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USE OF *Trichoderma* IN BIOLOGICAL CONTROL OF FOOT AND ROOT ROT OF LENTIL (*Lens culinaris* Medik)

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ABSTRACT

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A series of experiments were conducted to assess the effect of 14 isolates of *Trichoderma* spp. (*Trichoderma harzianum* and *T. viride*) for control of foot and root rot of lentil (*Lens culinaris* Medik) caused by *Fusarium oxysporum* Schlecht. The pathogenecity of 12 isolates of *F. oxysporum* and the mass production of an isolate of *T. harzianum* on 25 substrates were also studied. The isolates of *Trichoderma* spp. and *F. oxysporum* were collected from different locations of Bangladesh. *Trichoderma* isolates inhibited the growth of *F. oxysporum* from 45.87 to 92.07 % at 7 days after inoculation on agar plates. The isolate TG-2 of *T. harzianum* showed the highest inhibition of the pathogen in field condition. The lowest foot and root rot incidence (6.9%), highest seed germination (82.08%), maximum plant stand (93.12%) and the highest seed yield (3726.67 kg ha^{-1}) were recorded in plots where the isolate TG-2 was applied. In pathogenecity test, the highest foot and root rot incidence and the lowest plant stand of lentil were recorded in the pots where soil inoculation with the isolate FBg-1 of *F. oxysporum* was done. The highest colony forming unit (4.04 x 10^6/g) of *T. harzianum* (TG-2) was recorded in chickpea bran. The study concluded that the isolate TG-2 of *T. harzianum* can be used to control foot and root rot disease of lentil in Bangladesh and chickpea bran is a useful substrate for mass production of that isolate for soil application.

Key words: *Bio-control agent, Fusarium oxysporum, Soil-borne fungus*

INTRODUCTION

Lentil (*Lens culinaris* Medik) is an important food crop in Bangladesh. It contains 25.78% protein (Erskine and Witcombe, 1984) and 59% carbohydrate (Bakhsh *et al.* 1991). Lentil is traditionally used as a common item in daily diet of the people of Bangladesh. In Bangladesh lentil is cultivated in an area of 1,54,655 ha and the yearly production is about 1,22,000 mt (BBS 2009). However, the yield of lentil is much lower in Bangladesh compared to that of other lentil growing countries like Syria, Turkey, Canada, USA and Ethiopia (Hossain *et al.* 1999).

There are various reasons associated with low yield of lentil in Bangladesh, where diseases are considered as the major constraints. Various diseases may cause 30-40% yield loss in lentil (Begum 2003). Among the major diseases of lentil, foot and root rot is the most important one. The disease is caused by a soil borne fungus *Fusarium oxysporum* Schlecht and it may cause 100% seedling mortality in the field under monoculture and conducive weather conditions (Begum 2003). As there is no effective fungicide or resistant variety for the management of this disease, the farmers can not maintain the optimum plant population in the field and the yield of the crop is drastically reduced.

The biological management of soil-borne diseases is increasingly gaining stature as a possible practical and safe approach. The potential of the antagonistic micro-organisms in reducing the intensity of crop damage by the soil-borne plant pathogens has been reported (Lewis and Larkin, 1997). Several strains of *Trichoderma* spp. have been found to be effective as biocontrol agents of various soil-borne plant pathogenic fungi such as *Fusarium, Pythium, Rhizoctonia* and *Sclerotium* (Papavizas 1985). *Trichoderma* spp. are found in almost any tropical and temperate soil. The suppression of disease by *Trichoderma* is based on hyperparasitism, antibiosis, induced resistance in the host plant and competition for nutrients and space (Harman *et al.* 2004).

Little work has been done on the management of plant disease with biocontrol microorganism in Bangladesh. Yet information on biological control of foot and root rot of lentil is inadequate under Bangladesh condition. Therefore, the present study was undertaken in order to assess 14 isolates of *Trichoderma* in controlling foot and root rot of lentil. In our study, the efficacy of the isolates of *Trichoderma* sp. was assessed against *F. oxysporum* in both *in vitro* and field condition. In addition, 25 different substrates were evaluated in order to find out an inexpensive and readily available substrate for mass production of *Trichoderma* sp.

