Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 2, No. 1, p. 9-18, 2012
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Use of culture filtrates of *Trichoderma* strains as a biological control agent against *Colletotrichum capsici* causing Anthracnose fruit rot disease of chili

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Received: 02 January 2012
Revised: 17 January 2012
Accepted: 24 January 2012

Key words: Biological control, Culture filtrates, *Trichoderma*, anthracnose fruit rot, chili, germination percentages, yield components.

Abstract

Culture filtrates of five *Trichoderma* strains viz. *Trichoderma virens* IMI-392430, *T. pseudokoningii* IMI-392431, *T. harzianum* IMI-392432, *T. harzianum* IMI-392433 and *T. harzianum* IMI-392434 were used as seed treatments alone and in combination with culture filtrates of *Colletotrichum capsici*, to assay their efficacy in suppressing Anthracnose fruit rot disease and promoting chili plant growth and yield, under field conditions. A pot trial experiment was conducted at the Botanical Garden, Rajshahi University, Bangladesh from July 2006 to March 2007. Application of culture filtrates of *T. harzianum* IMI-392433 (T_8) significantly (p=0.05) suppressed the disease percentages (94.97 %) compared to *C. capsici* treatment and improved both plant growth and yield. The highest seed germination rate (100%) and the highest growth and yield (20.37gm/plant) were also recorded in the same treatment; while culture filtrates of *C. capsici* treatment (T_1) alone significantly decreased these values. The correlation matrix showed that yield of chili had significant and positive correlation with plant height (r = 0.979**), number of leaf per plant (r = 0.877 **), number of primary branch (r = 0.916 **), number of secondary branch (r = 0.889**), total number of leaf (r = 0.949**) and total number of flower (r = 0.953**) at maximum flowering time, root number (r = 0.970**), root length(r = 0.923**), total number of fruit (r = 0.980**), fruit length (r = 0.935**), fresh fruit weight (r = 0.967**), dry fruit weight (r = 0.920**), total number of seed per fruit (r = 0.868**) and hundred seed weight (r = 0.955**). The significant and negative correlation (r = - 0.671**) was observed with the yield and percentages of infected fruits. The results suggest that *T. harzianum* IMI-392433 has growth promoting effects and this strain may be used as an effective bio control agent to control Anthracnose fruit rot disease of chili.

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Introduction

Anthracnose incited by *Colletotrichum capsici* is one of the most damaging diseases of chili in Bangladesh. The fungus is both internally and externally seed-borne (Ramachandran *et al.*, 2007). Sowing such contaminated seeds results in pre emergent and post emergent damping-off of seedlings in nursery and field. These infected seedlings form the primary sources of inoculums. The fungus survives in an active form on the stems and branches causing die-back symptoms. This fungus causes severe damage on chili fruits in both pre and post harvest stages and these infections together account for more 50% of the crop losses (Pakdeevaraporn *et al.*, 2005). Fungicide is most commonly used as a treatment to manage, but there is a need for non-chemical methods of control to reduce the adverse effects of toxic chemicals on the environment. However, this may occasionally be ineffective due to the occurrence of resistant fungal populations (Katan *et al.*, 1989).

