



## **Efficacy of *Trichoderma* against *Colletotrichum capsici* Causing Fruit Rot Due to Anthracnose of Chilli (*Capsicum annum* L.)**

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### **Abstract**

Five *Trichoderma* species/strains, *Trichoderma virens* IMI-392430, *T. pseudokoningii* IMI-392431, *T. harzianum* IMI-392432, *T. harzianum* IMI-392433 and *T. harzianum* IMI-392434 were tested against anthracnose and fruit rot of chilli. Effect of *Trichoderma* species in suppressing anthracnose and fruit rot as well as the growth and yield of chilli were evaluated. Seven treatments consisting of five *Trichoderma* strains, one *Colletotrichum capsici* and control were used as seed treatments. Chilli seeds were treated with spore suspension or secondary metabolites of each *Trichoderma* species/strain and *C. capsici* separately. Mixture of suspension of each *Trichoderma* species/strain with *C. capsici* was also used as spore suspension or secondary metabolites. Percent fruit infection in the control treatment was found almost similar to the treatment that contained *T. viridae* and *T. pseudokoningii* spore suspension or secondary metabolites. *T. harzianum* strains alone suppressed fruit infection (%) significantly. Further all the *Trichoderma* species/strains reduced the fruit infection (%) than the diseased control even when seeds were treated with *Trichoderma* separately mixing with *C. capsici*. Spore suspension of *T. harzianum* IMI-392433 was found much more effective against *C. capsici* which suppressed 95.8% and 79.6 % fruit infection respectively under natural (without *C. capsici*) and high inoculum pressure of *C. capsici*. All the tested *Trichoderma* species/strains showed higher plant growth and increased fruit yield irrespective of rest of the treatments. It was found that *Trichoderma* strains control chilli fruit rot significantly but high inoculum pressure of *C. capsici* reduced fruit yield drastically. Among the treatments, spore suspension of *T. harzianum* IMI-392433 increased the fruit yield 83.6% and 76.5% per plant compared to spore suspension of *C. capsici* and control treatments, respectively. These results implied that *T. harzianum* IMI-392433 can effectively control fruit rot of chilli caused by *C. capsici* through host resistance and antifungal metabolite activity. The fruit yield was increased due to the influence of *T. harzianum* IMI-392433 on vigorous physiological growth of plants as well as efficacy against the disease.

**Keywords:** Anthracnose fruit rot, Biological control, chilli, *Colletotrichum capsici*, *Trichoderma*.

### **1. Introduction**

Chilli (*Capsicum annum* L.) is an important spice generally used for food preparation in south-east Asia including Bangladesh. It is

cultivated around 166 ha and produced 141 metric tons in 2000-2001 (Barua *et al.*, 2009). Anthracnose incited by *Colletotrichum* spp. is one of the severe diseases of chilli. Two species,

namely *C. capsici* and *C. gloeosporioides* were known earlier to cause Anthracnose in chilli. However *C. capsici* is the most predominant species in the major chilli growing areas in Bangladesh. The fungus is both internally and externally seed-borne (Ramachandran, 2007). It also survives on the stems and branches causing die-back symptoms. This fungus causes severe damage on chilli fruits in both pre- and post harvest stages and more than 50% of the crop losses observed (Pakdevaraporn *et al.*, 2005). In tropical countries, high moisture condition due to monsoon rain during June-October favors sporulation of *C. capsici* which enhances the fruit rot disease incidence and helps outbreak of the disease.

Fungicide is most commonly used as a treatment to manage, but there is a need for non-chemical methods of control to avoid the adverse effect of toxic chemicals on the environment. Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical methods (Baker and Paulilutz, 1996). Biological control of plant pathogens has been shown to have potential to control many diseases in plantations. *Chaetomium*, *Penicillium* and *Trichoderma* species are biological control agents that have the potential to control plant diseases (Soytong, 2005). The antagonistic activity of *Trichoderma* species against plant pathogens has been studied extensively (Hjeljord *et al.*, 2001; Etabarian, 2006). *Trichoderma* produced secondary metabolites such as harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-pentyl- $\alpha$ -pyrone, massoialactone, viridin, glioviridin, gliovirin, glisoprenins, heptelidic acid, pentyl pyrone, gliotoxin, trichorzianines and oxazole which have antifungal properties (Di Pietro *et al.*, 1993; Lee *et al.*, 1995; Chet *et al.*, 1997; Intana, 2003; Vey *et al.*, 2001).

A number of commercial formulations, based on *T. harzianum* and *T. virens*, are available for soil borne and foliar disease control of horticultural crops (Samuels, 1996; Etabarian, 2006). *T.*

*harzianum* isolate, T39, is the active ingredient of Trichodex, which is reported to control *Botrytis* grey mold on a range of crops (Elad, 1994). *T. harzianum* has been evaluated for the control of black seed rot disease of oil palm sprouted seeds in Nigeria (Eziashi *et al.*, 2007). Therefore, biological control becomes important in integrated disease management for improved plant production. This finding suggested that certain strains of *Trichoderma* can induce systemic and localized resistance to several plant pathogens (Yedidia *et al.* 1999, 2000).

In this study, we therefore, investigated the effect of *Trichoderma* strains to control anthracnose and fruit rot of chilli under low and high inoculum pressure of *C. capsici*. The growth and yield of chilli was also evaluated to elucidate the influence of *Trichoderma* against fruit rot of chilli.

## 2. Materials and Methods

The experiment was conducted at Botanical Garden of Rajshahi University, Rajshahi, Bangladesh in pot culture condition. The efficacy of *Trichoderma* species in controlling chilli fruit rot due to anthracnose caused by *Colletotrichum capsici* was evaluated and the growth and yield of chilli was also investigated.

