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Effect of times and levels of inoculum of *Trichoderma* for controlling root rot and collar rot of lentil

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ABSTRACT

The experiments were carried out during 2010 to 2011 to find out the time of application and level of inoculum of *T. harzianum* for controlling root rot (*F. solani)* and collar rot (*S. rolfsii*) of lentil (*Lens culinaris* Medik). *F. solani* and *S. rolfsii* and their antagonist, *Trichoderma* were collected from different pulses growing areas of Bangladesh. The experiments were carried out following CRD with five replications. Fourteen isolates of *Trichoderma* were tested against *F. solani* and *S. rolfsii* under Dual Culture Technique. The inhibition was ranged from 60.42 to 99.27% at 10 DAI against *F. solani* and from 64.07-99.41% at 6 DAI against *S. rolfsii*. The highest inhibition 99.27% against *F. solani* was found in isolate, Th-2 and 99.41% was found in isolate TG-2 against *S. rolfsii*. In the pot, the treatment of soil with the Th-2 isolate of *T. harzianum* at 2g/kg shown the highest germination (96.67%) and plant stand (81.67%) and the lowest root rot (15.52%), where the isolate of TG-2 of *T. harzianum* at 20g/kg of soil was observed better for controlling *S. rolfsii*. *T. harzianum* (Th-2) increased germination (17.86%), plant stand (171.43%), vigour index (33.27%) and yield (260.74%) over control against root rot. *T. harzianum* (TG-2) also increased germination (248%), plant stand (600%), vigour index (865.91%) and yield (1209.81%) of lentil over control against collar rot. The soil treatment with *Trichoderma* at 9, 6 and 3 days before sowing and also at the time of sowing showed significantly better effect in increasing germination, plant stand and reducing root rot and collar rot compared to control. The highest germination, seed yield and lower root rot was obtained by treating soil with *Trichoderma* before 9 days of sowing against both the pathogens.

Key words: Biological control, Collar rot, *Lens culinaris,* Root rot, Soil treatment, *Trichoderma*.

INTRODUCTION

Lentil (*Lens culinaris* Medik.) belongs to the family Leguminosae is an important pulse crop in semiarid regions of Iran, India, Turky and canadia and originated in the Fertile Crescent of the Near East, and dates back to the beginning of agriculture itself (Sabagh pour *et al.* 2004).

Lentils provide a good source of protein (20 to 35 per cent), but are limited in the amino acids methionine and cystine. (Kandel and Ashley, 2013) and 48% carbohydrate (Feedipedia, 2012). Out of eight kinds of pulse, lentil, being the rich source of vegetable protein, is a common item in the daily diet of the people of Bangladesh (Bakr, *et al.* 1997). It is the most important pulse crop in Bangladesh. Although, it ranks the second highest pulse crop in respect of production, but in respect of consumer's preference it ranks the top position among all the pulses (Ali *et al.* 2003). The yield of lentil is much lower (1.5t/ha; BARI, 2010) in Bangladesh compared to other countries like Syria, Turkey, Canada, USA and Ethiopia because of its poor management practices and lack of proper disease management (Hossain *et al.* 1999). It is specially honoured as a protein source in comparison with high cost animal protein and considered as poor man's meat (Begum, 1997).

Lentil is being grown in an area of 0.25 million hectares and production is about 0.23million metrics tonnes in Bangladesh (BBS, 2011). There are various causes associated with low yield of lentil in Bangladesh, where diseases are considered as the major constraints in production resulting about 30-40% yield loss (Begum, 2003). Root rot of lentil caused by *F. solani* and *S. rolfsii* (Dey *et al*.,1993) is a common disease in Bangladesh and also in almost all the lentil growing countries of the world. 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).

Biological control represents a natural and ecological approach to controlling diseases that reduces chemical inputs and their effects (Mukhopadhyay 1994). Biological control can be safer for human, the crop and the environment. So a primary significance is that incorporation of biological controls will allow a reduction in the use of chemical pesticides. Concerns about the health, safety and

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environmental effects of agricultural chemicals in our water, soil and food require that these inputs be minimized (Haque *et al.,* 1992). The biological management of soilborne diseases is increasingly gaining stature as a possible practical and safe approach (Patel and Anahosur, 2001).