Trichoderma isolates are known for their ability to control plant pathogens (Elad and Freeman, 2002). Intensive research into bio control with *T. harzianum* has been carried out under commercial conditions, and there have been some significant achievements in greenhouse crops and in vine yards (Elad and Shtienberg, 1995).The first bio control agent (BCA) to be commercialized, registered and used in greenhouse crops and vine yards was isolateT-39 of *T. harzianum* (TRICHODEX), which effectively controlled *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Cladosporium fulvum* diseases in greenhouse grown tomato and cucumber, and in vine yards (Elad, 2000). *T. harzianum* isolates were also reported to control Anthracnose disease of chili (Intana *et al.*, 2007). Fungal metabolites are substances discharged by fungi in their metabolic processes. The metabolites are products of some amino acids, cyclic peptides, aromatic, phenols, terpinoids and plant growth regulators (Madhosing, 1995). Strains of *Trichoderma* can produce antifungal metabolites (Viterbo *et al.*, 2002). They may also be competitors of fungal pathogens (Elad and Shtienberg, 1995), which promotes plant growth (Papavizas, 1985). In addition, a number of *Trichoderma* strains are able to secrete lytic enzymes such as chitinases and 1, 3-β-glucanases (El-Katatny *et al.*, 2001). These enzymes act as key enzymes in the lyses of phytopathogenic fungal cell walls during the antagonistic action of *Trichoderma* spp. Biological control of plant diseases by antifungal volatiles from fungal strains have been carried out under the greenhouse conditions (Koitabashi, 2005). Moreover, the use of culture filtrates of bio-agents mixed with fungicides to control some plant pathogens, have been reported (Fan and Tian, 2001; Yoshida *et al.*, 2001). Therefore, the present study was undertaken to evaluate the effect of culture filtrates of selective *Trichoderma* strains alone and their combination with culture filtrates of *Colletotrichum capsici* to control anthracnose fruit rot disease and to evaluate growth, yield and yield contributing character of chili.

Materials and Methods

To evaluate the efficacy of culture filtrates of *Trichoderma* strains in controlling of *C. capsici* caused Anthracnose fruit rot disease and investigate the growth and yield contributing character of chili the experiment was conducted at Botanical Garden of Rajshahi University, Rajshahi, Bangladesh during July 2006 to March 2007.

Seed collection

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

Sources of Trichoderma

Five *Trichoderma* strains viz. *T. virens* IMI-392430, *T. pseudokoningii* IMI-392431 and *T. harzianum* IMI-392432, *T. harzianum* IMI-392433 and *T. harzianum* IMI-392434 were used in this study, which was collected from Biotechnology and Microbiology Laboratory, Department of Botany, University of Rajshahi, Bangladesh. These strains were isolated and identified from decomposed garbage and soil by Rahman (2009)

and were previously verified by CABI Bioscience, Surrey, U.K.

Isolation and identification of pathogen from anthracnose disease of Chili

Anthracnose fungus was isolated by tissue transplanting method according to the method of Agrios (2005). Pathogen was isolated from the transitional zone of healthy and infected tissues on PDA medium.

Preparation and application of culture filtrates of Trichoderma strains and pathogen

200 ml of Richard's solution (KNO_3: 1.0g, KH_2PO_4:0.5g, $MgSO_47H_2O$: 0.25g, glucose: 34g, trace amounts of $FeCl_3$ in 1L distilled water, pH6.5) was prepared and poured into 500 ml conical flasks and autoclaved for 15 minute at 121 °C/1.05kg/cm² 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 50 ml of 30 days old filtrate of each *Trichoderma* strains and the treated seeds were dried under laminar air flow hood. In the same way treated and untreated seed were treated with 50 ml of culture filtrates of *C. capsici* about 20 minutes and dried inside the laminar air flow. After germination of the treated seeds, 50 ml of culture filtrates of both *Trichoderma* strains and *C. capsici* were poured separately in respective treatment onto the pot-soil 10 days interval up to harvesting. The control treatment was carried out using water.

Treatments

Experiment was designed with the following combinations.

T_0= control (untreated seeds), T_1 = culture filtrates of *C. capsici*, T_2= culture filtrates of *T. virens* IMI-392430, T_3= culture filtrates of *T. virens* IMI-392430 + culture filtrates of *C. capsici,* T_4= culture filtrates of *T. pseudokoningii* IMI-392431, T_5= culture filtrates of *T. pseudokoningii* IMI-392431 + culture filtrates of *C. capsici,* T_6= culture filtrates of *T. harzianum* IMI-392432, T_7= culture filtrates of *T. harzianum* IMI-392432 + culture filtrates of *C. capsici,* T_8= culture filtrates of *T. harzianum* IMI-392433, T_9= culture filtrates of *T. harzianum* IMI-392433 + culture filtrates of *C. capsici*, T_{10}= culture filtrates of *T. harzianum* IMI-392434, T_{11}= culture filtrates of *T. harzianum* IMI-392434 + culture filtrates of *C. capsici.*

Sterilization of soil

Soil was collected from the research field of Rajshahi University campus and sterilized with formaldehyde (formalin: water; 1:5 V/v) and covered with polythene. After 30 days of sterilization, soils were put in the earth pot (30 × 20 cm). To minimize the losses of excess water 2 cm hole was made from the bottom of the pot.

