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EFFECT OF SUBSTRATES TO FORMULATE *Trichoderma harzianum* BASED BIO-FUNGICIDE IN CONTROLLING SEEDLING DISEASE (*Rhizoctonia solani)* of BRINJAL

M. I. FARUK[1] AND M. L. RAHMAN[2]

Abstract

Efficacy of three different substrates viz., rice bran, wheat bran, grass pea bran and their combinations with mustard oilcake (MOC) were tested to formulate a suitable *Trichoderma harzianum* based bio-fungicide for controlling seedling disease of brinjal caused by *Rhizoctonia solani* in tray soil as well as in seedbed soil under net house condition of Bangladesh Agricultural Research Institute (BARI), Gazipur during 2010 to 2014. The results of three years experiments revealed that *T. harzianum* bio-fungicides formulated in five different combinations of substrates viz., (1) rice bran + wheat bran, (2) rice bran + mustard oilcake (MOC) (3) rice bran + grasspea bran, (4) rice bran + wheat bran + MOC and (5) rice bran + grasspea bran +MOC were equally effective to control the soil borne seedling disease of brinjal caused by *Rhizoctonia solani* in tray soil and seedbed condition. In addition, vegetative growth of brinjal seedlings viz., shoot length, shoot weight, root length and root weight were enhanced significantly by the *T. harzianum* bio-fungicides in *R. solani* inoculated seedbed condition.

Keywords: *Trichoderma harzianum*, *Rhizoctonia solani*, brinjal seedling.

Introduction

Quality food and nutrition are the serious challenges worldwide where vegetable can play a vital role in everyday diet in general. Among the vegetables, brinjal (*Solanum melongena* L.) is the major crop that achieves tremendous popularity over the last century in Bangladesh. It grows round the year in any space available for crop cultivation in the country and uses as multifarious item of every day dish (Rashid, 1976; Bose and Som, 1986). The productivity of brinjal in Bangladesh is 17.5 t/ha while in Japan 32 t/ha, Italy 28.2 t/ha and Turkey 30.2 t//ha (FAOSTAT, 2012). It is estimated that 10% of crops are lost due to plant diseases worldwide annually which incurs considerable financial losses to the farmers (Strange and Scott, 2005). The pathogen *Rhizoctonia solani* causing germination failure, damping off and seedling rot is the major constraint to brinjal cultivation (Najar *et al.*, 2011; Seema and Devaki, 2010). The management of this soil borne disease is difficult owing to long saprophytic survival ability of pathogen in soil (Dey, 2005). Suppression or elimination of soil borne inoculums is the only effective solution which may be achieved

[1]Senior Scientific Officer, Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh, [2]Director, Research Wing, BARI, Gazipur, Bangladesh.

through effective fungal antagonists. The beneficial microbe such as *Trichoderma harzianum* has been reported as a potential bio-control agent that effectively controlled the soil borne pathogens including *R. solani* (Elad *et al.*, 1980; Roy *et al*., 1989; Anand and Reddy, 2009). The *Trichoderma* spp. are considered as potential biocontrol and plant growth promoting agents for many crop plants (Verma *et al*., 2007; Bai *et al*., 2008; Savazzini *et al*., 2009). The native bio-control agents usually remain in the agricultural soil in low population density. Augmenting their density to higher stability level in soil through artificial inoculation may successfully control soil borne pathogens in brinjal seed bed. Many bio-product/farm household bio-products which are locally available could easily be used as substrates to promote *Trichoderma* population in the soil. Therefore, the present study was undertaken to find out the effective local substrates to formulate the best medium for mass culturing of *T. harzianum* to be used as effective bio-fungicides against seedling disease (*R. solani*) of brinjal under seed bed condition.