### 2.1. Sterilization of soil

Soil was collected from the research field of Rajshahi University campus and sterilized with formaldehyde (formalin: water; 1:50 v/v) and covered with polythene sheet for 24 hours. Then the soil was allowed to dry for disappearance of formaldehyde odor. After 30 days of sterilization, soil was put in the earthen pot (30 × 20 cm). To allow flowing of excess water, 2 cm hole was made from the bottom of the pot.

### 2.2. Seed collection

Chilli variety "Bogra local" was collected from Spices Research Centre, Bogra, Bangladesh. Disease free healthy seeds were selected for use in this experiment.

### 2.3. Sources of *Trichoderma*

Five *Trichoderma* strains namely; *T. virens* IMI-392430, *T. pseudokoningii* IMI-392431 and *T. harzianum* IMI-392432, *T. harzianum* IMI-392433 and *T. harzianum* IMI-392434 were collected from Biotechnology and Microbiology Laboratory, Department of Botany, Rajshahi University, Bangladesh. These strains were isolated from decomposed garbage and soil by Rahman (2009) and were previously verified by CABI Bioscience, Surrey, U.K.

### 2.4. Isolation of pathogen from anthracnose disease of Chilli

Isolates of *C. capsici* was isolated from infected fruit parts of chilli which was collected after proper noting down of the symptoms of the disease. Following standard phytopathological methods (Booth, 1971), pathogen was isolated from the transitional zone of healthy and infected tissues on Potato Dextrose Agar (PDA) medium. All isolates were tested for pathogenicity to chilli using Koch's Postulate. The most virulent isolate was chosen for further experimentation.

### 2.5. Preparation and application of spore suspension

Mycelial disc (5 mm dia.) of *Trichoderma* isolates and *C. capsici* were obtained from 4-5 days-old cultures and transferred to 50 ml potato dextrose agar in a 250-ml conical flask separately and incubated at 28 °C for 7 days (Panahian *et al.*, 2012; Cascino *et al.*, 1990). After incubation, 30 ml of sterile distilled water was added to each culture and the flasks were shaken at 50 rpm for 30 min in an orbital shaker. Then the content of each conical flask was filtered through sterile muslin cloth. The culture filtrate with the spores was collected and a concentration of  $4-5 \times 10^5$  spores/ml was obtained by dilution with sterilized distilled water. The spore concentration were examined and ensured under compound microscope (Olympus CX21).

For seed treatment, 10 to 15 seeds were dipped, for about 20 minutes in the spore suspension ( $5 \times 10^5$  spores/ml) obtained as mentioned and the treated seeds were dried inside the laminar air

flow cabinet. After that in the same way *Trichoderma* treated and untreated seeds were again dipped in the spore suspension ( $4 \times 10^5$  spores/ml) of 7 days old culture of *C. capsici* for about 20 minutes and then the treated seeds were dried under laminar air flow hood. After germination of the treated seeds, 50 ml of the conidial suspension of *Trichoderma* strains/species and *C. capsici* were poured individually and in mixture of both onto the pot-soil of respective treatments at 7 days interval up to harvesting.

### 2.6. Preparation and application of secondary metabolites

200 ml of Richard's solution (KNO<sub>3</sub>: 1.0g, KH<sub>2</sub>PO<sub>4</sub>:0.5g, MgSO<sub>4</sub>·7H<sub>2</sub>O: 0.25g, glucose: 34g, trace amounts of FeCl<sub>3</sub> in 1L distilled water, pH 6.5) was prepared and poured into 500 ml conical flasks and autoclaved for 15 minute at 121 °C/1.05kg/cm<sup>2</sup> pressure. Six pieces of agar discs (6 mm) were kept in a flask (with media) for each strains of *Trichoderma* and *C. capsici* separately with four replications. The flasks were incubated on a Gallenkamp orbital incubator at 100 rpm at 28 °C (Dennis and Webster, 1971). The culture filtrates were collected after 30 days of incubation. These were then concentrated to about 50% using a vacuum evaporator at 38-40 °C and finally filtered by sterilized membrane filter. For seed treatment, 10 to 15 seeds were dipped in the 30 days old filtrate secondary metabolites of each *Trichoderma* strains/species and the treated seeds were dried under laminar air flow hood. In the same way treated and untreated seeds were treated with secondary metabolites of *C. capsici* about 20 minutes and dried inside the laminar air flow. After germination of the treated seeds, 50 ml of secondary metabolites of both *Trichoderma* and *C. capsici* were poured individually and in mixture of both onto the pot-soil of respective treatments at 7 days interval up to harvesting.

### 2.7. Treatments and data analysis

Five *Trichoderma* species/strains and one virulent isolate of *C. capsici* were used in this experiment. *Trichoderma* species/strains were *T.*

*virens* IMI-392430, *T. pseudokoningii* IMI-392431, *T. harzianum* IMI-392432, *T. harzianum* IMI-392433, *T. harzianum* IMI-392434. Chilli plants were inoculated with *C. capsici* ( $4 \times 10^5$  spores/ml) and *Trichoderma* species/strains ( $5 \times 10^5$  spores/ml) in different suspension forms keeping one control (no treatment). Both *Trichoderma* species/strains and *C. capsici* were applied or inoculated separately as spore suspension and secondary metabolites. Moreover, spore suspension mixtures and secondary metabolites suspension mixtures of each *Trichoderma* species/strains with *C. capsici* were also applied as different treatment. Therefore, seven treatments, i.e. one *C. capsici* applied only as spores and secondary metabolites, while five *Trichoderma* were tested separately as spores, secondary metabolites and mixture of these spores or secondary metabolites with *C. capsici* in the chilli plant including non-treated one. Three replications of each treatment with factorial design of experiment were followed considering the suspension forms (or treatment types) as main plots and the causal pathogen and *Trichoderma* in the sub-plots. Statistical analysis was done with the help of computer package program SPSS (SPSS Inc., Chicago, IL, USA).

