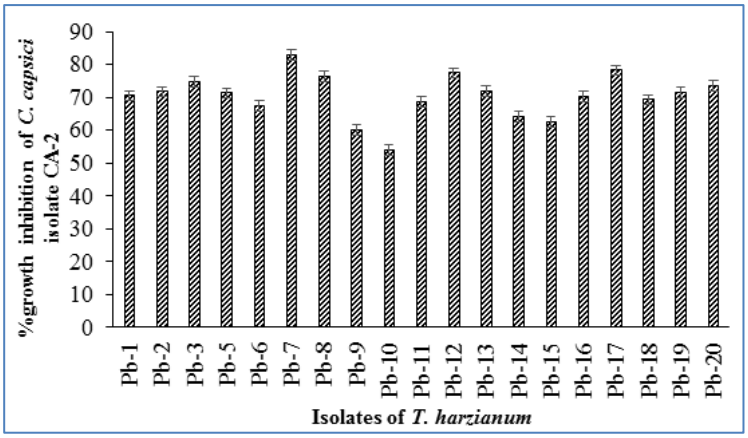

Fig-1: Screening of *T. harzianum* isolates against *C. capsici* isolate CA-2 by dual culture technique

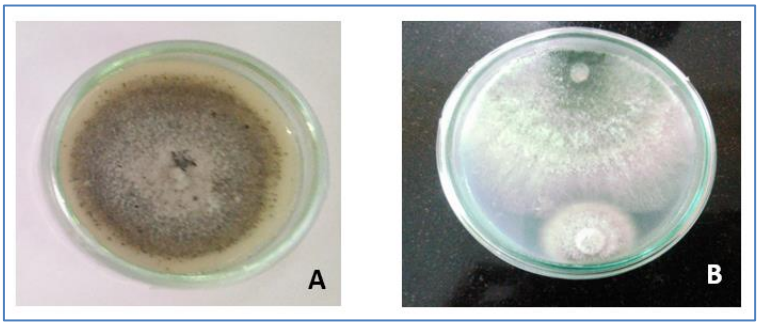

Plate-III: Radial growth inhibition of *C. capsici* isolate CA-2 by *T. harzianum* isolate Pb-7
A. Control plate of *C. capsici* isolate CA-2
B. *T. harzianum* isolate Pb-7 and *C. capsici* isolate CA-2

Effect of *T. harzianum* on seedling mortality of chilli

Immediately after sowing of chilli seeds, pre emergence and post-emergence seedling mortality were recorded during the plant growth (Plate IV). The highest 76.92% reduction of the seedling mortality over control -2 was observed at treatment T_8 where seed treated with spore suspension of *T. harzianum* isolate Pb-7 and field incorporated with bio-fortified composted poultry refuge followed by T_3 where seed treated only spore suspension of *T. harzianum* isolate Pb-7 (Table 2). Seedling mortality reduction in the treatment T_8 was found superior to all other treatments. However, suggestively reduction of anthracnose disease caused by *C. capsici* were also observed in the treatment T_4, T_5, T_6 and T_7 in comparison to the control-2.

Table-2: Effect of *T. harzianum* on seedling mortality of chilli

Treatments	% mortality			
	Pre-emergence mortality	Post-emergence mortality	Total mortality	% reduction over control-2
T_1	46.22	14.44	60.66 b	……
T_2	64.44	22.22	86.67 a	……
T_3	24.44	5.76	30.20 e	65.15 b*
T_4	31.11	6.67	37.78 e	56.41 b
T_5	34.44	12.89	47.33 d	45.54 c
T_6	38.89	14.00	52.89 c	8.98 d
T_7	33.58	7.78	41.36 d	52.28 c
T_8	16.67	3.33	20.00 f	76.92 a

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

T_1 = Untreated healthy seeds without pathogen (Control-1), T_2 = Seeds treated with *C. capsici* isolate CA-2 (Control-2), $T_3 = T_2$ + seeds treated with spore suspension of *T. harzianum* isolate Pb-7, $T_4 = T_2$ + seeds treated with extract of bio-fortified composted poultry refuge, $T_5 = T_2$ + field treated with bio-fortified composted poultry refuge, $T_6 = T_2$ + field treated with composted poultry refuge, $T_7 = T_2$ + field treated with wheat grain colonized *T. harzianum* isolate Pb-7, $T_8 = T_3 + T_5$.

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Plate-IV: Typical symptom of seedling mortality compared with control-2

Effect of *T. harzianum* on anthracnose disease incidence of chilli

Anthracnose disease caused by *C. capsici* was one of the most serious diseases of chilli production all over the Bangladesh. Disease incidence of the anthracnose disease was found from 18.18-67.86% (Table 3 and Plate V). The highest 73.71% reduction of the disease incidence was observed with the treatment T_8 where seed treated with spore suspension of *T. harzianum* isolate Pb-7 and field incorporated with bio-fortified composted poultry refuge followed by T_3 where seed treated only spore suspension of *T. harzianum* isolate Pb-7. Disease incidence reduction in the treatment T_8 was found superior to all other treatments. On the other hand, significantly reduction of disease incidence caused by *C. capsici* was also observed in the treatment T_4, T_5, T_6 and T_7 in comparison to the control-2. The reduction of the disease incidence to the treatment T_3 and T_4, T_5 and T_7 were identical but inferior to the treatment T_8. Treatment T_8 was appeared to the most superior to reducing the anthracnose disease of chilli.