Seed germination and vigour index

All treated seeds were sown separately in each pot where the soil was previously inoculated with culture filtrates of *C. capsici* (50 ml/pot). For control untreated seeds were shown in un-inoculated soil as a positive control. At least 10 seeds were sown in each pot. After 5 days of germination, seeds germination percentages were recorded. Vigour index for each treatment was determined according to the following formula Abdul-Baki and Anderson, (1973). Vigour index = [Mean of root length (cm) + Mean of shoot length (cm)] × percentages of seed germination.

Collection of data on yield and yield contributing characters

Yield and yield contributing data were collected at different stages of plant growth after sowing. Observation were recorded for plant height, leaf number, primary and secondary branch, number of flower and number of leaves at the maximum flowering time, root number, root length, total number of fruit, fresh fruit weight, dry fruit weight, total number of seed per fruit, 100 seed weight, yield per plant and percentage of infected fruits.

Percentage of infected fruits

Percentage of infected fruits was recorded by adopting the grading formula of Siddaramaiah *et al.* (1978).

Percentage of infected fruits =

$$\frac{\text{Total no. of infected fruits}}{\text{Total no. of fruits.}} \times 100$$

Experimental design and statistical analysis

The experiment was carried out following Randomized Block Design (RBD) with three replicates and 10 chili plants were used in each replicates. Data on growth yield and yield contributing characters were recorded and statistically analyzed with the help of computer package program SPSS (SPSS Inc., Chicago, IL, USA) for DMRT test.

Results and discussion

Germination percentages and vigour index

Seed germination and the vogour index were significantly ($p \leq 0.05$) affected by the treatments. The highest percentage of seed germination (100%) and vigour index (725) were recorded for the seeds treatments with *T. harzianum* IMI-392433 (T_8) and the lowest was recorded for *C. capsici* (T_1) treatment alone. Seed germination was drastically reduced for T_1 (*C. capsici*) and control. These results revealed that *T. harzianum* might promote chili seed germination. *Trichoderma* spp. have evolved numerous mechanisms like mycoparasitism, production of inhibitory substances, inactivation of pathogen enzymes and induction of resistance to attack other fungi and enhance and root growth (Ozbay *et al.*, 2004). Seedling vigour was found to be higher when seeds were treated with *Trichoderma* IMI-392433 (T_8), whereas control (T_0) and *C. capsici* (T_1) showed the worst seedling vigour. Consistent with the results, Mukhtar (2008) observed the highest vigour index when okra seeds were treated with spore suspension of *T. harzianum*. Lo and Lin (2002) screened *Trichoderma* strains on plant and root growth of bitter gourd, loofah and cucumber and noted that *Trichoderma* strains significantly increased seedling height by 26 to 61%, root exploration by 85-209%, leaf area by 27-38% and root dry weight by 38 to 62% after 15 days of showing. Shake (2006) observed the highest percentage of seed germination and vigour index when rice seeds were treated with *T. harzianum*. Watanabe (1993) were treated thirteen strains of *Trichoderma* species for seed germination and growth promotion effect on tomato and egg plants and found that *T. viride* (IAM 5141) was most effective for the growth of tomato plant and *T. polysporum* was the most effective for seed germination in egg plant. Begum *et al.* (2010) were evaluated five *Trichoderma* strains to assay their efficacy in suppressing *Alternaria* fruit rot disease of chili and promoting chili plant growth and yield and observed that application of spore suspension of *T. harzianum* IMI-392432 significantly suppressed the disease and improved highest seed germination percentage, vigour index, growth and yield.

Table 1. Seed germination (%) and vigour index of chili under different treatments.

