Mycobiology 38(2) : 113-117 (2010)
DOI:10.4489/MYCO.2010.38.2.113

Biological Control of Alternaria Fruit Rot of Chili by *Trichoderma* Species under Field Conditions

Most. Ferdousi Begum*, M. A. Rahman and M. Firoz Alam

Biotechnology and Microbiology Laboratory, Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh

(Received February 17, 2010. Accepted February 22, 2010)

***Trichoderma* strains were evaluated under field conditions to assay their efficacy in suppressing *Alternaria* fruit rot disease and promoting chili plant growth. The experiment was conducted at the Botanical Garden, Rajshahi University, Bangladesh from July 2006 to March 2007. Application of *Trichoderma harzianum* IMI 392432 significantly ($p = 0.05$) suppressed the disease compared to *Alternaria tenuis* (T2) treatment and improved both growth and yield. The treatment T4 (*T. harzianum* IMI-392432 + *A. tenuis*) was most effective in reducing disease percentage (72.27%) compared to *A. tenuis* (T1) treatment. The highest seed germination rate (85.56%) and the highest growth and yield (12.5 g/plant) was also recorded in the same treatment (T4), followed by T5 (*T. harzianum* IMI-392433 + *A. tenuis*), T6 (*T. harzianum* IMI-392434 + *A. tenuis*), T2 (*T. virens* IMI-392430 + *A. tenuis*), and T3 (*T. pseudokoningii* IMI-392431 + *A. tenuis*) treatment, while single treatment with *A. tenuis* significantly decreased these values.**

KEYWORDS : Alternaria-fruit rot, Biological control, Chili, *Trichoderma*, Vigor index, Yield components

Chili (*Capsicum annum* L.) is an important spice in Bangladesh, in 2000~2001, the total area under chili cultivation was recorded as 166 ha, with a total production of 141 metric tons [1]. *Alternaria* fruit rot is seed-borne, widespread and highly destructive disease that infects chili plants, and yield loss caused by these diseases has been recorded up to 100 percent under congenial environment conditions [2]. Biological control represents a natural and ecological approach to disease control that reduces chemical input and their effects [3]. The fungi *Trichoderma* has been an exceptionally good model to study biocontrol because it is ubiquitous, easy to isolate and culture, grows rapidly on many substrates, affects a wide range of plant pathogens, is rarely pathogenic on higher plants, acts as a mycoparasite, competes well for food and growth sites, produces antibiotics and has an enzyme system capable of attacking a wide range of plant pathogens [4]. Furthermore, *Trichoderma* inhibit or degrade pectinases and other enzymes that are essential for plant-pathogenic fungi, such as *Botrytis cinerea*, to penetrate leaf surfaces [5].

Although some chemicals are known to control *Alternaria tenuis,* they are not always effective. Furthermore, because chili is a vegetable crop, using chemicals for disease control is nonideal in view of the residue problems. Biocontrol of plant pathogens using antagonistic fungi and bacteria, therefore, assumes more significance. Among antagonistic fungi, *Trichoderma harzianum* has shown promise as a biocontrol agent [6]. Although *Trichoderma* species are probably the most widely used fungi in biological control of plant pathogens, no *in vivo* experiment has been conducted to test whether it can control *Alternaria* fruit rot of chili. Therefore, the objectives of this investigation were to assay the effects of *Trichoderma* strains against fruit rot pathogen *A. tenuis.* Plant growth, yield components and disease percentage were recorded to evaluate their performance under field conditions.

Materials and Methods

To evaluate the efficacy of *Trichoderma* species at controlling *Alternaria* fruit rot disease in chili, experiments were conducted at the Botanical Garden of Rajshahi University, Rajshahi, Bangladesh from July 2006 to March 2007.

Seed collection. Local chili variety "Bogra" was collected from the Spices Research Centre, Bogra, Bangladesh. Pathogen-free healthy seeds were selected for use in this experiment.

Sources of *Trichoderma*. Five *Trichoderma* strains, including *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 the Biotechnology and Microbiology Laboratory, Department of Botany, Rajshahi University, Bangladesh. These strains were previously verified [7] by CABI Bioscience, Surrey, UK.