In India, *T. harzianum* significantly reduce the growth of *S. rolfsii* (Upadhyay and Mukhopadhyay, 1983) and in Pakistan *T. harzianum* overlapped the pathogen and suppressed growth by 63.6% (Iqbal *et al.* 1995). In Poland, *T. harzianum* were used for biological control of *Fusarium oxysporum, F. solani* and *F. culmorum* on lentils (Sadowski and Kryziak, 1991). In pot trials, *T. harzianum* was effective against *F. solani* in a 1:2 Mixture (Deshmukh and Raut 1992). As a consequence, plant growth may be increased (Burr *et al*. 1978 and Baker, 1988). Addition of *T. harzianum* to pathogen infested soil gave 45% control of foot rot disease of soybean and yield of the crop increased from 1.5 to 1.8g/ plant (Deb and Dutta, 1991). This antagonist increased the percentage of seedling emergence, plant height, fresh and dry weight, number of nodules, total N and total protein content of the plants. *T. harzianum* produces IAA and certain vitamins which seemed to play a role in yield increase (Ychia *et al. 1994*). However, intensive work on the management of soil-borne diseases with microbial antagonists had been carried out in India and other developed countries (Lewis and Larkin, 1997).

The major limitations of biological control by *Trichoderma* spp. is mass production of inoculum, proper time of application and inoculum level. To overcome these limitations and to successful use of *T. harzianum* as an antagonist to *F. solani* and *S. rolfsii* has led us to undertake the present piece of research work.

MATERIALS AND METHODS

Some experiments were carried out at the laboratory and the field of Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh, during 2010 to 2011 following CRD with five replications at laboratory and at pot. Diseased plant samples of root rot and collar rot were collected from different cropping area of Bangladesh. Then isolation, identification, purification and preservation of the related organism of *Trichoderma*, *Fusarium* and *Sclerotium* isolates were done following the method as used by Begum (2003) and Rifai (1969). Antagonistic effect of different isolates of *T. harzianum* against *F. solani* and *S. rolfsii* were studied following dual culture method. In this method, PDA plates were inoculated with a 6mm block of *Trichoderma* isolates at one side and pathogens (*F. solani* and *S. rolfsii*) on other side following the method as used by Sultana (1999). The distance between inoculums blocks in plates were 7 cm. Control plates were contained pathogens only at the center of the PDA plates. The plates were then incubated at 28 ± 1 ^{0}C and observation was taken regularly to see the growth. The isolate of pathogen after come in contact with the *T. harzianum* were inhibited. The per cent inhibition was calculated following the formula as suggested by Sundar *et al*. (1995):

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

Where,

X = Mycelial growth of pathogen alone without antagonist (Control)

Y = Mycelial growth of pathogen along with the antagonist

In pot, soil was collected from the field laboratory of the Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh and dried. Decomposed cow dung from the Dairy Farm of BAU, Mymensingh was added to it. The dried soil was mixed uniformly with well-decomposed cowdung at 2:1 (soil : cowdung) and sterilised with formalin (40%) at the rate of 5 ml formalin diluted with 20 ml of water for 4kg soil. The formalin treated soils were covered with polythene sheet for 48-hrs and then exposed to 48-hrs aeration before pot filling with 4kg/pot (Hossain, 2000). A total of 110 pots for different experiments were filled with the treated soil. *F. solani*, *S. rolfsii* and *T. harzianum* was grown on chickpea bran following the method of Khan (1998). The chickpea bran was soaked in water at the ratio of 3:4 (w/v) in a 2000 ml beaker. Chickpea bran (250 g) was taken in a 2000 ml beaker and the beaker was closed tightly with cotton wrapped by brown paper and autoclaved for 30 minutes. The sterilized chickpea bran was inoculated with 15-20 blocks (6 mm) of *F. solani* that previously grown on PDA and incubated at 25±1^{0}C for 15 days where the beaker was shaken once at every three days during incubation for uniform growth of the fungus. The chickpea culture of *F. solani* and *S. rolfsii* were then mixed with pot soil at the rate of 0.1% weight basis of dry soil before sowing seed (Khan *et al*. 1998). The inoculum was applied upto 6cm depth in the pot soils. Chickpea bran culture of *T. harzianum* was mixed with each pot soil @ 2g/kg soil after five days of seed sowing. The culture media of chickpea bran was contained spores 4.8 x10^7/g. Six different levels of inoculum of *T. harzianum* (Th-2) in case of *F. solani* and *T. harzianum* (TG-2) in case of *S. rolfsii* were used. The treatments were used as follows: Control (Pathogen alone), 0.02g (Th-2/TG-2), 0.20g (Th-2/ TG-2), 2.0g (Th-2/TG-2), 20.0g (Th-2/TG-2), 200.0g (Th-2/TG-2).