### 2.8. Sampling and data collection

Data on shoot length, number of primary branch, number of secondary branch and number of roots were recorded from 10 plants in each replication. These were measured at 90 days after sowing. Observations were also recorded for number of infected and total fruits per plant, dry fruit weight and yield per plant. Fresh fruit was dried in an oven at 95 °C for two hours first and then 72 hours at 65 °C. Then dry weight was determined using electronic balance. Percentage of infected fruits was calculated through simple arithmetic calculation considering the recorded rotted fruits out of the total fruits observed.

The percentage of fruit yield increment was calculated from the difference of fruit yield between a specific treatment and non-treated

one. Similar calculation was also done in case of disease increment or decrement.

## 3. Results and Discussion

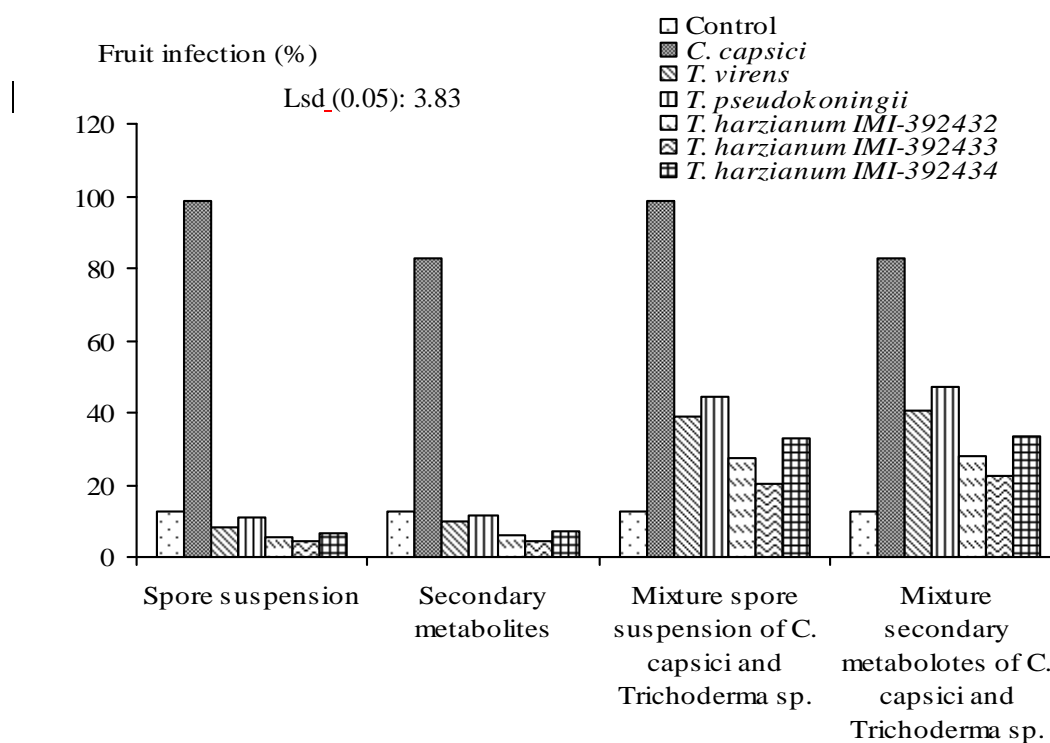
### 3.1. Percentage of fruit infection

The highest percentage of infected fruit was recorded in spore suspension of *C. capsici* (98.4%) and the lowest was recorded in spore suspension of *T. harzianum* IMI-392433 (4.2%) (Fig. 1). In control (no treatment), a remarkable percentage (12.5%) of infected fruit was also observed. Here infection source might be the seeds, or the environment or both. Application of spore suspension of *T. harzianum* IMI-392433 significantly ( $p \leq 0.05$ ) suppressed the fruit infection 95.9% and 74.7% compared to the spore suspension of *C. capsici* and control respectively (Fig 1). Compare to the control the disease reduction was 66.2 and 65.4% respectively in spore suspension and secondary metabolites of *T. harzianum*. Wharton et al. (2012) reported that *T. harzianum* provided control of seed piece decay (caused by *Phytophthora infestans*) reducing disease incidence and severity on average by 73% and 86% respectively under optimal conditions and these were similar like fludioxonil + mancozeb, which reduced disease incidence and severity on average by 73% and 85.5% respectively. Antifungal metabolites extracted from *T. harzianum* showed markedly high inhibitory activities on spore germination and germ-tube growth of *C. capsici* (Rahman et al., 2013). In this study, biochemical analysis of antifungal secondary metabolites applied or produced after the application of spore suspension of *Trichoderma*, were not done. However, previous reports indicated that *Trichoderma* can produce harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-pentyl- $\alpha$ -pyrone, massoilactone, viridin, glioviridin, gliovirin, glisoprenins, heptelidic acid, pentyl pyrone, gliotoxin, trichorzianines and oxazole which can increase growth of plants and induce resistance to disease (Di Pietro et al., 1993; Lee et al., 1995; Chet et al., 1997; Intana, 2003; Vey et al., 2001). *Trichoderma* inhibited the growth and

pathogenicity of *C. capsici* in some other different ways like parasitism, competition for food and space, degradation of pathogenic enzymes like pectinase etc. through antibiotics, antifungal toxic metabolites, lytic enzymes etc. (Zimand *et al.*, 1996). Biswas and Das (1999) reported that *Trichoderma* was found effective when used as seed coating against seedling disease. Prasad *et al.* (2002) found that soil treated with *T. harzianum* showed 61.5% disease control in chickpea while Kashem *et al.* (2005) observed <30% disease control in lentil seed.