Isolation of *A. tenuis*. *A. tenuis* was isolated from infected fruit parts of chili which were collected after proper

*Corresponding author <E-mail : ferdrita@yahoo.com>

recording of the symptoms of the disease. Following standard phytopathological methods [8], pathogen was isolated from the transitional zone of healthy and infected tissues on potato dextrose agar (PDA) medium. The pathogenicity of the *A. tenuis* isolate was confirmed on local chili cultivar. All the cultures were stored at 4°C for further study.

Preparation and application of spore suspensions. Mycelial discs (5 mm diameter) of *Trichoderma* isolates and *A. tenuis* were obtained from 4~5 days old culture and separately transferred to 50 mL PDA in a 250-mL conical flask and incubated at 28°C. 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, containing the spores, was collected, and a concentration of 5×10^5 spores/mL was obtained by dilution with sterilized distilled water. For seed treatment, 10 to 15 seeds were dipped in the spore suspension (5×10^5 spores/mL) of 4~5 days old *Trichoderma* strains for about 20 min, and the treated seeds were dried by laminar air flow. After that, both *Trichoderma* treated and untreated seeds were again dipped in the spore suspension (3×10^5 spores/mL) of 7 days old culture of *A. tenuis* for about 20 min and then dried by laminar air flow. After germination of the treated seeds, the pot soil was treated with 30 mL of conidial suspension (combination of *Trichoderma* strains and *A. tenuis*) according to respective treatment. The treatment was continued up to harvesting with seven days interval.

Treatments. The experiments were designed with the following combinations:

T0 = Control (untreated soil and untreated seeds)
T1 = *A. tenuis* (3×10^5 spores/mL)
T2 = *T. virens* IMI-392430 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL)
T3 = *T. pseudokoningii* IMI-392431 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL)
T4 = *T. harzianum* IMI-392432 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL)
T5 = *T. harzianum* IMI-392433 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL)
T6 = *T. harzianum* IMI-392434 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL)

Sterilization of soil. Soil was collected from the research field of Rajshahi University 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 pots (30 × 20 cm). To minimize the loss of excess water, a 2 cm hole was made from the bottom of each pot.

Seed germination and vigour index. All treated seeds were sown separately in each pot with soil previously inoculated with *A. tenuis* (3×10^5 spores/mL). Untreated seeds were sown in un-inoculated soil as a positive control. At least 10 seeds were sown in each pot. After 7 days of germination, seed germination percentages were recorded. Vigour index for each treatment was determined according to the following formula from Abdul-Baki and Anderson [9].

Vigour index = [Mean of root length (cm) + Mean of shoot length (cm)] × percentage of seed germination

Collection of data on yield and yield contributing characteristics. Yield and yield contributing data were collected at different stages of plant growth after sowing. Observations were recorded for plant height, leaf number, node number, primary and secondary branch numbers, number of flowers at maximum flowering, number of leaves at maximum flowering, total number of fruit, fresh fruit weight, dry fruit weight, total number of seeds, yield per plant and percentage of infected fruit.

Percentage of infected fruit. Percentage of infected fruit was recorded by the following formula:

$$\text{Percentage of infected fruit} = \frac{\text{No. of infected fruit}}{\text{Total No. of fruit}} \times 100$$

Experimental design and statistical analysis. The experiment was carried out following Randomized Block Design with three replications. Data on growth yield and yield

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

Treatment	% of seed germination[a]	Shoot length[a] (cm)	Root length[a] (cm)	Vigour index[a]
T0	47.78 d	2.01 d	1.84 e	179.33 f
T1	35.56 e	1.69 e	1.5 f	113.67 g
T2	68.89 b	2.56 c	2.34 c	335.67 d
T3	62.22 c	2.34 c	2.1 d	277.56 e
T4	85.56 a	3.42 a	3.23 a	569.67 a
T5	80 a	3.09 b	3.05 a	492.67 b
T6	73.33 b	2.87 b	2.77 b	415 c

Values within a column followed by the same letters are not significantly different ($p < 0.05$) by DMRT analysis.
T0 = Control, T1 = *Alternaria tenuis* (3×10^5 spores/mL), T2 = *Trichoderma virens* IMI-392430 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T3 = *T. pseudokoningii* IMI-392431 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T4 = *T. harzianum* IMI-392432 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T5 = *T. harzianum* IMI-392433 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T6 = *T. harzianum* IMI-392434 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL).
[a]Values are the means of three replications.