Treatments	% of seed germination	Shoot length (cm)	Root length (cm)	Vigour index
T_0	51 k	1.98 b	2.21 ab	213.69 k
T_1	39 l	1.95 b	2.16 ab	123.3 l
T_2	89 d	2.86 ab	2.78 ab	501.96 d
T_3	57 i	2.21 ab	2.32 ab	258.21 i
T_4	87 e	2.73 ab	2.53 ab	457.62 e
T_5	54 j	2.12 ab	2.26 ab	236.52 j
T_6	96 b	3.53 ab	2.92 ab	619.20 b
T_7	68 g	2.46 ab	2.45 ab	333.88 g
T_8	98 a	3.98 a	3.08 a	691.88 a
T_9	70 f	2.59 ab	2.49 ab	355.60 f
T_{10}	93 c	3.37 ab	2.85 ab	578.46 c
T_{11}	62 h	2.29 ab	2.41 ab	291.40 h

Growth characters

Plant height, leaf number, primary branch number, secondary branch number after 60 days and root number, root length after 90 days and leaf and flower number at maximum flowering time were highest for T_8 (*T. harzianum* IMI-392433) and lowest for T_2 (*C. capsici*) treatment, with a significant difference ($p<0.05$). These results indicated that *T. harzianum* has growth-promoting effects on chili. Earlier work on cabbage supports this result, that is, the regular application of *T. harzianum* increased cabbage growth, leaf area, shoot and root dry weight (Rabeendran *et al.*,

2000). Growth promoting effects by *T. harzianum* has been reported in other crops (Chang *et al.*, 1986; Windham *et al.*, 1986) and by *T. koningii* (Windham *et al.*, 1986), but growth promotion has not been demonstrated by *T. virens* on cotton (Hanson, 2000).

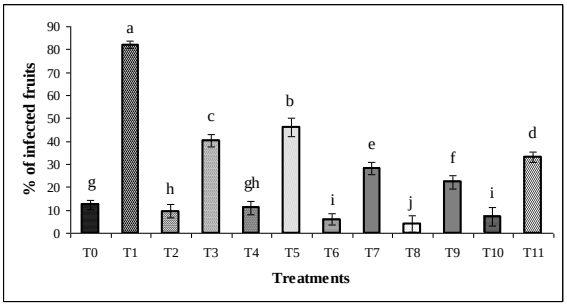

Fig. 1. Effect of *Trichoderma* strains on percentages of infected chili fruit. Bar marked by the same letters are not significantly different ($p<0.05$) by DMRT analysis.

T_0= control (untreated seeds), T_1 = *C. capsici*, T_2= *T. virens* IMI-392430, T_3= *T. virens* IMI-392430 + *C. capsici*, T_4= *T. pseudokoningii* IMI-392431, T_5= *T. pseudokoningii* IMI-392431 + *C. capsici*, T_6= *T. harzianum* IMI-392432, T_7 = *T. harzianum* IMI-392432 + *C. capsici*, T_8 = *T. harzianum* IMI-392433, T_9= *T. harzianum* IMI-392433 + *C. capsici*, T_{10}= *T. harzianum* IMI-392434, T_{11}= *T. harzianum* IMI-392434 + *C. capsici*.

Table 2. Effect of seed treatment with Trichoderma strains on chili growth characteristics after 6 months of sowing.