The effect of spore suspension and secondary metabolites irrespective of treatments were similar on the disease incidence. But disease incidence was significantly higher under high

inoculum pressure i.e., when spore suspension or secondary metabolites of *Trichoderma* species/strains was mixed with *C. capsici*. In this case, the disease incidence ranged from 20.4-33.6% in the *T. harzianum* strains and 38.6-48.0% in other tested *Trichoderma* species across the treatment types. On the other hand *C. capsici* treatment caused the highest fruit infection in spore suspension (98.4%) followed by secondary metabolite (82.7%). These results indicated that both conidial spores of *C. capsici* as well as its secondary metabolite effectively incited the disease, while, spores of *T. harzianum* strains and its secondary metabolites alone suppressed the disease effectively (average 65.8% disease reduction).



**Fig. 1.** Effect of *Trichoderma* strains on percentages of infected chilli fruit. Bar marked by the same letters are not significantly different ( $p < 0.05$ ) by DMRT analysis.

The disease development by the pathogen and suppression of the disease by *Trichoderma* were dependent on the production level of their spores/secondary metabolites in the substrate level. Because, under high inoculum level of *C. capsici* the disease incidence increased and extent of disease control by *Trichoderma* depends to some extent on disease pressure. Many reports are available for successful use of antifungal metabolite extracted from *Trichoderma* spp. to control *S. rolfsii* causing disease on vegetables (Maiti et al., 1991), *P. aphanidermatum* causing wilt of cotton and watermelon (Ordentlich et al., 1992) and damping-off of cucumber (Intana, 2003) and *Phytophthora* sp. causing various plant diseases (Wilcox et al., 1992). This research indicated an additional successful use of antifungal metabolites from both *T. harzianum* wild type and mutant strains in controlling anthracnose on chilli fruits caused by *C. capsici*.

However, the spore and the secondary metabolites of *T. harzianum* strains influence

and increase the growth of shoot, number of primary branches, number of secondary branches and number of roots, leaf and flower (detail data are not presented). Vigor of the plant and its disease resistance might also be increased with the metabolites of *T. harzianum* strains especially in *T. harzianum* IMI-392433. Recent studies indicated that some strains enhance plant growth and development. Yedidia et al. (2001) showed that treatment of cucumber plants in soil with *T. harzianum* (T-203) resulted in large increases in root area and cumulative root length, and significant increases in dry weight, shoot length, and leaf area over that of the untreated control.

### 3.2. Shoot and root growth

The shoot length of the chilli plant among the treatments varied significantly ( $p < 0.05$ ) (Table 1). The shoot length was similar and lower in both the control (14.48 cm) and *C. capsici* treatments (spore-13.98 cm, secondary metabolite-14.47 cm).

**Table 1.** Effect of *Trichoderma* on the shoot length (cm) of chilli

Treatments*	Treatment types			
	Spore suspension	Secondary metabolites	Mixture spore suspension of <i>C. capsici</i> and ** <i>Trichoderma</i> sp.	Mixture secondary metabolites of <i>C. capsici</i> and ** <i>Trichoderma</i>
Control	14.48	14.48	14.48	14.48
<i>C. capsici</i>	13.98	14.47	13.98	14.47
<i>T. virens</i>	29.98	29.48	21.88	19.93
<i>T. pseudokoningii</i>	28.78	27.54	18.67	17.69
<i>T. harzianum</i> IMI-392432	31.91	31.86	24.59	24.43
<i>T. harzianum</i> IMI-392433	32.95	32.83	26.56	25.46
<i>T. harzianum</i> IMI-392434	30.89	30.48	23.58	22.67
Lsd (0.05)	0.93			

\*Number of spores of *C. capsici* and *Trichoderma* sp. were  $4 \times 10^5$  and  $5 \times 10^5$  spores/ml suspension, respectively.

\*\**Trichoderma* sp. was not mixed with treatment *C. capsici* in no forms of spore suspension or secondary metabolites.

Control: No treatment either with *C. capsici* and or *Trichoderma* sp.

*Trichoderma* species, exhibited a significant increase in the height and dry weight of pepper seedlings using 1/16 dilution in cell-free culture broths. These results proved that *Trichoderma* species produce substances in the cell-free culture filtrate responsible for these effects. Thus, they have a phytohormonal effect on pepper seedlings exhibiting the same growth enhancement as natural plant hormones (Brenner, 1981). The secondary metabolites are key facilitators of many of these positive effects. They act as microbe-associated molecular patterns (MAMPS) and as auxin-like analogues at low concentrations (Vinale *et al.*, 2008). The number of primary branch, secondary branch per shoot and number of roots per plant at 90 days after sowing were found similar to the results of shoot length irrespective of treatment types. Only the effects of spore suspension on these parameters are shown in Table 2. *T. harzianum* IMI-392433 produced the highest number of primary branch, secondary branch and roots of chilli plants among the treatments.

### 3.3. Number of fruits per plant

The number of fruits/plant were found similar in case of spore suspension (5.13) and secondary metabolites (5.14) treated treatments of *C.*

*capsici* (Table 3). The total number of fruits per plant ranged 22.32-38.96 and 20.18-36.39, respectively in spore suspension and secondary metabolites of *Trichoderma* treatments. But the number of fruits per plant was lower (10.46-18.88) when spore suspension or (10.23-16.49) when secondary metabolites of *Trichoderma* was applied in mixing with *C. capsici*. This was because of high inoculum pressure of *C. capsici* and/or influenced by the activity of *Trichoderma*. Probably metabolites produced from *Trichoderma* were mainly used for the inactivation of *C. capsici* spores or secondary metabolites (Di Pietro *et al.*, 1993; Lee *et al.*, 1995; Chet *et al.*, 1997; Intana, 2003; Vey *et al.*, 2001). Hence, the physiological growth was not influenced much by *Trichoderma* and thereby less numbers of fruits were observed in the mixture of spore suspensions or secondary metabolites of *Trichoderma* and *C. capsici*. Further, under low or natural inoculum pressure, *Trichoderma* produced much more number of fruits because of its role in the higher physiological growth. It means, *Trichoderma* played its role in two ways. It might help the plant immunue system that resulted vigorous physiological growth of plants.