Materials and Method

An experiment was conducted in the plastic tray and three other experiments in seed bed conditions of the net house of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur during the period from 2010 to 2014 to find out the suitable carrier material for mass culturing of *Trichoderma harzianum* and thereby formulation of effective bio-fungicides against *Rhizoctonia solani* causing seedling disease of brinjal. A pure culture of bio-control agent *T. harzianum* (TM14) was grown on Potato Dextrose Agar (PDA) medium which was subsequently used as an inoculum of bio-fungicide that multiplied on rice bran, wheat bran, grasspea bran and their combination with mustard oilcake (MOC). The treatment combinations of the study were T_1= Rice bran, T_2= Wheat bran, T_3= Grasspea bran, T_4= Rice bran + Wheat bran (1:1), T_5=Rice bran + Grasspea bran (1:1), T_6= Rice bran + MOC (1:1), T_7= Rice bran + Wheat bran + MOC (1:1:1), T_8= Rice bran + Grasspea bran + MOC (1:1:1), T_9= Wheat bran + Grasspea bran + MOC (1:1:1), T_{10}= Rice bran + Wheat bran + Grass pea bran+ MOC (1:1:1:1), T_{11}=Seed treatment with Provax and T_{12}= Control. According to the treatment combinations 600 g of individual or combination of substrate materials were taken separately in 1000 ml Erlenmeyer flasks and sterilized in an autoclave at 121^0C for 15 minutes. After cooled down the substrate was inoculated individually with 5 mm diameter mycelia disc of five-day old culture of *T. harzianum* grown on PDA and finally incubated at 25±2 ^{0}C for 15 days. After incubation the colonized substrates were removed from the flasks and air dried and preserved in refrigerator at 10 ^{0}C. Besides, the pathogenic fungus *R. solani* was multiplied on sterilized barley grains in 1000 ml Erlenmeyer flask at temperature of 25±2 ^{0}C for 15 days.

In plastic tray experiment both pathogenic fungus *R. solani* and *T. harzianum* bio-fungicide were used at the rate of @) 20 g/kg soil where the control treatment received *R. solani* only and the seeds of BARI Bagun-7 were sown in the tray soil @100 seeds per tray. The experiment was laid out in completely randomized design (CRD) with six replications. Intercultural operations were done to grow the crop (Anon., 2007).

In seed bed trials the colonized barley grains were incorporated in the seed bed soils @100 g/m^2 soil. Inoculated seed bed soil was allowed to multiply the pathogen *R. solani* for 10 days with proper soil moisture. The *T. harzianum* bio-fungicides were incorporated to the seed bed and kept for 7 days with proper soil moisture for establishment of *T. harzianum* in the soils. The control bed received only *R. solani*. The seeds of BARI Bagun-7 were sown in the seed bed @ 200 seeds per treatment. The experiment was laid out in completely randomized design (CRD) with four replications. Necessary intercultural operations were done as per recommendation of the crop (Anon., 2007).

Data were collected on percent seed germination, seedling mortality, shoot height, shoot weight, root length and root weight of brinjal seedlings. The percent data were converted into arcsine transformation values before statistical analysis. The data were analyzed statistically and means were separated by Duncun's New Multiple Range Test (DMRT).

Results and Discussion

Screening of substrates based *T. harzianum* bio-fungicides in plastic tray

The seedling emergence of brinjal was significantly higher and ranged from 76% (grasspea bran) to 95% (rice bran + mustard oilcake) due to the carrier based on *T. harzianum* bio-fungicide treatments whereas untreated control tray gave lower seedling emergence (71%) (Table 1). Pre-emergence and post-emergence seedling mortality was also varied among the treatments and the highest seedling mortality of 47% was recorded in control trays. Soil treatment with *T. harzianum* bio-fungicides reduced seedling mortality of 25.53% –76.60% as compared to untreated control (Table 1). The result showed that *T. harzianum* treated tray soil gave higher amount of healthy seedlings (65% - 89%) while untreated control tray soil produced only 53% healthy seedling in *R. solani* inoculated soil. The overall performance of the bio-fungicide *T. harzianum* with respect to seedling emergence, seedling mortality and healthy seedlings of brinjal under *R. solani* inoculated condition was better.