Five different times of application of *T. harzianum* (Th-2/TG-2) were tested against *F. solani* and *S. rolfsii*. The treatments were used as follows: (1) Soil treatment at 9 DAS, (2) Soil treatment at 6 DAS, (3) Soil treatment at 3 DAS, (4) Soil treatment 0 DAS and (5) Control (Pathogen alone/ without *Trichoderma*). Data were collected on the following parameters: Germination (%), Pre-emergence seedling rot (%), Root rot (%), Plant stand (%), Shoot length plant^{-1} (cm), Root length plant^{-1}(cm), shoot weight plant^{-1}(g), shoot weight pot^{-1}(g)

RESULTS AND DISCUSSION

Antagonistic potentiality of fourteen isolates of *Trichoderma harzianum* against *F. oxysporum* was studied. Isolates of *T. harzianum* showed wide variation in growth inhibition of *F. solani* that varied from 60.42 to 99.27% at 10 DAI. The highest inhibition was found in the isolate of Th-2 (99.27%) (Table 1). Michrina *et al*. (1995) obtained 46.98% growth inhibition of *F. culmorum* with *T. harzianum* in dual culture test. Michalikova and Michrina (1997) reported the greatest inhibition rate of the radial growth of *F. culmorum* (55-58%) with *T. harzianum*. Karunanithi and Usman (1999) observed *Trichoderma* spp. highly inhibitory to *F. oxysporum* in dual culture. Ngueko and Xu (2002) found that mycelial growth of *F. solani* and *F. oxysporum* was inhibited with *T. harzianum*. Tian *et al.* (2001) determined the antagonism between *T. viride* to *F. oxysporum in vitro* with a covering rate of 97%. Ngueko and Xu (2002) reported that the *T. harzianum* reduced the mycelial growth of *F. oxysporum* with 52%-87%. Similar findings were obtained by Sultana and Hossain (2000).

Among the fourteen isolates of *T. harzianum* the highest (99.41%) inhibition against *S. rolfsii* was found in TG-2 (Table 2). Pranab *et al.* (2002) recorded 61.5% inhibition of *S. rolfsii* by *T. harzianum*. The findings of the present study clearly elucidated two isolates of *T. harzianum* (Th-2 and TG-2) as potent biocontrol agent for *F. solani* and *S. rolfsii*. These two isolates are used to find the effective doses and time of its application against *F. solani* and *S. rolfsii*.

Application of different levels of inoculum (0.00g, 0.02g, 0.20g, 2.00g, 20.00g and 200.00g) of chickpea bran culture (in per kg of soil) of *Trichoderma* against root rot (*F. solani*) and collar rot (*S. rolfsii*) showed significant effect in reducing diseases of lentil. In *F. solani* inoculated soil, the maximum germination (96.67%), plant stand (81.67%), shoot length (10.5 cm) and the minimum Pre-emergence seedling rot and root rot were recorded in the inoculum level of 2g/kg of soil. The lowest germination, highest root rot and lowest plant stand were recorded in untreated control (Table 3 & Fig.1). Results indicated that per cent germination over control increased with increasing level of inoculums of TG-2 with being the highest in 200g of soil. Tian *et al.* (2001) applied the *Trichoderma* strain T41 at 1 x 10^8/g to the rhizosphere of tomato plants at 2g/plant and then inoculated with a spore suspension of the pathogen at 1 x 10^5/ml, 1 day later and recorded disease control by 86.53%. Osuinde *et al*. (2002) investigated that the spore concentration 10^6 spores/ml of *Trichoderma* species prevented wilt (*Fusarium oxysporum)* disease development in the tomato seedlings. In *S. rolfsii* inoculated soil, the maximum plant stand and the highest shoot length, root length and shoot weight as