contributing characteristics were recorded and statistically analyzed by DMRT test with the help of the computer package program SPSS (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Germination percentage and vigour index. Seed germination and the vigour index were significantly ($p \leq 0.05$) affected by the treatments (Table 1). The highest percentage of seed germination (85.56%) and vigour index (569.67) were recorded for the seeds treated with *T. harzianum* IMI-392432 (T4), and the lowest was recorded for pathogen treatment alone (T1). Seed germination was drastically reduced for T1 (*A. tenuis*) 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 plant and root growth [10]. Seedling vigour was found to be higher when seeds were treated with *T. harzianum* IMI-392432 (T4) spore suspension, whereas control (T0) and *A. tenuis* (T1) showed the worst seedling vigour. Consistent with these results, Mukhtar [11] observed the highest vigour index when okra seeds were treated with *T. harzianum*. Lo and Lin [12] 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% 15 days of showing. Shake [13] observed the highest percentage of seed germination and vigour index when rice seeds were treated with *T. harzianum*.

Growth analysis. After 30, 60, and 90 days, plant height, leaf number, node number, primary branch number, secondary branch number, and leaf and flower numbers at maximum flowering were highest for T4 (*T. harzianum* IMI-392432) and lowest for T1 (*A. tenuis*) treatment, with

Table 2. Effect of seed treatment with *Trichoderma* strains on chili growth characteristics

Treatment	Plant height[a] (cm)			No. of leaf[a]			No. of node			No. of primary branch[es]		No. of secondary branch[es]		No. of leaf at the maximum flowering stage[a]	No. of flower at the maximum flowering stage[a]
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS*		
T0	3.31 d	12.96 d	22.22 e	5.89 e	54.89 cd	88.44 d	3.11 de	15 e	54.22 de	2.89 e	3.56 f	29.22 e	42.44 cd	39.89 e	8.56 e
T1	2.53 e	8.14 e	16.91 f	4 f	42.11 d	13.89 e	2.22 e	11.33 f	45.44 e	2.22 f	2.44 g	20.44 f	33.44 d	23.78 f	4.89 e
T2	5.28 b	16.99 bc	25.08 cd	7.67 cd	70.67 b	117.33 bc	4.67 bc	19.56 d	88.44 bc	4.22 d	5 d	53 c	77.56 b	79.89 d	29 c
T3	4.28 c	14.7 cd	23.26 de	6.56 de	63.22 bc	100.56 cd	3.78 cd	16.89 e	74.67 cd	3.44 e	4.22 e	38.89 d	54.67 c	69.22 d	20.44 d
T4	6.92 a	23.17 a	37.64 a	13 a	89.44 a	161.11 a	6.78 a	28.33 a	133.44 a	7.33 a	9.11 a	81.33 a	95.22 a	159.11 a	55.89 a
T5	5.96 b	19.57 b	30.6 b	9.44 b	78 ab	131.56 b	5.56 b	25.78 b	107.78 b	6.22 b	7.22 b	74.78 ab	87.78 ab	130.78 b	44.56 b
T6	5.49 b	17.71 b	27.64 c	9.22 bc	75.44 ab	121 b	5 b	23.44 c	96.67 bc	5 c	6 c	68.56 b	81.56 ab	99.44 c	32.11 c

Values within a column followed by the same letters are not significantly different ($p < 0.05$) by DMRT analysis.
T0 = Control, T1 = *Alternaria tenuis* (3×10^5 spores/mL), T2 = *Trichoderma virens* IMI-392430 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mLl), T3 = *T. pseudokoningii* IMI-392431 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T4 = *T. harzianum* IMI-392432 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T5 = *T. harzianum* IMI-392433 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T6 = *T. harzianum* IMI-392434 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL).
[a]Means of three replications.

Table 3. Effect of *Trichoderma* strains on chili yield and yield contributing characteristics

Treatment	Total no. of fruit[sa]	Fresh fruit weight[a] (gm)	Dry fruit weight[a] (gm)	No. of seed/Fruit[a]	100 seed weight[a] (gm)	Yield (gm)/Plant[a]
T0	4.22 ef	6.29 e	0.19 e	89.11 e	0.44 cd	3.17 de
T1	3 f	3.84 f	0.13 e	80.56 e	0.37 d	1.95 e
T2	7.67 d	8.41 cd	0.45 cd	140.56 c	0.54 bcd	6.62 bc
T3	5.78 de	6.97 de	0.35 d	118.33 d	0.48 bcd	4.84 cd
T4	20.78 a	14.59 a	0.88 a	189.11 a	0.74 a	12.5 a
T5	16.44 b	10.92 b	0.66 b	177 ab	0.63 ab	10.76 a
T6	12.11 c	8.59 c	0.53 c	158.56 bc	0.55 abc	8.24 b