MATERIALS AND METHODS

Collection and identification of *Trichoderma* isolates

Nine isolates of *Trichoderma harzianum* were collected from different pulse and oilseed growing areas of Bangladesh. Four isolates of *T. harzianum* and one isolate of *T. viride* were obtained from the Department of Plant Pathology, Bangladesh Agricultural University, Bangladesh (Table 1).

Diseased plants showing typical symptoms of foot and root rot were collected from fields and isolation of *Trichoderma* was done following the method as used by Begum (2003). The roots were cut into small pieces

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(1.0 cm) and surface sterilization was done with 1:1000 mercuric chloride solution for 1 minute. The roots were washed thoroughly with sterilized water and then dried between folds of filter paper. The sterilized root pieces were transferred into the media of Potato Dextrose Agar PDA and incubated at 28±1^{0}C for 15 days. The fungi grown on PDA were observed under compound microscope (x40) and identified following the key of Barnett (1980). Pure culture of *Trichoderma* was made in PDA plates following the hyphal tip culture technique (Tuite 1969) and preserved at 5^{0}C for further use.

Collection and identification of isolates of *Fusarium oxysporum*

Twelve isolates of *Fusarium oxysporum* were obtained from diseased plant samples collected from different locations of Bangladesh (Table 2). The plants showing typical foot and root rot symptoms were washed initially with tap water to remove sand and soil particles and cut into small pieces (1.0 cm) along with healthy and dead tissues. Surface sterilization was done with 1:1000 mercuric chloride solution for 1 minute. The plant pieces were washed thrice with sterilized water and placed on sterilized filter paper to remove excess water adhering to the pieces. Thereafter, five pieces were placed on PDA plates aseptically maintaining equal distances. The plates were incubated at 28 ± 1^{0}C for 7 days and regular observation was done to see the growth of fungi from the plant pieces. The fungus was then purified by hyphal tip culture technique. The isolated fungus was identified following the key outlined by Booth (1971). The pure culture of *F. oxysporum* was preserved in PDA slants at 5 ±1^{0}C as stock culture.

Pathogenecity test of *Fusarium oxysporum* to lentil

The pathogenecity of the isolates of *F. oxysporum* was tested on a lentil cultivar.in the glasshouse. The dried soil was mixed with well-decomposed cowdung at 2:1 (soil: cowdung, w/w) and sterilized with formalin (40%). The treated soil was covered with polythene sheet for 48 hrs and thereafter exposed for 48 hrs prior to pot filling @ 2kg soil/pot. The pot soil was inoculated with *F. oxysporum* previously grown on chickpea bran @ 40 g/pot. Inoculation was done 3 days before seed sowing. The control pots were not inoculated with the pathogen. There were four replications for each isolate as well as for the control. The pot soil was moistened to 50% water holding capacity. Fifteen seeds of lentil were sown in each pot. Foot and root rot incidence was recorded at 5, 10, 15, 20, 25 and 30 days after sowing. Data on seed germination, pre- and post-emergence death of seedling and plant stand were recorded. *F. oxysporum* was confirmed after re-isolation of the pathogen from the dead seedlings.

Production of colony forming unit of *Trichoderma harzianum* in different substrates

To develop an inexpensive and readily available substrate for mass production of *T. harzianum*, 25 different substrates were evaluated following the technique described by Dubey and Patel (2002); (Table 5). Moistened substrate (10g) was sterilized at 121^{0}C with 15 psi for 30 minutes for two successive days in 500 ml Erlenmeyer flask. The substrate in a flask was inoculated with 10 mycelial discs (5mm diameter each) of *T. harzianum* (TG-2) previously grown on PDA for 3 days. The flasks were incubated at 25 ± 1^{0}C for 20 days with intermittent hand shaking on every third day. The colony forming units per gram of substrate were counted.