Table 1. Effect of different carrier based *T. harzianum* bio-fungicides on the emergence and mortality of brinjal seedling in *R. solani* inoculated soils in the plastic tray

Name of substrates for *T. harzianum*	Seedling emergence (%)	Pre-emergence mortality (%)	Post-emergence seedling mortality (%)	Total seedling mortality (%)	Seedling mortality reduction (%) over control	Total healthy seedling (%)
Rice bran	82	18	5	23	51.06	77
Wheat bran	81	19	13	32	31.91	68
Grasspea bran	76	24	11	35	25.53	65
Rice bran + Wheat bran	88	12	12	24	48.94	76
Rice bran + Grasspea bran	85	15	7	22	53.19	78
Rice bran + MOC	95	5	6	11	76.6	89
Rice bran + Wheat bran + MOC	86	14	13	27	42.55	73
Rice bran + Grasspea bran +MOC	92	8	8	16	65.96	84
Wheat bran + Grasspea bran + MOC	85	15	8	23	51.06	77
Rice bran + Wheat bran + Grasspea bran+ MOC	91	9	8	17	63.83	83
Seed treatment with Provax	86	14	11	35	25.53	65
Untreated Control	71	29	18	47	-	53

Efficacy of substrates based *T. harzianum* bio-fungicides and provax in seed bed

The emergence of brinjal seedling in *R. solani* inoculated soils was sharply enhanced by the application of different carrier material based *T. harzianum* bio-fungicides and Provax in seedbed (Table 2). The individual as well as combined application of *T. harzianum* bio-fungicides was found superior in seedling emergence as compared to the untreated control during three consecutive years. The seedling emergence of brinjal ranged from 55.67% to 65.67%, 71.67% to 80.67%, and 60% to 72%, respectively in consecutive three years due to the

application of different bio-fungicides in seed bed while lower seedling emergence (49.33%, 56.67%, and 50%) was recorded from the control seedbed. The trend of pre-emergence mortality was almost similar among the bio-fungicide treatments over the years that ranged from 39.68-44.33% in 1st year trial, 19.33-28.33% in 2nd year and 28-40% in 3rd year trial (Table 2). The pre-emergence seedling mortality in the control treatments was 50.67, 43.33, and 50% during 1st, 2nd and 3rd year trials, respectively. The effect of Provax was inferior or similar to that of *T. harzianum* bio-fungicide in respect of emergence and mortality of brinjal seedling in seedbed. The results of three years trial indicated that the effects of single as well as mixed carrier based *T. harzianum* bio-fungicides were very much similar in increasing seedling emergence and reducing pre-emergence mortality of brinjal seedling caused by *R. solani* under seedbed conditions.

Table 2. Effect of different carrier material based *T. harzianum* bio-fungicides on the emergence and pre-emergence mortality of brinjal seedling in *R. solani* inoculated seed bed soil

Name of substrates	Seedling emergence of brinjal in consecutive three years (%)			Pre-emergence mortality in consecutive three years (%)		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran	59.00	77.67	60.00	41.00	22.33	40.00
Wheat bran	59.33	74.33	61.00	40.67	25.67	39.00
Grasspea bran	56.00	71.67	69.00	44.00	28.33	31.00
Rice bran + Wheat bran	60.33	74.33	67.00	39.68	25.67	33.00
Rice bran + Grass pea bran	57.67	75.00	67.00	42.33	25.00	33.00
Rice bran + Mustard oilcake	55.67	80.67	69.00	44.33	19.33	31.00
Rice bran + Wheat bran + MOC	58.00	79.67	68.00	42.00	20.33	32.00
Rice bran + Grasspea bran + MOC	57.67	76.00	70.00	42.33	24.00	30.00
Wheat bran + Grass pea bran + MOC	57.33	74.33	72.00	42.67	25.67	28.00
Wheat bran + Grass pea bran+ Rice bran + MOC	65.67	72.33	66.00	44.33	27.67	34.00
Seed treatment with Provax	63.67	77.67	59.00	46.33	22.33	41.00
Control	49.33	56.67	50.00	50.67	43.33	50.00