Values within a column followed by the same letters are not significantly different ($p < 0.05$) by DMRT analysis.
T0 = Control, T1 = *Alternaria tenuis* (3×10^5 spores/mL), T2 = *Trichoderma virens* IMI-392430 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T3 = *T. pseudokoningii* IMI-392431 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T4 = *T. harzianum* IMI-392432 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T5 = *T. harzianum* IMI-392433 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T6 = *T. harzianum* IMI-392434 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL).
[a]Means of three replications.

a significant difference ($p < 0.05$) (Table 2). These results indicate that *T. harzianum* IMI 392432 has growth-promoting effects on chili. Growth promoting effects by *T. harzianum* has been reported in other crops [14, 15] and by *T. koningii* [15], but growth promotion has not been demonstrated by *T. virens* on cotton [16].

Yield and yield contributing characters. The highest total number of fruit, fresh fruit weight, dry fruit weight, and number of seeds per fruit, 100 seed weight and yield/plant was highest for T_4 treatment and lowest for *A. tenuis* (T1) treatment (Table 3). *T. harzianum* IMI-392432 increased yield and yield contributing characteristics by 74.77% for total number of fruit, 58.33% for fresh fruit weight, 74.26% for dry fruit weight, 40.25% for number of seeds/fruit, 33.33% for 100 seed weight and 73.01% for yield/plant compared to *A. tenuis* (T2) treatment. The results reveal that the yield and yield contributing characteristics were significantly affected by the application of *T. harzianum* IMI-392432. With *T. harzianum* treatment of the seeds, many workers found much higher yields compared to control. Sultana [17] obtained up to 81.60% higher len-

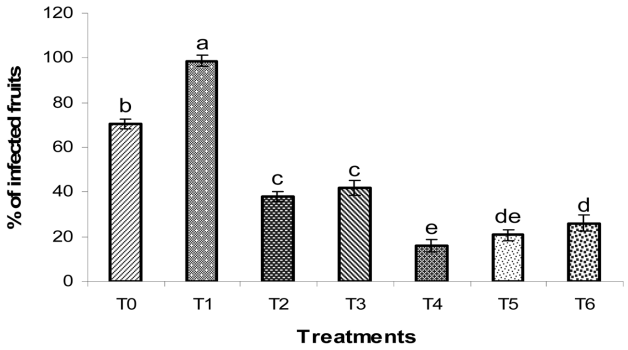

Fig. 1. Effect of *Trichoderma* strains on percentages of infected chili fruit. Bars marked by the same letters are not significantly different ($p < 0.05$) by DMRT analysis. T0 = Control, T1 = *Alternaria tenuis* (3×10^5 spores/mL), T2 = *T. virens* IMI-392430 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T3 = *T. pseudokoningii* IMI-392431 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T4 = *T. harzianum* IMI-392432 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T5 = *T. harzianum* IMI-392433 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL), T6 = *T. harzianum* IMI-392434 (5×10^5 spores/mL) + *A. tenuis* (3×10^5 spores/mL).