Treatments	Plant height (cm) 60 DAS	No. of leaf 60 DAS	No. of primary branch 60 DAS	No. of secondary branch 60 DAS	Root number after 90 DAS	Root length after (cm) 90 DAS	No. of leaf at the maximum flowering time	No. of flower at the maximum flowering time
T_0	8.97 i	30.94 j	2.18 d	16.33 k	35.91 i	9.16 e	66.95 j	12.49 gh
T_1	8.31 i	26.72 k	2.14 d	14.96 k	33.81 j	8.36 e	62.71 k	8.78 i
T_2	18.13 d	60.28 c	3.99 bcd	51.21 d	51.24 d	13.58 bc	98.91 d	33.96 c
T_3	11.26 h	42.21 h	2.36 cd	31.28 i	37.12 hi	11.79 cd	71.26 i	12.26 h
T_4	15.36 e	56.81 d	3.54 cd	47.57 e	48.23 e	12.99 bc	92.48 e	28.45 d
T_5	11.81 gh	39.76 i	2.19 d	28.28 j	36.37 i	10.12 de	68.21 j	14.14 g
T_6	22.42 b	65.82 a	5.64 ab	58.96 b	57.29 b	16.94 a	110.36 b	40.86 a
T_7	13.15 fg	48.88 f	2.99 cd	39.64 g	40.91 g	12.28 c	84.59 g	20.92 e
T_8	24.46 a	67.32 a	6.86 a	62.46 a	60.16 a	17.29 a	116.54 a	42.13 a
T_9	14.61 ef	52.46 e	3.24 cd	43.62 f	44.32 f	12.65 bc	88.54 f	22.45 e
T_{10}	20.22 c	62.28 b	4.23 bc	55.24 c	53.29 c	14.29 b	104.46 c	36.84 b
T_{11}	12.14 gh	45.58 g	2.54 cd	34.76 h	38.41 h	11.86 cd	76.27 h	17.46 f

Values within a column followed by the same letters are not significantly different ($p<0.05$) by DMRT analysis.

T_0= control (untreated seeds), T_1 = *C. capsici*, T_2= *T. virens* IMI-392430, T_3= *T. virens* IMI-392430 + *C. capsici*, T_4= *T. pseudokoningii* IMI-392431, T_5= *T. pseudokoningii* IMI-392431 + *C. capsici*, T_6= *T. harzianum* IMI-392432, T_7 = *T. harzianum* IMI-392432 + *C. capsici*, T_8 = *T. harzianum* IMI-392433, T_9= *T. harzianum* IMI-392433 + *C. capsici*, T_{10}= *T. harzianum* IMI-392434, T_{11}= *T. harzianum* IMI-392434 + *C. capsici*.

Yield and yield contributing characters

The highest total number of fruit, fruit length, fresh fruit weight, dry fruit weight and number of seeds per fruit, 100 seed weight and yield/plant was highest for T_8 (*T. harzianum* IMI-392433) treatment and the lowest for T_2 (*C. capsici*) treatment (Table 3). *T. harzianum* IMI-392433 increased yield and yield contributing characters by 85.87 % for total number of fruit, 61.36 % for fruit length, 82.47 % for fresh fruit weight, 63.27 % for dry fruit weight, 67.54 % for number of seeds/fruit, 43.75 % for 100 seed weight and 89.59 % for yield/plant compared to *C. capsici* (T_1)treatment. The results revealed that the yield and yield contributing characteristics were significantly affected by the application of *T. harzianum* IMI-392433. With *T. harzianum* treatment of the seeds, many workers found much higher yields compared to control. Sumitra and Gaikward (1995) opined that *T. harzianum* increased shoot and root length of pigeon pea in *Trichoderma* treated plots. Harman (2000) reported that *T. harzianum* (T22), when applied as a seed treatment on potatoes, frequently increased both size and yield. Bal

and Altintas (2006)observed that *T. harzianum* has positive effect in the early yield of tomato plant (*Lycopersicon esculentum*), which produced 527g/plant in comparison to the control with 374g/plant. Da Luz *et al.* (1998) also observed that yields of wheat seeds infected with *Pyrenophora triticirepentis*, were significantly increased after application of *T. virens* (1.66 kg/ha). Poldma *et al.* (2002) conducted an experiment on cucumber production in a greenhouse, to determine the effect of *T. viride* on yield and observed that *T. viride* produced the highest yield.

Table 3. Effect of Trichoderma strains on yield and yield contributing characteristics of chili after 6 months of sowing.