The post emergence seedling mortality of brinjal in *R. solani* inoculated soils in seed bed was significantly reduced by the application of different carrier material based *T. harzianum* bio-fungicides and Provax (Table 3). The individual as well as combination of mixed carrier material based *T. harzianum* bio-fungicides were found superior in reduction of post emergence seedling mortality as compared to

the untreated control. The highest seedling mortality of 17.33%, 25.33% and 21%, respectively was recorded in untreated control seedbed during the consecutive three years. Seedling mortality of brinjal ranged from 4.67%-7.33% in 1st year, 6.67%-12.67% in 2nd year and 7.67% - 9.33% in 3rd year due to the application of *T. harzianum* bio-fungicides were recorded (Table 3). Provax treated seedbed showed similar seedling mortality as observed in the bio-fungicides treated beds every year. The effect of different carrier material based *T. harzianum* bio-fungicides was much encouraging that reduced post-emergence seedling mortality of brinjal from 57.70% -73.05% in 1st year, 49.98% -73.67% in 2nd year and 55.57% - 63.48% in 3rd year over untreated control in *R. solani* inoculated seedbeds (Table 3).

Table 3. Efficacy of various carrier material based *T. harzianum* bio-fungicides on the post-emergence mortality of brinjal seedling in *R. solani* inoculated soils in seed bed

Name of substrates	Post-emergence seedling mortality in consecutive three years (%)			Seedling mortality reduction over control in consecutive three years (%)		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran	7.00 bc (15.31)	12.67 b (20.84)	9.33 b (17.05)	59.61	49.98	55.57
Wheat bran	6.33 bc (14.58)	10.00 b (17.71)	9.00 b (17.44)	63.47	60.52	57.14
Grasspea bran	7.33 b (15.72)	11.00 b (19.36)	9.33 b (16.75)	57.70	56.57	55.57
Rice bran + Wheat bran	5.67 bc (13.77)	10.67 b (18.89)	7.67 b (16.05)	67.28	57.88	63.48
Rice bran + Grasspea bran	5.33 bc (13.36)	8.00 b (16.41)	8.33 b (16.75)	69.24	68.42	60.33
Rice bran + Mustard oilcake	6.00 bc (14.19)	8.67 b (17.05)	9.00 b (16.75)	65.38	65.77	57.14
Rice bran + Wheat bran + MOC	5.33 bc (13.38)	6.67 b (14.95)	8.67 b (16.02)	69.24	73.67	58.71
Rice bran +Grasspea bran +MOC	5.33 bc (13.41)	9.00 b (17.21)	8.33 b (17.08)	69.24	64.47	60.33
Wheat bran + Grasspea bran + MOC	7.00 bc (15.33)	9.00 b (17.39)	9.33 b (17.78)	59.61	64.47	55.57
Wheat bran + Grasspea bran+ Rice bran + MOC	4.67 c (12.48)	8.67 b (16.96)	9.00 b (16.77)	73.05	65.77	57.14
Seed treatment with Provax	6.00 bc (14.21)	9.33 b (17.49)	8.33 b (17.75)	65.38	63.17	60.33
Control	17.33 a (24.59)	25.33 a (30.17)	21.00 a (27.48)	-	-	-

Values in a column having same letter did not differ significantly (p=0.05) by LSD.
Arcsine transformed values were within the parentheses.