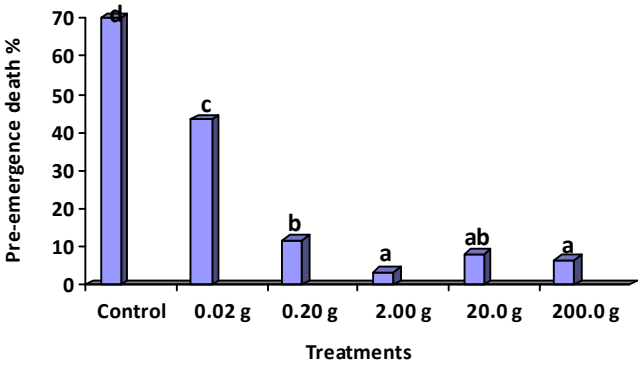

FIG 1: Effect of different levels of inoculum of *T. harzianum* (Th-2) on Pre-emergence seedling rot of lentil inoculated with *F. solani*

TABLE 1: Effect in terms of inhibition (%) of different isolates of *T. harzianum* against *F. solani* following dual culture method *in vitro*

Trichoderma Isolates	% inhibition			
	3 DAI	5 DAI	7 DAI	10 DAI
Th-1	3.92	51.68	63.64	80.65
Th-2	11.67	74.55	90.13	99.27
TBg-1	8.22	66.75	83.98	92.91
TBg-2	3.17	26.98	45.87	59.32
TL-1	5.14	70.35	81.00	90.61
TL-2	0.00	45.28	52.48	62.37
TL-3	10.35	63.36	81.42	92.32
TM-1	3.74	25.47	46.19	60.98
TM-2	3.29	21.03	46.14	60.43
TR-1	0.00	26.85	47.37	61.72
TR-2	2.24	24.21	46.32	60.46
TR-3	1.11	26.60	50.03	61.50
TG-2	10.38	76.72	92.07	98.60
TChi-1	7.39	40.52	52.52	70.02
LSD(P≤0.01)	7.12	8.592	6.294	5.621

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

TABLE 2: Effect in terms of inhibition (%) of different isolates of *T. harzianum* against *S. rolfsii* following dual culture method *in vitro*

Trichoderma isolates	% inhibition			
	3 DAI	4 DAI	5 DAI	6 DAI
Th-1	6.04	55.96	73.84	81.76
Th-2	5.88	66.36	86.66	97.16
TBg-1	4.40	67.86	89.20	96.10
TBg-2	0.55	31.46	53.30	66.42
TL-1	6.95	59.75	72.49	82.08
TL-2	4.47	49.83	63.40	77.06
TL-3	6.37	63.7	73.77	80.83
TM-1	0.05	34.25	54.34	72.49
TM-2	0.00	37.80	54.76	70.15
TR-1	3.83	32.90	57.64	70.20
TR-2	5.64	48.78	63.69	73.32
TR-3	0.52	46.26	62.79	74.88
TG-2	6.02	66.44	91.97	99.41
TChi-1	4.23	30.75	53.32	64.07
LSD(P≤0.01)	1.617	4.332	5.137	4.614

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

TABLE 3: Effect of different levels of inoculum of *T. harzianum* (Th-2) on germination and Root rot, plant stand and plant growth when inoculated with *F. solani*

Treatments(Th-2)	Germination (%)	Root rot (%)	Plant stand (%)	Shoot length plant^{-1} (cm)	Root length plant^{-1} (cm)	Shoot weight Plant^{-1} (g)	Shoot weight Pot^{-1} (g)
Control (without Th-2)	30.00	72.22	8.33	3.75	2.50	0.38	0.38
0.02g (Th-2)	56.67	55.88	25.00	8.50	5.25	0.86	3.21
0.20g (Th-2)	88.33	37.74	55.00	9.50	5.50	1.27	10.56
2.0 g (Th-2)	96.67	15.52	81.67	10.50	6.00	1.71	21.11
20.0g (Th-2)	91.67	16.36	76.67	10.25	6.25	2.07	23.52
200.0g(Th-2)	91.67	16.82	77.92	10.00	6.00	1.82	21.17
$LSD_{(0.01)}$	14.74	19.19	13.49	4.19	3.24	0.17	8.84

Data represent the means of five replications

well as minimum collar rot were recorded in 20g/kg of soil. The lowest germination, highest collar rot and lowest plant stand were recorded in untreated control (Table 4 & Fig. 2). Xu *et al.* (1993) observed in soil treatment with 0.6% (w/w) T_{82} bran culture (10^7 cfu/g) reduced incidence of diseases caused by *S. rolfsii* by 46.5%, 20 d after inoculation with the pathogens. Cuevas *et al.* (2001) showed that *Trichoderma* controlled *S. rolfsii*.