Effect of *Trichoderma* spp. on *Fusarium oxysporum* in *in vitro*

A fungal disc (6mm diameter) of *Trichoderma* was placed at one side and that of *F. oxysporum* was placed on the opposite side of PDA plates. The distance between the two inoculum discs were 7 cm. Control plates contained only *F. oxysporum* disc at the center of the plates. There were five replications for the treatments and control. The plates were then incubated at 28 ± 1^{0}C and regular observation was done. The percent inhibition of *F. oxysporum* was calculated following the formula suggested by Sundar *et al.* (1995).

$$\text{Inhibition (\%)} = \frac{X - Y}{X} \times 100$$

Where,

X = Mycelial growth of pathogen *(F. oxysporum*) without *Trichoderma*

Y = Mycelial growth of pathogen with *Trichoderma*

Mycelial mat from the lysed zone were transferred to freshly prepared PDA plates and incubated at room temperature (28^{0}C) for a week to observe the ability of *F. oxysporum* to grow further.

Field evaluation of *Trichoderma* isolate for controlling *F. oxysporum*

Two isolates of *Trichoderma* sp. that showed better performance to reduce the growth of *F. oxysporum* in *in vitro* test were evaluated in the field soil naturally infested with *F. oxysporum.* The land was prepared by four ploughings and cross ploughings. The land was then left exposed to natural weathering for ten days and finally ploughed down and properly leveled before sowing.

The experiment was laid out in a randomized complete block design with three replications. The unit plot size was 2m x 2m and there were 6 rows (30 cm apart) in each plot. There were three treatments: (i) *Trichoderma viride* (Tv), (ii) *Trichoderma harzianum* (TG-2) and (iii) control (*F. oxysporum* alone) in the experiment.

The isolates of *T. viride* and *T. harzianum* were grown on chickpea bran. The chickpea bran was soaked in water at the ratio of 3:4 (w/v). Soaked chickpea bran (500 g) was taken in a beaker (2L) and autoclaved at 121^0C for 30 minutes at 15 psi. The sterilized chickpea bran was inoculated with 20 blocks (6 mm diameter each) of *Trichoderma* previously grown on PDA and incubated at room temperature (28^0C) for 15 days. The inoculum of *Trichoderma* was applied in the field in rows @ 10g m^{-1} at the time of seed sowing. The seed rate was 25 kg ha^{-1}. Number of seeds for each unit plot was counted before sowing.

Soil was moistened when necessary. Weeding was done three times during the crop growing period. No chemical pesticide was applied for controlling pests and diseases. Data on seedling emergence, foot and root rot disease incidence, plant growth and yield attributes were recorded at different stages of crop growth.

RESULTS AND DISCUSSION

Effect of *Trichoderma* spp. on *Fusarium oxysporum* in *in vitro*

All the isolates of *Trichoderma* spp. inhibited the growth of *F. oxysporum* in PDA plates. Percentage of inhibition induced by *Trichoderma* isolates at 3, 5 and 7 days after co-inoculation with *F. oxysporum* ranged from 0 to 11.67 %, 21.03 to 76.72% and 45.87 to 92.07 %, respectively (Table 3). The isolate TG-2 of *T. harzianum* was found to be the most effective in inhibiting the growth of *F. oxysporum* followed by the isolate Tv of *T. viride*. Inhibition of *F. oxysporum*, *F. solani* and *F. culmorum* by *Trichoderma* isolates were observed in dual culture tests by several workers (Ngueko and Xu, 2002). However, *Trichoderma* isolates showed wide range of variations in growth inhibition of *F. oxysporum* in our test that varied from 45.87 to 92.07% at 7 days after inoculation. Different activities of both volatile and non-volatile substances released by *Trichoderma* isolates can be a reason for this variation (Chakraborty and Chatterjee, 2008).