Shoot length and shoot weight of seedlings were increased significantly (p=0.05) by the *T. harzianum* bio-fungicides in the *R. solani* inoculated seed bed soil (Table 4). In 1st year trial, the shoot length of brinjal seedlings ranged from 15.87 cm to 18.80 cm due to *T. harzianum* bio-fungicides and minimum shoot length (13.40 cm) was obtained from the control bed. Similarly, application of individual and mixed carrier material based *T. harzianum* bio-fungicides gave higher shoot length ranged from 7.67 cm – 10.60 cm in 2nd year and 6.77 cm-9.07 cm in 3rd year, while shorter shoots were observed in Provax and untreated control seedbed (Table 4). The shoot weight of individual brinjal seedling was enhanced up to 8.72 g by the *T. harzianum* bio-fungicide where minimum shoot weight of 5.87 g was recorded from the control bed during the 1st year trial. Similarly, maximum shoot weights of 6.80 g, and 7.80 g were recorded from the bio-fungicide treated seedbeds of 2nd and 3rd year trials where control beds gave shoots of 3.28 g and 4.37 g, respectively. Seed treatment with chemical fungicide Provax gave comparatively lower shoot weight of brinjal seedling in all the years.

Table4. Effect of different carrier material based *T. harzianum* bio-fungicides on the shoot growth of brinjal seedling in *R. solani* inoculated seed bed soil

Name of substrates	Shoot length in consecutive three years (cm)			Shoot weight in consecutive three years (gplant^{-1})		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran	16.43 cd	8.07 bcd	9.07 a	8.34 ab	5.05 c	6.17 b
Wheat bran	16.80 bcd	8.13 bcd	6.77 bc	8.69 ab	4.87 cd	6.60 b
Grasspea bran	17.27 bc	7.67 cd	7.73 ab	8.57 ab	4.87 cd	6.70 b
Rice bran + Wheat bran	15.87 cd	8.40 bc	8.90 a	8.36 ab	6.76 a	7.80 a
Rice bran + Grasspea bran	18.80 a	8.22 bcd	8.60 a	8.72 a	6.80 a	7.80 a
Rice bran + Mustard oilcake	16.67 bcd	8.97 bc	8.57 a	8.53 ab	6.10 ab	7.73 a
Rice bran + Wheat bran + MOC	16.20 cd	9.27 ab	8.97 a	8.31 ab	6.63 a	7.50 a
Rice bran+ Grasspea bran +MOC	17.03 bc	10.47 a	8.73 a	8.35 ab	6.52 a	7.70 a
Wheat bran + Grasspea bran + MOC	16.67 bcd	9.43 ab	7.83 ab	8.45 ab	5.35 bc	7.43 a
Wheat bran + Grasspea bran+ Rice bran + MOC	18.00 ab	10.60 a	8.63 a	8.16 ab	5.10 c	7.77 a
Seed treatment with Provax	15.33 d	6.86 de	5.97 cd	7.51 b	4.19 d	5.43 c
Control	13.40 e	5.80 e	5.00 d	5.87 c	3.28 e	4.37 d

Values in a column having same letter did not differ significantly (p=0.05) by LSD.

The root length was enhanced up to 6.80 cm by wheat bran based bio-fungicide followed by rice bran + grass pea bran (6.73 cm) and the shortest root was found in untreated control in 1st year trials (Table 5). The root length ranged from 6.80 cm – 8.60 cm in 2nd year and 6.60 cm- 7.93 cm in 3rd year trials while the shorter roots were recorded from Provax and untreated control treatments. The *T. harzianum* based bio-fungicides also augmented the root weights of brinjal seedlings as compared to untreated control.

Table 5. Role of various carrier material based *T. harzianum* bio-fungicides on the root growth of brinjal seedling in *R. solani* inoculated seed bed soil

Name of substrates	Root length in consecutive three years (cm)			Root weight in consecutive three years (mgplant^{-1})		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran	6.57 ab	7.00 abc	6.73 c	400	440 a	430 bc
Wheat bran	6.80 a	7.13 abc	6.87 bc	350	410 ab	450 abc
Grasspea bran	6.57 ab	6.80 abc	6.60 c	390	410 ab	460 abc
Rice bran + Wheat bran	6.40 ab	7.13 abc	7.93 a	380	420 ab	520 ab
Rice bran + Grasspea bran	6.73 a	7.80 ab	7.37 abc	400	430 ab	500 abc
Rice bran + Mustard oilcake	6.30 ab	8.47 a	7.60 ab	390	450 a	520 ab
Rice bran+ Wheat bran + MOC	6.43 ab	8.00 ab	7.70 a	400	440 a	550 a
Rice bran+ Grasspea bran +MOC	6.50 ab	8.60 a	7.73 a	380	440 a	480 abc
Wheat bran + Grasspea bran + MOC	6.30 ab	8.40 a	7.67 a	390	450 a	490 abc
Wheat bran + Grasspea bran+ Rice bran + MOC	6.70 a	8.57 a	7.63 ab	400	430 ab	500 abc
Seed treatment with Provax	5.80 bc	5.73 c	5.53 d	370	370 bc	420 bc
Control	5.13 c	5.53 c	4.87 d	330	320 c	380 c