Treatments	Total no. of fruit	Fruit length (cm)	Fresh fruit weight (gm) 5fruit/plant	Dry fruit weight (gm) 5fruit/plant	Total number of seed/fruit	100 seed weight (gm)	Yield (gm)/plant
T_0	7.48 i	3.42 cd	2.99 f	0.38 k	80.12 k	0.56 j	2.94 gh
T_1	5.14 j	3.18 d	2.61 f	0.36 l	76.36 l	0.54 k	2.12 h
T_2	24.94 d	6.54 ab	11.26 b	0.78 d	211.51 d	0.82 d	10.36 d
T_3	10.86 h	3.91 cd	3.88 ef	0.52 i	93.39 i	0.61 i	3.58 gh
T_4	20.18 e	5.28 bc	9.54 c	0.76 e	201.31e	0.77 e	7.54 e
T_5	10.23 h	3.54 cd	3.28 f	0.46 j	88.21 j	0.57 j	3.12 gh
T_6	32.94 b	7.54 a	13.86 a	0.96 b	226.18 b	0.92 b	16.39 b
T_7	13.24 g	4.42 cd	5.28 de	0.64 g	106.49 g	0.69 g	4.78 fg
T_8	36.39 a	8.23 a	14.89 a	0.98 a	235.26 a	0.96 a	20.37 s
T_9	16.49 f	4.84 bcd	6.43 d	0.69 f	192.53 f	0.73 f	5.92 ef
T_{10}	28.98 c	7.21 a	11.96 b	0.89 c	220.13 c	0.87 c	13.71 c
T_{11}	12.86 g	4.12 cd	4.18 ef	0.61 h	99.78 h	0.65 h	3.98 gh

Values within a column followed by the same letters are not significantly different ($p<0.05$) by DMRT analysis.

T_0= control (untreated seeds), T_1= *C. capsici*, T_2 = *T. virens* IMI-392430, T_3= *T. virens* IMI-392430 + *C. capsici*, T_4= *T. pseudokoningii* IMI-392431, T_5= *T. pseudokoningii* IMI-392431 + *C. capsici*, T_6 = *T. harzianum* IMI-392432, T_7 = *T. harzianum* IMI-392432 + *C. capsici*, T_8 = *T. harzianum* IMI-392433, T_9= *T. harzianum* IMI-392433 + *C. capsici*, T_{10}= *T. harzianum* IMI-392434, T_{11}= *T. harzianum* IMI-392434 + *C. capsici*.

Table 4. Correlation matrix among different parameters of Chili as influenced by treatments.

Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1.000															
2	0.94**	1.000														
3	0.90**	0.783**	1.000													
4	1.00**	0.99**	0.792**	1.000												
5	0.98**	0.949**	0.875**	0.953**	1.000											
6	0.95**	0.931**	0.926**	0.935**	0.930**	1.000										
7	0.9**	0.969**	0.843**	0.974**	0.991**	0.931**	1.000									
8	0.97**	0.958**	0.844**	0.959**	0.993**	0.916**	0.99**	1.000								
9	0.99**	0.949**	0.872**	0.954**	0.993**	0.939**	0.98**	0.98**	1.000							
10	0.94**	0.86**	0.962**	0.871**	0.927**	0.943**	0.90**	0.916**	0.92**	1.000						
11	0.97**	0.931**	0.901**	0.933**	0.992**	0.931**	0.972**	0.98**	0.98**	0.955**	1.000					
12	0.96**	0.98**	0.812**	0.990**	0.971**	0.940**	0.98**	0.975**	0.97**	0.885**	0.951**	1.000				
13	0.90**	0.932**	0.752**	0.932**	0.953**	0.839**	0.95**	0.94**	0.92**	0.840**	0.936**	0.937**	1.000			
14	0.97**	0.96**	0.850**	0.973**	0.993**	0.937**	0.99**	0.991**	0.98**	0.913**	0.978**	0.990**	0.950**	1.000		
15	-0.70**	-0.77**	-0.55**	-0.74**	-0.75**	-0.68**	-0.767	-0.77**	-0.73**	-0.64**	-0.71**	-0.74**	-0.74**	–0.75**	1.000	
16	0.97**	0.877**	0.916**	0.889**	0.970**	0.923**	.0.94**	0.95**	0.98**	0.935**	0.967**	0.920**	0.86**	0.955**	-0.671**	1.000

** Significant at 1% level. 1=60 days of plant height, 2= 60 days of leaf number, 3= 60 days of primary branch, 4= 60 days of secondary branch, 5= 90 days of root number, 6= 90 days of root length, 7= No. of leaf at the maximum flowering time, 8= No. of flower at the maximum flowering time, 9= Total no. of fruit, 10= Fruit length, 11= Fresh fruit weight, 12= Dry fruit weight, 13= Total number of seed per fruit, 14= 100 seed weight, 15= Percentages of infected fruits, 16= Yield /plant.