Pathogenecity test of *Fusarium oxysporum* to lentil

Fusarium oxysporum significantly ($P\geq0.01$) influenced the incidence of foot and root rot disease and plant stand. The highest foot and root rot incidence (53%) was recorded for the isolate of FBg-1 followed by FL-6 (39%) and the lowest was recorded in non-inoculated control pots (Table 4). The plant stand ranged from 33 to 98%, where the lowest and the highest record was made in the isolate of FBg-1 and control pots, respectively. Sultana and Hossain (1999) found 5.17% pre-emergence death of lentil due to *F. oxysporum,* while Begum *et al.* (1998) found the highest 58% pre-emergence death of black gram (*Vigna mungo* L.) due to *F. oxysporum.*

Production of colony forming unit of *Trichoderma harzianum* in different substrates

Colony forming units (CFU) of *T. harzianum* (TG-2) on different substrates ranged from 0.05 x 10^6 to 4.04 x 10^6 CFU/g. The highest CFU (4.04 x 10^6/g) was recorded in chickpea bran followed by barley grain (3.17 x 10^6/g) and the lowest was recorded in chopped straw (0.05 x 10^6/g) (Table 5). Nutrient source from culture media like carbon (glucose and sucrose) and nitrogen (tryptone) can greatly influence the growth of *Trichoderma* (Rossi-Rodrigues *et al*. 2009). The chemical analysis of the substrates could explain the reason why the tested *Trichoderma* isolate grew better on chick pea bran than other substrates. However, as a substrate to grow *Trichoderma*, chickpea bran is inexpensive and readily available in Bangladesh. Therefore, use of chickpea bran for commercial production of *Trichoderma* would be economical.

Field evaluation of *Trichoderma* isolates for controlling *Fusarium oxysporum*

Foot and root rot incidence, seed germination and the survival of plants were significantly ($P\geq0.05$) influenced by the application of *T. harzianum* (TG-2) and *T. viride* (Tv) in soil (Table 6). The lowest foot and root rot incidence (6.9%) was recorded in plots where the isolate TG-2 was incorporated to the soil. The maximum foot and root rot incidence (39.0%) was recorded in untreated control plots. The highest seed germination (82.1%) was obtained in plots where the isolate TG-2 was applied followed by the isolate Tv (74.1%). The highest plant stand (93.1%) was recorded in soil incorporated with TG-2 and the lowest was in untreated control. The field where this experiment was conducted had been utilized for lentil cultivation for the last three consecutive years and the occurrence of foot and root rot was a normal phenomenon there. However, *T. harzianum* (TG-2) decreased foot and root rot incidence in lentil by 82.36%. *Trichoderma* spp. were successfully used for controlling foot and root rot diseases of lentil (Prasad *et al.* 2002). In our experiment, 31.57 % more germination of lentil was recorded for the application of *T. harzianum* (TG-2) compared to control. Inoculation with *Trichoderma* isolates reduced the incidence of root rot and increased seed germination in lentil (Vyas and Mathur, 2002).

Shoot weight, root weight, number of pod, weight of pod and yield were significantly influenced by application of the isolates of *T. harzianum* (TG-2) and *T. viride* (Tv) in soil (Table 7). The highest shoot weight (665g m^{-2}), root weight (21g m^{-2}), number of pod (11320 pod m^{-2}), weight of pod (370g m^{-2}) and yield (3726 kg ha^{-1}) were

recorded with the isolate TG-2 of *T. harzianum* and the lowest was in untreated control plots. Prasad *et al.* (2002) found significantly higher seed yield when field soil was treated with *T. harzianum* and *T. viride* against root rot of chickpea. Increased root development and yield were also observed in betelvine, gladiolous, sunflower, mustard, *Chrysanthemum*, tomato, maize, sugarcane, groundnut and chickpea by the application of *T. harzianum* (Singh *et al*. 2007).

The *in-vitro* and *in-vivo* assessments of the effects of 14 isolates of *Trichoderma* spp. against *F. oxysporum* revealed that two isolates of *Trichoderma* spp., TG-2 and Tv significantly reduced the incidence of foot and root disease of lentil and increased the survival of plant and seed yield. Among these two isolates, the performance of the isolate TG-2 of *T. harzianum* was better than the isolate Tv of *T. viride*. Therefore, the isolate TG-2 can be used as a bio-fungicide for the environment friendly management of foot and root rot in order to increase lentil yield in Bangladesh.