**Table 2.** The number of primary branch, secondary branch and roots per plant in spore suspension of different *Trichoderma* species/strains.

Treatments*	Number of primary branch/plant	Number of secondary branch/plant	Number of roots/plant
Control	2.69 f	65.51 f	35.91 f
<i>C. capsici</i>	2.54 g	64.43 g	32.37 g
<i>T. virens</i>	5.71 d	97.36 d	52.14 d
<i>T. pseudokoningii</i>	4.54 e	93.48 e	50.38 e
<i>T. harzianum</i> IMI-392432	6.96 b	104.91 b	58.37 b
<i>T. harzianum</i> IMI-392433	7.89 a	108.87 a	62.52 a
<i>T. harzianum</i> IMI-392434	5.89 c	101.48 c	55.38 c

Means following the same letter in a column did not differ significantly among the treatment types by DMRT at 5%. \*Number of spores of *C. capsici* and *Trichoderma sp.* were  $4 \times 10^5$  and  $5 \times 10^5$  spores/ml suspension, respectively. \*\**Trichoderma sp.* was not mixed with treatment *C. capsici* in no forms of spore suspension or secondary metabolites. Control: No treatment either with *C. capsici* and or *Trichoderma sp.*

**Table 3.** The number of fruits produced per plant in different treatment types of *Trichoderma*

Treatments*	Treatment types			
	Spore suspension	Secondary metabolites	Mixture spore suspension of <i>C. capsici</i> and ** <i>Trichoderma sp.</i>	Mixture secondary metabolites of <i>C. capsici</i> and ** <i>Trichoderma</i>
Control	7.48	7.48	7.48	7.48
<i>C. capsici</i>	5.13	5.14	-	-
<i>T. virens</i>	26.34	24.94	11.19	10.86
<i>T. pseudokoningii</i>	22.32	20.18	10.46	10.23
<i>T. harzianum</i> IMI-392432	34.26	32.94	14.18	13.24
<i>T. harzianum</i> IMI-392433	38.96	36.39	18.88	16.49
<i>T. harzianum</i> IMI-392434	30.48	28.98	13.12	12.86
Lsd (0.05)			7.06	

\*Number of spores of *C. capsici* and *Trichoderma sp.* were  $4 \times 10^5$  and  $5 \times 10^5$  spores/ml suspension, respectively. \*\**Trichoderma sp.* was not mixed with treatment *C. capsici* in no forms of spore suspension or secondary metabolites. Control: No treatment either with *C. capsici* and or *Trichoderma sp.*

**Table 4.** Dry weight (g) of fruit in different treatment types of *Trichoderma*

Treatments*	Treatment types			
	Spore suspension	Secondary metabolites	Mixture spore suspension of <i>C. capsici</i> and ** <i>Trichoderma sp.</i>	Mixture secondary metabolites of <i>C. capsici</i> and ** <i>Trichoderma</i>
Control	0.38 (2.99)	0.38 (2.99)	0.38 (2.99)	0.38 (2.99)
<i>C. capsici</i>	0.32 (2.54)	0.36 (2.61)	-	-
<i>T. virens</i>	0.82 (11.43)	0.78 (11.26)	0.56 (3.96)	0.52 (3.88)
<i>T. pseudokoningii</i>	0.76 (10.36)	0.76 (9.54)	0.48 (3.54)	0.46 (3.28)
<i>T. harzianum</i> IMI-392432	0.97 (13.96)	0.96 (13.86)	0.66 (5.89)	0.64 (5.28)
<i>T. harzianum</i> IMI-392433	0.99 (15.17)	0.98 (14.89)	0.71 (6.83)	0.69 (6.43)
<i>T. harzianum</i> IMI-392434	0.91 (12.84)	0.89 (11.96)	0.63 (4.26)	0.61 (4.18)
Lsd (0.05)			0.03	

\*Number of spores of *C. capsici* and *Trichoderma sp.* were  $4 \times 10^5$  and  $5 \times 10^5$  spores/ml suspension, respectively. \*\**Trichoderma sp.* was not mixed with treatment *C. capsici* in no forms of spore suspension or secondary metabolites. Control: No treatment either with *C. capsici* and or *Trichoderma sp.* Each figure indicates the dry weight of five fruits (g). Figures within parenthesis indicate fresh weight (g) of five fruits.

The other way could be the inactivation of the pathogen and/or its metabolites. Under low or natural inoculum pressure of *C. capsici*, the first mechanism showed more active that resulted good physiological growth of chilli plants. The second mechanism was more activate under high

inoculum pressure of *C. capsici*, where anti-pathogenic activity of *Trichoderma* might suppressed the mycelial growth of *C. capsici* as well as influenced the physiological growth of chilli plant. All the *Trichoderma* species/strains produced higher number of fruits compare to the



control and *C. capsici* treatments in low or natural inoculum pressure. But all *Trichoderma* species/strains produced similar number of fruits by above mentioned treatments except for *T. harzianum* IMI-392433 under epidemic condition. The total number of fruit per plant was the highest in all the treatments of *T. harzianum* IMI-392433. Among the treatments, spore suspension of *T. harzianum* IMI-392433 increased the number of fruits 76.7% and 67.8% per plant compared to spore suspension of *C. capsici* and control treatments respectively.