Percentage of infected fruit

The highest percentage of infected fruit was recorded of *C. capsici* (T_1) treatment and the lowest was recorded of *T. harzianum* (T_8) treatment. In control (T_0), a remarkable percentage of infected fruit was also observed; in this case infection may be due to the seeds or environment. Application of culture filtrates of *T. harzianum* IMI-392433 was significantly ($p \leq 0.05$) suppressed the disease (94.97 %) compared to the *C. capsici* treatment (Fig 1). During plant pathogens attack, *Trichoderma* secreted many cell wall-degrading enzymes such as endochitinase, chitobiosidase, N-acetyl-β-glucosaminidase and glucan 1, 3-β-glucosidase. These enzymes strongly inhibit spore germination (or cell replication) and germ tube elongation (Tronsmo and Hjeljord, 1997). *Trichoderma* spp. can also produce different antibiotics against fungal phytopathogens. Among these antibiotics, the production of gliovirin, gliotoxin, viridin, pyrones, peptaibols and others have been described (Vey *et al*., 2001). Gliovirin from *T. virens* was active against *Pythium ultimum*, gliotoxin from *T. virens* was very active against *Rhizoctonia solani* (Howell and Stipanovic, 1995). There are many reports of successful use of antifungal metabolite extracted from *Trichoderma* spp. to control disease causing fungi such as *Sclerotium rolfsii* causing disease on vegetables (Maiti *et al*., 1991), *Pythium aphanidermatum* causing wilt of cotton and watermelon (Ordentlich *et al*., 1992), *Ceratocystis paradoxa* causing pine apple disease of sugarcane (Rahman *et al*., 2009) and damping-off of cucumber (Intana *et al*., 2007) and *Phytophthora* sp. causing various plant diseases (Wilcox *et al*., 1992). This research indicated an additional successful use of antifungal metabolites from *Trichoderma* strains in controlling anthracnose on chili fruits caused by *C. capsici*.

Correlation matrix

The correlation matrix among different plant parameters are presented in Table 4. The correlation matrix showed that yield per plant of chili had significant and positive correlation with plant height ($r = 0.979^{**}$), number of leaf per plant ($r = 0.877^{**}$), number of primary branch ($r = 0.916^{**}$), number of secondary branch ($r = 0.889^{**}$), total number of leaf ($r = 0.949^{**}$) and total number of flower ($r = 0.953^{**}$) at maximum flowering time, root number ($r = 0.970^{**}$), root length ($r = 0.923^{**}$), total number of fruit ($r = 0.980^{**}$), fruit length ($r = 0.935^{**}$), fresh fruit weight ($r = 0.967^{**}$), dry fruit weight ($r = 0.920^{**}$), total number of seed per fruit ($r = 0.868^{**}$) and hundred seed weight ($r = 0.955^{**}$). The significant and negative correlation ($r = -0.671^{**}$) was observed with the yield and percentages of infected fruits. This results indicated that yield of chili were dependence on plant height, number of leaf, number of primary branch, number of secondary branch, root number, root length, number of leaf at maximum flowering time, number of flower, total number of fruit, fruit length, fresh fruit weight, dry fruit weight, number of seed per fruit and hundred seed weight.

From the above findings it may be concluded that culture filtrates of *T. harzianum* IMI-392433 is more effective at controlling *C. capsici* for fruit rot disease of chili, and the strain also showed promising results on chili seed germination, growth and yield characteristics. The results suggest that this strain may be used as an effective bio control agent to control Anthracnose fruit rot disease of chili.

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