CONCLUSION

In our study the evaluation of fourteen isolates of *Trichoderma* revealed that the isolate TG-2 (an isolate of *T. harzianum*) can be used to control foot and root rot disease of lentil in Bangladesh. In addition, chickpea bran is a useful substrate for mass production of the isolate TG-2 for soil application. Thus soil application of *T. harzianum* provides a way to increase production of lentil without environmental pollution.

Table 1. Isolates of *Trichoderma* spp. collected from different sources and locations of Bangladesh

Sl. no.	Isolates	Isolate code	Sources	Locations
1	*Trichoderma harzianum*	Th	Stock culture	BAUL, Mymensingh
2	*Trichoderma viride*	Tv	Stock culture	BAUL, Mymensingh
3	*Trichoderma harzianum*	TBg-1	Blackgram	BAUL, Mymensingh
4	*Trichoderma harzianum*	TBg-2	Blackgram	Rahmatpur, Barishal
5	*Trichoderma harzianum*	TL-1	Lentil	BAUL, Mymensingh
6	*Trichoderma harzianum*	TL-2	Lentil	Tajhat, Rangpur
7	*Trichoderma harzianum*	TL-3	Lentil	Ishurdi, Pabna
8	*Trichoderma harzianum*	TM-1	Mungbean	Ishurdi, Pabna
9	*Trichoderma harzianum*	TM-2	Mungbean	Godagari, Rajshahi
10	*Trichoderma harzianum*	TR-1	Rice	Islumpur, Patuakhali
11	*Trichoderma harzianum*	TR-2	Rice	Sathkhira
12	*Trichoderma harzianum*	TR-3	Rice	BINA farm, Mymensingh
13	*Trichoderma harzianum*	TG-2	Groundnut	BAUL, Mymensingh
14	*Trichoderma harzianum*	TChi-1	Chickpea	Magura

BAUL = Bangladesh Agricultural University Laboratory
BINA = Bangladesh Institute of Nuclear Agriculture

Table 2. Isolates of *Fusarium oxysporum* collected from different hosts and locations of Bangladesh

Sl. no.	Isolates	Isolate code	Host plants	Locations
1	*Fusarium oxysporum*	FL-1	Lentil	BINA farm, Mymensingh
2	*Fusarium oxysporum*	FL-2	Lentil	BINA farm, Mymensingh
3	*Fusarium oxysporum*	FL-3	Lentil	Ishurdi, Pabna
4	*Fusarium oxysporum*	FL-4	Lentil	Tajhat, Rangpur
5	*Fusarium oxysporum*	FL-5	Lentil	Tajhat, Rangpur
6	*Fusarium oxysporum*	FL-6	Lentil	Magura
7	*Fusarium oxysporum*	FChi-1	Chickpea	BINA farm, Mymensingh
8	*Fusarium oxysporum*	FChi-2	Chickpea	Magura
9	*Fusarium oxysporum*	FChi-3	Chickpea	BINA farm, Mymensingh
10	*Fusarium oxysporum*	FBg-1	Blackgram	Ishurdi, Pabna
11	*Fusarium oxysporum*	FG-1	Groundnut	BINA farm, Mymensingh
12	*Fusarium oxysporum*	FM-1	Mungbean	BINA farm, Mymensingh

BINA = Bangladesh Institute of Nuclear Agriculture

Table 3. Percent inhibition of growth of *Fusarium oxysporum* induced by *Trichoderma* spp. on agar plates

***Trichoderma* isolate code**	**Mean inhibition (%)**		
	3 DAI	**5 DAI**	**7 DAI**
Th	3.92	51.68	63.64
Tv	11.67	74.55	90.13
TBg-1	8.22	66.75	83.98
TBg-2	3.17	26.98	45.87
TL-1	5.14	70.35	81.00
TL-2	0.00	45.28	52.48
TL-3	10.35	63.36	81.42
TM-1	3.74	25.47	46.19
TM-2	3.29	21.03	46.14
TR-1	0.00	26.85	47.37
TR-2	2.24	24.21	46.32
TR-3	1.11	26.60	50.03
TG-2	10.38	76.72	92.07
TChi-1	7.39	40.52	52.52
LSD(P≥0.01)	7.12	8.59	6.29