Effect of *T. harzianum* on anthracnose disease severity of chilli

Severity of the anthracnose disease was observed from 15.27 to 72.26 % (Table 3). The highest reduction of the disease severity (78.87%) was observed in the treatment T_8 where seed treated with spore suspension of *T. harzianum* isolate Pb-7 and field treated with bio-fortified composted poultry refuge followed by T_3 where seed treated only spore suspension of *T. harzianum* isolate Pb-7. Significantly reduction of disease severity caused by *C. capsici* was also found in the treatment T_4, T_5, T_6 and T_7 in comparison to the control-2. The reduction of the disease severity to the treatment T_3 and T_4, T_5 and T_7 were identical but inferior to the treatment T_8. Treatment T_8 was also appeared to the most superior in reducing the anthracnose disease of chilli.

Table-3: Effect of *T. harzianum* on anthracnose disease incidence and severity of chilli

Treatments	Disease incidence (%)	Disease reduction (%)	Disease severity (%)	Severity reduction (%)
T_1	51.85 b	…….	59.50 b	…..
T_2	67.86 a	…….	72.26 a	…..
T_3	26.67 e	60.70 b	23.41 e	67.60 b*
T_4	29.03 e	57.22 b	28.03 e	61.20 b
T_5	36.63 d	46.02 c	32.91 d	54.46 c
T_6	41.38 c	39.02 d	36.90 c	48.93 d
T_7	33.33 d	50.88 c	31.94 d	55.80 c
T_8	18.18 f	73.71 a	15.27 f	78.87 a

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

T_1 = Untreated healthy seeds without pathogen (Control-1), T_2 = Seeds treated with *C. capsici* isolate CA-2 (Control-2), T_3 = T_2 + seeds treated with spore suspension of *T. harzianum* isolate Pb-7, T_4 = T_2 + seeds treated with extract of bio-fortified composted poultry refuge, T_5 = T_2 + field treated with bio-fortified composted poultry refuge, T_6 = T_2 + field treated with composted poultry refuge, T_7 = T_2 + field treated with wheat grain colonized *T. harzianum* isolate Pb-7, T_8 = T_3 + T_5.

Plate-V: Anthracnose disease of chilli caused by *C. capsici*

Effect of *T. harzianum* on plant height and number of branch of chilli

The plant height and number of branch were recorded randomly taken five plants from each replication of all the treatments attained after certain maturity. The highest 63.4 cm plant height was observed with the treatment T_8 where seed treated with spore suspension of *T. harzianum* isolate Pb-7 and field incorporated with bio-fortified composted poultry refuge followed by T_3, T_4, T_5, T_6 and T_7 in comparison to the control-2 (Table 4). Plant height in the treatment T_3 and T_4 were identical while plant height of the treatment T_5 and T_7 were also found statistically non-significant difference. Similarly, the highest number of the branch was observed with the treatment T_8 followed by T_3. The increase of branching in the treatments T_3, T_4, T_5 and T_7 were identical but superior to the treatment T_6 while the treatment T_8 was superior in comparison to all other treatments.

Effect of *T. harzianum* on yield of chilli

The highest 5.00 tha^{-1} yield was recorded in the treatment T_8 (where seed treated with spore suspension of *T. harzianum* isolate Pb-7 and field mixed with bio-fortified composted poultry refuge) which was significantly maximum in comparison to all other treatments (Table 4). The second highest 4.00 tha^{-1} yield was recorded in the treatment T_3 which was significantly identical with the treatment T_4. The lowest 1.31 tha^{-1} chilli was produced in the treatment T_2 where only pathogen inoculated. The increase yield of the treatment T_3 and T_4 were identical while the treatment T_5 and T_7 were found statistically non-significant difference. All the treatments were superior to the control-1 and control-2.

The results are also in agreement with the other investigators whose were also observed the disease reduction and increased of yield of other different crops by the application of *Trichoderma*-fortified compost [7, 30-31].

Table-4: Effect of *T. harzianum* on yield contributing characters of chilli

Treatments	Plant height (cm)	No. of branching/plant	No. of fruit/plant	Yield (tha^{-1})	Yield increased over control-2 (%)
T_1	51.68 e	9.00 d	160.00 d	1.72 e	….
T_2	50.94 f	8.00 e	130.04 de	1.31 e	….
T_3	62.00 b	14.00 b	275.56 ab	4.00 b	67.50 b
T_4	61.00 b	13.00 b	268.56 ab	3.74 b	64.86 b
T_5	58.28 c	12.00 b	230.34 bc	3.00 c	56.67 c
T_6	55.07 d	10.00 c	200.54 c	2.64 d	50.00 d
T_7	59.44 c	12.00 b	235.24 bc	3.10 c	58.06 c
T_8	63.40 a	16.00 a	300.26 a	5.00 a	74.00 a

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

T_1 = Untreated healthy seeds without pathogen (Control-1), T_2 = Seeds treated with *C. capsici* isolate CA-2 (Control-2), T_3 = T_2 + seeds treated with spore suspension of *T. harzianum* isolate Pb-7, T_4 = T_2 + seeds treated with extract of bio-fortified composted poultry refuge, T_5 = T_2 + field treated with bio-fortified composted poultry refuge, T_6 = T_2 + field treated with composted poultry refuge, T_7 = T_2 + field treated with wheat grain colonized *T. harzianum* isolate Pb-7, T_8 = T_3 + T_5.

CONCLUSION

Based on the present study it could be concluded that integration of *T. harzianum* based treatment at different formulation was appeared to be an excellent bio-agent in controlling anthracnose disease of chilli caused by *C. capsici* as well as significantly increased of growth and yield. Moreover, *T. harzianum* would be a novel revolution in our agriculture for sustainable crop production without affecting the ecology.

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