### 3.4. Fruit weight (dry)

Fruit weight (dry) was found higher in all the *Trichoderma* species/strains treatments including spore suspension and secondary metabolite treatments (Table 4). Further mixture suspension

of *Trichoderma* with *C. capsici* showed lower fruit weight compare to the spore suspension or secondary metabolite of *Trichoderma* species/strains. The highest dry weight was recorded in *T. harzianum* IMI 329433 followed by IMI-392432 in all the treatment types. The lowest dry weight was observed in diseased control followed by control. Among the treatments, spore suspension of *T. harzianum* IMI-392433 increased the dry weight of fruit 49.6% and 44.5% compared to spore suspension of *C. capsici* and absolute control treatments, respectively (Table 4).

### 3.5. Fruit yield

The yield per plant among the treatments ranged from 1.98-22.12 g in spore suspension and 2.12-20.37 g in secondary metabolites (Fig. 2).

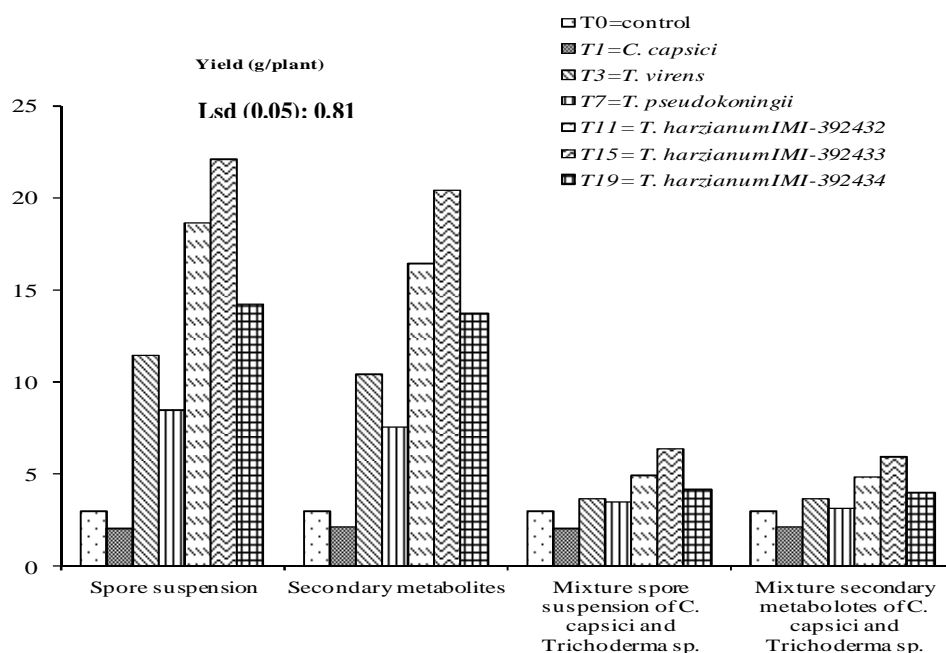


Fig. 2. Dry weight of fruit yield of Chilli in different treatments of *Trichoderma* species/strains

The highest yield in the spore suspension (22.12 g) was similar to secondary metabolite (20.37 g) treatments of *T. harzianum* IMI-392433. The lowest yield was recorded in the *C. capsici* followed by control treatments of both spore suspension and secondary metabolites. All *T. harzianum* strains produced higher yield compare to two other *Trichoderma* species *T. virens* and *T. pseudokoningii* across the treatment types. Further, compare to the treatment of *Trichoderma* species/strains as spore suspension or secondary metabolites, fruit yield was lower under high inoculum pressure i.e., when *Trichoderma* applied in mixing with *C. capsici*. Here, mixture of spore suspension treatment produced 3.48-6.34 g while mixture of secondary metabolites produced 3.12-5.92 g fruit per plant.

Among the treatments, spore suspension of *T. harzianum* IMI-392433 increased the fruit yield 83.6% and 76.5% per plant compared to spore suspension of *C. capsici* and control treatments, respectively. The results reveal that the yield was significantly influenced by the application of *T. harzianum* IMI-392433. Many workers found higher yields compared to control when seeds treated with *T. harzianum*. Sultana (1999) obtained up to 81.60% higher seed yield of lentil when they were treated with *T. harzianum* for controlling foot and root rot. Da Luz et al. (1998) also observed that yields of wheat seeds infected with *Pyrenophora tritici-repentis*, were significantly increased after application of *T. virens*.

*Trichoderma* species have shown direct effects on the chilli plant host. These effects include increased growth, yield as well as immunity against disease. Harman et al. (2004) also mentioned these effects including increased nutrient uptake, germination percentage, stimulation of plant immunity, defense against biotic and abiotic threats, and increased efficiency of fertilizer uptake. *Trichoderma* which is endophytic plant symbionts conferred inductin of increased nitrogen use efficiency in plants and reduce N application rate by 30-50%

without reduction in yield (Harman 2011, Shores et al. 2010). Therefore, the increase in plant height, shoot and root dry weight and yield was due to higher N use efficiency by the plants treated with *Trichoderma* spp. *Trichoderma* spp. is one of the important groups of rhizosphere microorganisms, which can impart some beneficial effects on promoting plant growth and development (Harman et al., 2004; Qi and Zhao, 2013). The *Trichoderma* species have also been known to be used by plants as biological control agents for controlling different species of plant fungus diseases for decades (Harman et al., 2004).