DAI = Days After Inoculation
Data represent the means of five replications

Table 4. Pathogenecity of different isolates of *Fusarium oxysporum* on lentil cultivar Barimasur-4

***Fusarium* isolates code**	**Foot and root rot incidence (%)**	**Plant stand (%)**
FL-1	18	63
FL-2	11	63
FL-3	24	54
FL-4	11	59
FL-5	15	69
FL-6	39	52
FChi-1	23	61
FChi-2	38	48
FChi-3	21	67
FBg-1	53	33
FG-1	11	69
FM-1	16	64
Control	1	98
LSD(P≥0.01)	17.12	21.24

Data represent the means of four replications

Table 5. Production of colony forming units of *Trichoderma harzianum* (TG-2) in different subsatrate

Substrate	**Ratio**	**CFU (x10^{6})/g substrate**
Chopped straw-water	1:2 (w/v)	0.09
Chopped straw-urea-sucrose-water	20:1:1:40 (w/v)	0.10
Chopped straw-sucrose-water	20:1:40(w/w/v)	0.05
Wheat bran-water	3:4(w/v)	1.53
Wheat-water	1:2(w/v)	0.14
Wheat bran-Peat soil -water	3:3:4(w/w/v)	1.16
Rice bran (fine)-water	3:4(w/v)	1.61
Rice bran-Sucrose-water	20:1:40(w/w/v)	0.88
Rice broken grain-water	1:2(w/v)	0.93
Saw dust-water	1:1(w/v)	0.50
Saw dust-sucrose-water	20:1:20(w/v)	0.16
Sand-Cowdung-water	2:1:2(w/w/v)	0.09
Saw dust-Cowdung-water	1:1:2(w/w/v)	0.56
Soybean bran-water	3:4(w/v)	0.80
Grass pea bran-water	3:4(w/v)	2.00
Blackgram bran-water	3:4(w/v)	2.75
Chickpea bran-water	3:4(w/v)	4.04
Maize bran-water	3:4(w/v)	1.86
Chickpea-water	1:1.5(w/v)	1.29
Blackgram-water	1:1.5(w/v)	1.19
Mungbean-water	1:1.5(w/v)	0.88
Lentil-water	1:1.5(w/v)	1.16
Grass pea-water	1:1.5(w/v)	0.17
Anchor-water	1:1.5(w/v)	0.10
Barley-water	1:1.5(w/v)	3.17
LSD(P≥0.01)		0.61

CFU = Colony Forming Unit
Data represent the means of three replications

Table 6. Effect of soil treatment with *Trichoderma* on foot and root rot incidence, seed germination and plant stand of lentil under naturally infested soil with *Fusarium oxysporum*

Trichoderma isolates	Foot and root rot incidence (%)	Seed germination (%)	Plant stand (%)
Trichoderma viride (Tv)	12.3 (-68.4)	74.1 (+46.9)	87.7 (+43.7)
T. harzianum (TG-2)	6.9 (-82.4)	82.1 (+62.7)	93.1 (+52.7)
Control	39.0	50.4	61.0
LSD (P≥0.05)	22.8	6.7	6.7

Data in parenthesis indicate per cent increase (+) or decrease (-) over control
Data represent the means of three replications

Table 7. Effect of *Trichoderma* isolates on shoot weight, root weight, pod number, pod weight and yield of lentil under naturally infested soil with *Fusarium oxysporum*

Trichoderma isolates	Shoot weight (gm^{-2})	Root weight (gm^{-2})	Number of pod m^{-2}	Weight of pod (gm^{-2})	Yield (kg ha^{-1})
Trichoderma viride (Tv)	534	16.3	8289	300	2798
T. harzianum (TG-2)	665	21.8	11320	370	3726
Control	175	5.1	2815	87	1874
LSD (P≥0.05)	199.4	7.6	4184.0	112.5	1348.0

Data represent the means of three replications

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