Values in a column having same letter did not differ significantly (p=0.05) by LSD.

The overall effects of the carrier based *T. harzianum* bio-fungicides on the emergence, mortality, growth and development of brinjal seedlings under *R. solani* inoculated soils as revealed from the results of three years trials indicated that five different combination of carriers materials viz. (1) rice bran + wheat bran, (2) rice bran + grass pea bran (3) rice bran + MOC, (4) rice bran + wheat bran + MOC, and (5) rice bran + grass pea bran +MOC were superior for brinjal seedling disease (*R. solani*) management in seed bed condition.

Results of the present study revealed that *T harzianum* bio-fungicides were effective to control seedling disease of brinjal caused by *R. solani* in the tray and in seedbed condition. Many researchers reported that soil amendment with formulated *Trichoderma* was effective in controlling seedling disease of many crops especially the fungal pathogens such as *R. solani, Fusarium oxysporum* and *S. rolfsii* (Lo *et al.,* 1996; Tran, 1998; Bari *et al.,* 2000; Shamsuzzaman *et al.,* 2003; Benítez *et al*., 2004; Clear and Valic, 2005; Ngo *et al.,* 2006; Shalini *et al.,* 2006; Dubey *et al*., 2007; Rojo *et al*., 2007). The effect of *Trichoderma* spp. as bio-control agents against phytopathogenic fungi *Rhizoctonia* spp. and *Fusarium* spp. was also reported by other investigators (Poddar *et al*., 2004; Rojo *et al.,* 2007). Harman *et al.* (2004) reported that the biocontrol agents *Trichoderma* spp. influenced seed germination, seedling vigor and increased shoot and root growth as well as productivity of brinjal. The growth promotion in plant with special reference to the length and weight of their shoots and roots was enhanced due to *Trichoderma* spp. soil amendment (Samolski *et al*., 2012; Harman *et al*., 2012; Hermosa *et al*., 2012). Similarly, enhanced root length and root weight of many plants were also reported by the application of *Trichoderma* spp. (Chang *et al*., 1986; Kleifeld and Chet, 1992; Azarmi *et al*., 2011). Elad *et al*. (1980) reported that incorporation of *T. harzianum* bio-fungicide in the pathogen-infested soils significantly reduced bean seedling diseases caused by *R. solani* and *Sclerotium rolfsii*. Meah *et al.* (2004) reported the formulated *T. harzianum* grown on peat soil based black bran was found to be effective in controlling some of the nursery diseases like damping off, tip over and seedling blight of eggplant and also promoted seed germination. The present study revealed that rice bran based *Trichoderma* bio-fungicide gave better seed germination, reduced seedling mortality and increased growth of brinjal seedling. Sangeetha *et al.* (1993) found rice bran as the best substrate for the formulation of *Trichoderma* which gave 35% higher seed germination in brinjal and wheat. The disease incidence of brinjal, water melon and cotton was reported to be reduced considerably by the application of *T. harzianum* (Sivan and Chet, 1986). Tehroni and Nazari (2004) observed *T. harzianum* as an effective remedy against damping-off of cucumber. Shoresh *et al*. (2005) stated *Trichoderma* spp. as effective bio-control agents for a number of soil borne plant pathogens and induced a potentate state in the plant enabling it to be more resistant to subsequent pathogen infection.

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