#### 4. Conclusions

From the above findings and discussion it may be concluded that the number of fruits and yield of chilli can be increased with the application of spore suspension of *T. harzianum* IMI-392433 which produces vigorous plant growth, increases the number of primary branches, secondary branches and roots. These attributes was due to beneficial effects of *T. harzianum* IMI-392433 that might be increased nutrient uptake and phytohormonal effect which act as auxin-like analogues at low concentration in chilli plants (Brenner, 1981, Vinale et al., 2008). It may also effectively control fruit rot of chilli caused by *C. capsici* through inactivation of fungal spores or secondary metabolites as well as host resistance (Di Pietro et al., 1993; Lee et al., 1995; Chet et al., 1997; Vey et al., 2001; Intana, 2003). Therefore, *T. harzianum* IMI-392433 may be used as an effective bio control agent to control *C. capsici* that causes fruit rot of chilli in Bangladesh.

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## References

- Abdul-Baki A. and Anderson JD. 1973. Vigour determination of soybean seed by multiple criteria. *Crop Sci.* 3: 630-633.
- Baker R. Pautilz, TC. 1996. Theoretically basis for microbial interactions leading to biological control of soil borne plant pathogens. In: Principles and practice of managing soil borne plant pathogens. R. Hall (Ed.). The American Phytopathological Society. St. Paul. MN. Pp: 50-79.
- Barua H., Rahman MM., Masud MM. 2009. Effect of storage containers environment at different storage period on the quality of chilli seeds. *Int. J. Sustain. Crop. Prod.* 4(4): 28-32.
- Begum MF., Rahman MA., Alam MF. 2010. Biological control of *Alternaria* fruit rot of chilli by *Trichoderma* species under field conditions. *Mycobiology* 38 (2):113-117.
- Biswas KK., Das ND. 1999. Biological control of pigeon pea wilt caused by *Fusarium udum* with *Trichoderma* spp. *Annals. Plant. Protection. Sci.* 7(1): 46-50.
- Booth C. 1971. The genus *Fusarium*. Commonwealth Mycol. Inst. Kew, Surrey, England.
- Brenner ML. 1981. Modern methods for plant growth substance analysis. *Ann Rev Plant Phys.* 32: 511-538
- Cascino JJ., Harris, R. F., Smith C. S., and Andrews J. H. 1990. Spore Yield and Microcycle Conidiation of *Colletotrichum gloeosporioides* in Liquid Culture. *Applied and Environmental Microbiology*, p. 2303-2310.
- Chang YC., Baker R., Kleifield O., Chet I. 1986. Increased growth of plants in the presence of the biological control agent *T. harzianum*. *Plant. Disease.* 70:145-148.
- Chet I., Inbar J., Hadar I. 1997. Fungal antagonists and mycoparasites. In *The Mycota IV: Environmental and microbial relationships.* ed. D.T. Wicklow, B. Söderström. 165-184. Springer-Verlag, Berlin.
- Da Luz WC., Bergstrom GC., Stockwell C.A. 1998. Seed applied bioprotectants for control of seed-borne *Pyrenophora tritici-repentis* and agronomic enhancement of wheat. *Can. J. Plant. Pathol.* 19: 384-386.
- Di Pietro A., Lorito M., Hayes CK., Broadway RM. Haman EL. 1993. Endochitinase from *Gliocladium virens*: isolation, characterization, and synergistic antifungal activity in combination with gliotoxin. *Phytopathology* 83:308-313.
- Dennis C., Webster J. 1971. Antagonistic properties of species-group of *Trichoderma* I. Production of non-volatile antibiotics. *Trans. Brit. Mycol. Soc.* 57:25-39.
- Elad Y. 1994. Biological control of grape grey mold by *Trichoderma harzianum*. *Crop. Prot.* 13: 35-38.
- Etabarian HR. 2006. Evaluation of *Trichoderma* isolates for biological control of charcoal stem rot in melon caused by *Macrophomina phaseolina*. *J. Agric. Sci. Technol.* 8:243-250
- Eziashi EI., Omamor IB Odigie EE. 2007. Antagonism of *Trichoderma viridie* and effects of extracted water soluble compounds from *Trichoderma* species and benlate solution on *Ceratocystis paradoxa*. *African J. Biotech.* 6:388-392
- Hanson L E. 2000. Reduction of verticillium wilt symptoms in cotton following seed treatment with *Trichoderma virens*. *The Journal of Cotton Science.* 4: 224-231.
- Harman GE. 2000. Myths and dogmas of biocontrol. Changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Dis.* 84: 377-393.