The soil treatment with *Trichoderma* at 9, 6 and 3 days before sowing and also at the time of sowing showed significantly better effect in increasing germination, plant stand and reducing root rot and collar rot compared to control. The antagonist *Trichoderma* grows rapidly and they attack the pathogenic organism. They produce some toxic substances and enzyme that are harmful for pathogenic fungi. The highest germination, seed yield was obtained by treating soil with *Trichoderma* at 9 days before sowing against both the pathogens (Table 5 and 6). The lowest pre-emergence seedling rot was obtained by treating soil with *Trichoderma* at 9 days before sowing against both the pathogens and highest was in control (Fig. 3 and 4). Prasad *et al.* (2002a) showed that soil application of *T. harzianum* one week before sowing was more effective in reducing root rot. Pranab *et al*. (2002) observed that in field conditions, soil application of *Trichoderma* spp. inoculum at the time of transplanting, reduced disease incidence caused by *S. rolfsii* and increased dry mass of roots and shoots and yield. Osuinde *et al.* (2002) found that tomato seedlings were effectively protected from infection when the pathogen *F. oxysporum* and *Trichoderma*

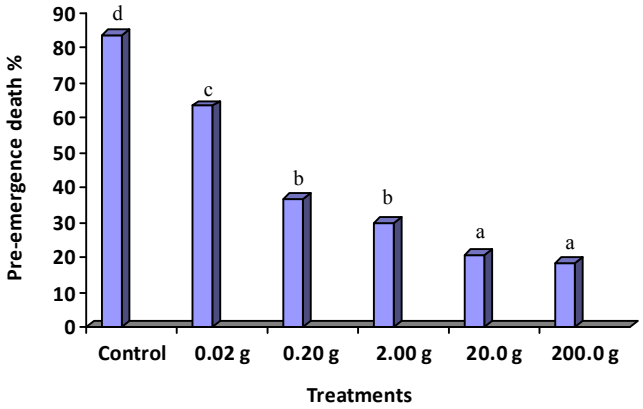

FIG 2: Effect of different levels of inoculum of *T. harzianum* (TG-2) on Pre-emergence seedling rot of lentil inoculated with *S. rolfsii*

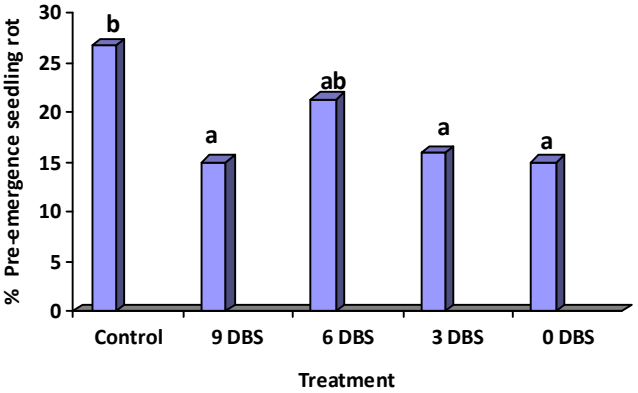

FIG 3: Effect of different times of application of *T. harzianum* (Th-2) on Pre-emergence seedling rot of lentil inoculated with *F. solani*

TABLE 4: Effect of different levels of inoculum of *Trichoderma harzianum* (TG-2) on germination, collar rot, plant stand and plant growth in pot trials of lentil when inoculated with *S. rolfsii*

Treatment	Germination (%)	% Increase in germination	Collar rot (%)	Plant stand (%)	Shoot length plant^{-1} (cm)	Root length plant^{-1} (cm)	Shoot weight plant^{-1} (g)	Shoot weight pot^{-1} (g)
Control	16.67	-	59.17	6.67	5.78	3.75	0.70	8.09
0.02g (TG-2)	36.67	119.9	56.55	16.67	8.45	5.43	1.17	20.29
0.20g (TG-2)	63.33	279.9	58.66	23.33	10.58	6.15	1.38	36.38
2.0 g (TG-2)	70.00	319.9	31.59	48.33	10.60	6.63	1.54	77.68
20.0g (TG-2)	79.33	375.9	29.17	56.10	10.68	6.95	2.34	87.08
200.0g (TG-2)	81.67	389.9	31.54	56.00	10.20	6.67	1.98	87.78
$LSD_{(0.01)}$	20.10	-	22.47	25.69	3.89	2.43	0.91	41.01