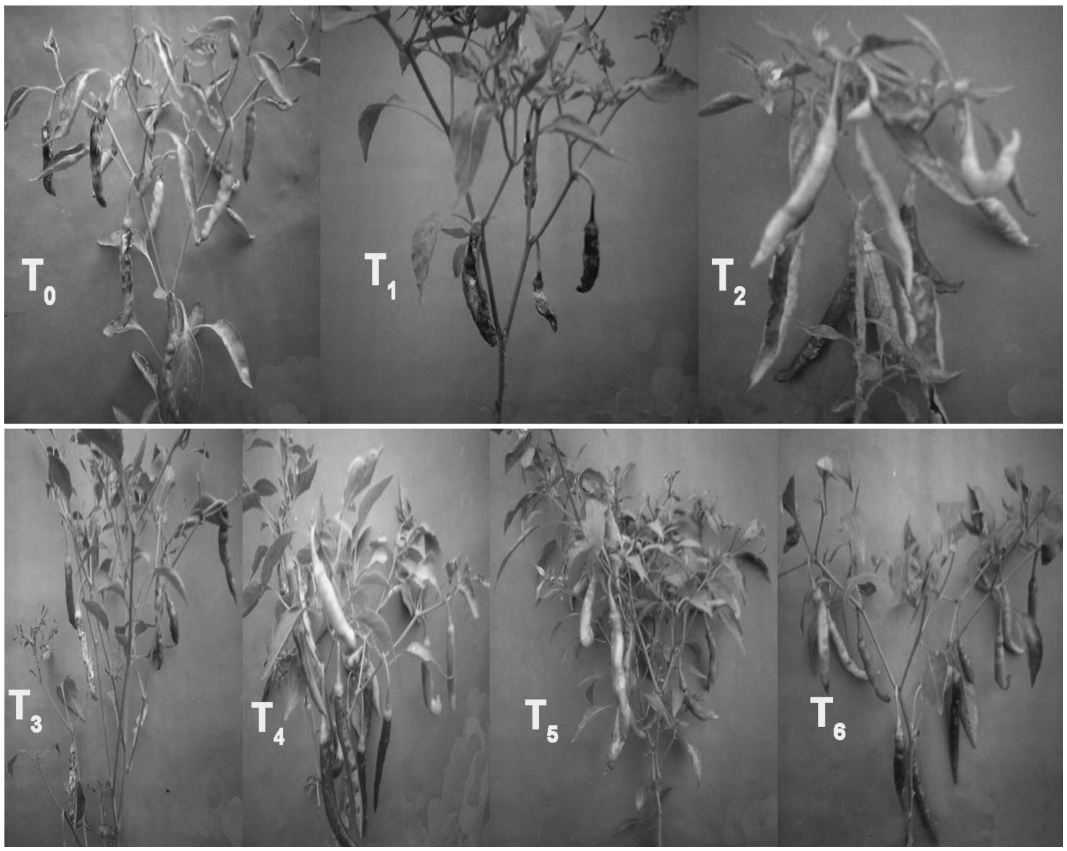

Fig. 2. Photographs show the effects of *Trichoderma* strains on suppressing *Alternaria* fruit rot of chili; T0 = infected fruit of chili in control; T1 = infected fruit of chili treated with *A. tenuis*; T2 = healthy fruit of chili treated with *T. virens* IMI-392430 + *A. tenuis*, T3 = infected fruit of chili treated with *T. pseudokoningii* IMI-392431 + *A. tenuis*, T4 = healthy fruit of chili treated with *T. harzianum* IMI-392432 + *A. tenuis*, T5 = healthy fruit of chili treated with *T. harzianum* IMI-392433 + *A. tenuis*, T6 = healthy fruit of chili treated with *T. harzianum* IMI-392434 + *A. tenuis*.

til seed yield when they were treated with *T. harzianum*. Sumitra and Gaikward [18] opined that *T. harzianum* increased shoot and root length in *Trichoderma* treated plots. Harman [19] reported that *T. harzianum* (T22), when applied as a seed treatment on potatoes, frequently increased both size and yield. da Luz *et al.* [20] also observed that yields of wheat seeds infected with *Pyrenophora triticirepentis*, were significantly increased after application of *T. virens* (1.66 kg/ha).

Percentage of infected fruit. The highest percentage of the lowest percentages of infected fruit was recorded in T4 treatment (Fig. 1). In the control (T0), a remarkable percentage of infected fruit was also observed; in this case infection may be due to the seeds or environment. Application of *T. harzianum* IMI 392432 was significantly ($p \leq 0.05$) suppressed the disease (72.27%) compared to the *A. tenuis* (T1) treatment (Fig. 2). Biswas and Das [21] reported that against seedling disease *Trichoderma* is superior as seed coating. Prasad *et al*. [22] found that soil treated with *T. harzianum* showed 61.5% disease control in chickpea while Kashem *et al*. [23] observed seed < 30% disease control in lentil.

From the above findings it may be concluded that *T. harzianum* IMI-392432 is more effective at controlling *Alternaria* fruit rot disease in chili, and this strain also showed promising results on chili germination, growth and yield characteristics. The results suggest that this strain may be used as an effective biocontrol agent to control fruit rot disease of chili.

Acknowledgements

The authors are highly grateful to the Ministry of National Science Information and Communication Technology, Bangladesh for financial support. The authors also express their special thanks to the Spices Research Centre, Bogra, Bangladesh.

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