- Harman GE., Howell CR., Viterbo A., Chet I., Lorito M. 2004. Trichoderma species-opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol.* 2:43-56.
- Harman GE. 2011. Multifunctional Fungal Plant Symbionts: New tools to enhance plant growth and productivity. *New Phytology.* 189, 647-649.
- Hjeljord LG., Stensvand A. and Transmo A. 2001. Antagonism of nutrient-activated conidia of *Trichoderma harzianum* (airoviridae) P1 against *Botrytis cinera*. *Phytopathol.* 91: 1172-1180.
- Intana W. 2003. Selection and development of *Trichoderma* spp. for high glucanase, antifungal metabolite producing and plant growth promoting isolates for biological control of cucumber damping-off caused by *Pythium* spp. [Ph.D Thesis] Kasetsart University, Thailand.
- Kashem MA., Naznin HA., Islam MM., Jalaluddin M., Hossain I. 2005. Effect of different methods of application of *Trichoderma* in controlling foot and root rot of lentil. *Bangladesh. J. Seed. Sci. Tech.* 9 (1&2): 39-42.
- KüçüK C., Kivanç M. 2003. Isolation of *Trichoderma* spp. and determination of their antifungal, biochemical and physiological features. *Turk. J. Biol.* 27: 247-253.
- Lo CT., Lin CY. 2002. Screening strains of *Trichoderma* spp. for plant growth enhancement in Taiwan. *Plant Pathol Bull.* 11:215-20.
- Lee C., Chung M., Lee H., Kho Y. 1995. MR-93A, a new oxazole from *Trichoderma harzianum* KCTC 0114BP. *J. Nat. Prod.* 58:1605-1607.
- Maiti D., Dasgupta B., Sen C. 1991. Antagonism of *Trichoderma harzianum* and *Gliocladium virens* isolates to *Sclerotium rolfsii* and biological control of stem rot of groundnut and betel vine. *Journal of Biological Control* 5, 105-109.
- Mukhtar I. 2008. Influences of *Trichoderma* species on seed germination in okra. *Mycopath.* 6(1&2):47-50.
- Ordentlich A., Z Wiesman HE., Gottlieb M., Cojocarú Chet I. 1992. Inhibitory furanone produced by the biocontrol agent *Trichoderma harzianum*. *Phytochemistry.* 31:485-486.
- Ozbay N., Newman SE. 2004. Biological control with *Trichoderma* spp. with emphasis on *T. harzianum*. *Pak J Biol Sci* 7:478-84.
- Panahian Gh., Rahnama K., Jafari M. 2012. Mass production of *Trichoderma* spp and application. *Intl. Res. J. of Applied and Basic Sciences.* Vol., 3 (2), 292-298.
- Pakdeevaporn P., Wasee S., Taylor PWJ., Mongkolporn O. 2005. Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in Capsicum. *Plant Breeding*, 124(2):206-208.
- Prasad RD., Rangeswaran R., Anuroop CP., Rashmi HJ. 2002. Biological control of wilt and root rot of chickpea under field conditions. *Ann. Plant.Protect.Sci.* 10(1):72-75.
- Qiu ZB., Li JT., Zhang MM., Bi ZZ., Li ZL. 2013. He-Ne laser pretreatment protects wheat seedlings against cadmium-induced oxidative stress. *Ecotoxicol. Environ. Saf.* 88 135-141.
- Rahman MA. 2009. Screening of *Trichoderma* spp. and their efficacy as a bioconversion agent of municipal solid waste through appropriate technique of solid state fermentation. Ph.D thesis, Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh.
- Rahman MA., Razvy MA., Alam MA. 2013. Antagonistic activities of *Trichoderma* strains against chilli anthracnose pathogen. *IJMM* 1(1): 7-22. (<http://www.innspub.net>)
- Ramachandran N, Madhavi RK, Rathnamma K. 2007. Current Status of Chilli

- Anthracnose in India. Abstracts of the First International Symposium on Chilli Anthracnose. National Horticultural Research Institute, Rural Development of Administration, Republic of Korea.
- Samuels GJ. 1996 *Trichoderma*: A review of biology and systematics of the genus. *Mycol. Res.* 100: 923-935.
- Shake MU. 2006 Studies on sheath blight disease of Rice (*Oryza sativa* L.) caused by *Rhizoctonia solani* and its control. M. Sc. thesis. Department of Botany, University of Rajshahi, Rajshahi, Bangladesh.
- Shoresh., M., Mastouri F., Harman GE. 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. *Annual Review of Phytopathology*. 48:21-43.
- Shoresh M, Mastouri, F., and Harman G. E. 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. *Annual Review of Phytopathology*. 48, 21-43.
- Soytong K, Srinon W, Rattanacherdchai K, Kanokmedhakul S and Kanokmedhakul K. 2005. Application of antagonistic fungi to control anthracnose disease of grape. *Journal of Agricultural Biotechnology* 1: 33-41.
- Sultana N. 1999. Biological control of foot and root rot of lentil with *Trichoderma harzianum*. MS thesis, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh., Bangladesh.
- Sumitra R., Gaikward SJ. 1995. Cheeking *Fusarium* wilt of pigeon pea by Biological Means. *J. Soil. Crop*. 5(2): 163-165.
- Vey A., Hoagland RE., Butt TM. 2001. Toxic metabolites of fungal bio control agents. In: Butt T.M., Jackson C., Magan N. (eds): Fungi as bio control agents: Progress, problems and potential. CAB International, Bristol. 311-346.
- Vinale F., Sivasithamparam K., Ghisalberti EL., Marra R., Barbetti M.J., Li H., Lorito M. 2008. A novel role for *Trichoderma* secondary metabolites in the interactions with plants. *Physiol Mol Plant Pathol*. 72(1-3): 80-86.
- Watanabe, N. 1993. Promoting effect of *Trichoderma* spp. on seed germination and plant growth in vegetable. Mem. Inst. Sci. Tech. Meiji. Univ. 32(2):9-18.
- Wharton PS., Kirk WW. , Schafer RL., Tumbalam P. 2012. Evaluation of biological seed treatments in combination with management practices for the control of seed-borne late blight in potato. *Biological Control*. 63 (3): 326-332.
- Wilcox WF, Harman GE, Di Pietro A. 1992. Effect of gliotoxin on growth, sporulation, and zoospore motility of seven *Phytophthora* spp. *in vitro*. *Phytopathology*. 82, 11-21.
- Windham MT., Elad Y. , Baker R. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. *Phytopathology*. 76: 518-521.
- Yedidia I, Benhamou N., Chet I. 1999. Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. *Appl. Environ. Microbiol*. 65: 1061-1070.
- Yedidia I, Benhamou N., Kapulnik Y., Chet I. 2000. Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-203. *Plant Physiol. Biochem*. 38:863-873.
- Yedidia I, Srivastva AK., Kapulnik Y., Chet I. 2001. Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. *Plant Soil* 235:235-242.
- Zimand G., Eland Y., Chet I. 1996. Effect of *Trichoderma harzianum* on *Botrytis cinerea* pathogenicity. *Phytopathology* 86: 1255-1260.