Data represent the means of five replications

TABLE 5: Effect of soil treatment with *T. harzianum* (Th-2) in different times of application on germination, vigour index, collar rot and root rot, plant stand and seed yield of lentil when pot soil inoculated with *F. solani*

Soil treatment	Germination(%)	Vigour index(%)	Root rot(%)	Plant stand(%)	Seed weightpot^{-1} (g)
Control	73.33	1089.24	25.00	75.00	4.31
9 DBS	85.00	1513.57	7.84	92.16	7.23(67.75)
6 DBS	78.33	1340.02	6.38	93.62	5.67(31.55)
3 DBS	83.33	1445.56	8.00	92.00	5.28(22.51)
0 DBS	85.00	1515.83	7.84	92.16	5.12(18.79)
$LSD_{(Pe"0.01)}$	10.50	NS	15.63	15.63	
$LSD_{(Pe"0.05)}$					1.69

DBS = Days Before Sowing
NS = Not significant
Data in parentheses indicate per cent increase over control
Data represent the means of five replications

TABLE 6: Effect of soil treatment with *Trichoderma harzianum* (TG-2) at different times of application on germination, vigour index, collar rot and root rot, plant stand and seed yield of lentil when pot soil inoculated with *S. rolfsii*

Soil treatment	Germination(%)	Vigour indexat 15 days(%)	Collar rot(%)	Plant stand(%)	Seed weightpot^{-1} (g)
Control	55.00	807.40	39.39	60.61	3.60
9 DBS	88.33	1737.22	9.43	90.57	8.75(143.06)
6 DBS	75.00	1125.00	11.11	88.89	5.16(43.33)
3 DBS	81.67	1429.17	10.20	89.80	5.52(53.33)
0 DBS	85.00	1473.33	17.65	82.35	6.49(80.28)
LSD(Pe"0.01)	NS	NS	12.51	12.51	1.69

DBS = Days Before Sowing

Data in parentheses indicate per cent increase over control
Data represents the means of five replications

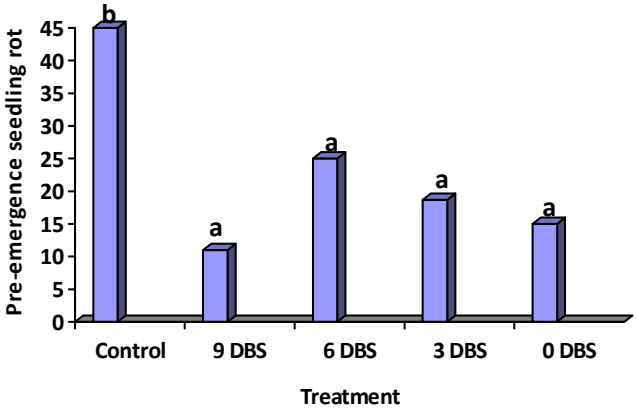

FIG 4: Effect of different times of application of *T. harzianum* (TG-2) on Pre-emergence seedling rot of lentil inoculated with *S. rolfsii*

species were inoculated simultaneously. Control of wilt was less effective when spores of *F. oxysporum* were allowed to grow one day ahead of spores of *Trichoderma* species. Even there was no protection when the spores of *F. oxysporum* were allowed to grow two days ahead of *Trichoderma* species. Hassan *et al.* (2002) expressed that root rot and wilt caused by *Fusarium sp.* was lowest when *T. harzianum* was applied 5 days before soil infestation. Cuevas *et al.* (2001) found that control of pathogenic activity of *S. rolfsii* as shown by per cent seed germination and per cent survival of seedlings in plots was most effective where *Trichoderma* was given 2 weeks and 1 week before seed sowing with 30 g carrier rice bran pellets. In fine, soil treatment with *T. harzianum* had been selected for showing better antagonistic performance.

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