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Efficacy of Substrate Based Trichoderma Harzianum Bio-Fungicides in Controlling Seedling Disease (Fusarium Oxysporum) of Brinjal in Seed Bed

M. I. Faruk[1], M. L. Rahman[2]

[1]Senior Scientific Officer, Plant Pathology Division, BARI
[2]Chief Scientific Officer, Training and Communication Wing, BARI

Abstract: Efficacy of individual and mixed substrates i.e. rice bran, wheat bran, grass pea bran and their combination with mustard oilcake (MOC), based *Trichoderma harzianum* bio-fungicides were tested against seedling disease (*Fusarium oxysporum*) of brinjal in plastic tray and seed bed during 2009 to 2014 in the net house of Bangladesh Agricultural Research Institute (BARI). The results of plastic tray and seed bed experiments revealed that four combinations of substrate based *T. harzianum* bio-fungicides such as (1) rice bran + wheat bran, (2) rice bran + wheat bran + mustard oil cake (MOC), (3) rice bran + grasspea bran, and (4) rice bran + grasspea bran +MOC were suitable for controlling the soil borne seedling disease (*F. oxysporum*) of brinjal in tray soil as well as seed bed soil conditions.

Keyword: Rice bran, wheat bran, grasspea ban, mustard oilcake, *Trichoderma harzianum*, *Fusarium oxysporum*, brinjal.

Introduction

Bangladesh is yet to be self-sufficient in quality food and nutrition which are the major challenges to achieve a healthy and prosperous country like other developing countries of the world. In the country vegetables play a vital role in everyday diet to a huge population in general. Eggplant vernacularly known as brinjal (*Solanum melongena* L.) is the major and popular vegetable available round the year in Bangladesh. Brinjal grows round the year in the country and provides vegetable to the consumers and stable financial assistance to the growers. The productivity of brinjal in Bangladesh is low (17.5 t/ha) as compared to that of Japan (32 t/ha), Italy (28.2 t/ha), Turkey (30.2 t//ha) and other countries (FAOSTAT, 2012). About 10% crops are lost worldwide annually due to plant diseases which lead to considerable economic discrepancy to the farmers of underdeveloped countries (Strange and Scott 2005). Among the diseases germination failure and seedling mortality caused by the soil borne pathogen *Fusarium oxysporum*, are the major constraints of brinjal especially in seed bed (Najar *et al.*, 2011). Conventional disease management with fungicide is less effective to control the soil borne *F. oxysporum*. On the other hand, application of chemical fungicides is expensive and also hazardous to health and environment (Brown and Hendrix 1980, Punja *et. al.* 1982). The beneficial microbes such as *Trichoderma harzianum* has been reported as a bio-control agent that effectively controlled the soil borne pathogens (Elad *et al*., 1983; Roy, 1989). The native bio-control agents usually remain in low population density in most of the agricultural soil, so up- scaling of their density to a higher stability level in soil through artificial inoculation is necessary for successful management of soil borne pathogens in brinjal seed bed. The major limitation is the lack of appropriate mass culturing techniques and inadequate information on the suitable substrate materials of *T. harzianum* (Harman *et al*., 1991). The *T. harzianum* has been formulated as bio-fungicides in various substrates like wheat bran, rice bran, maize bran, sawdust (Das *et al*., 1997); rice straw, chickpea bran, grass pea bran, rice course powder, black gram bran (Shamsuzzaman *et al*., 2003); cow dung, poultry manure, ground nut shell, black ash, coir waste, spent straw from mushroom bed, talc, vermiculite (Rettinassababady and Ramadoss, 2000); and jaggery, groundnut cake, neem cake, niger cake,

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M. I. Faruk (Correspondence)
mifaruk2012@yahoo.com
+88-02-9294605; +88-01732587563

pongamia (Shamarao *et al*., 1998). All of these substrate materials are available in Bangladesh but their potentialities to use in the formulation of *T. harzianum* bio-fungicide have not yet been studied in the country. Therefore, the present study was undertaken to find out the effective local substrates to formulate the suitable medium for mass culturing of *T. harzianum* to be used as effective bio-fungicides against *F. oxysporum* causing germination failure and seedling mortality of brinjal under seed bed condition.

Materials and Methods

One experiment was conducted in the plastic tray and three experiments were conducted under seed bed conditions in the net house of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur during the period 2009 to 2014 to find out the suitable substrate material for mass culturing of *T. harzianum* and thereby formulation of an effective bio-fungicides to control soil borne seedling disease (*F. oxysporum*) of brinjal.

A pure culture of *T. harzianum* (TM7) was grown in potato dextrose agar (PDA) medium which was used as inoculum of bio-fungicide. Three substrates such as rice bran, wheat bran, grass pea bran and their combination with mustard oilcake (MOC) were used in the experiment for mass multiplication of *T. harzianum.* Thus the treatments (substrates) of the experiment 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 + Mustard oilcake (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} = Untreated control.

According to the treatment combinations 600 g of individual or combination of substrate materials were taken separately in 1000 ml Erlenmeyer flask. The flask with substrate materials were sterilized in an autoclave at 121^0C for 15 minutes and cooled down to make it ready for inoculation. The sterilized substrate was inoculated individually with 5 mm diameter mycelia disc of five-day old culture of *T. harzianum* grown on PDA and then incubated at room temperature (25±2 ^{0}C) for 15 days. After incubation the colonized substrates were removed from the flasks and air dried and finally preserved in refrigerator at 10 ^{0}C. The tray soils as well as seed bed soils were inoculated with *Fusarium oxysporum* to make it sick soil. The inoculum of *T. harzianum*, colonized on different substrates, were incorporated with the *F. oxysporum* sick soils @100 g/m^2 soil and kept for 7 days with proper soil moisture to establish *T. harzianum* in the soils. The untreated control did not receive any colonized substrate of *T. harzianum* except the inoculum of *F. oxysporum*.

The seeds of BARI Bagun-7 were sown both in the plastic tray and seed bed experiments using 200 seeds per treatment. The experiments were laid out in completely randomized design (CRD) with four replications. Proper weeding, irrigation and intercultural operations were done in time to raise the brinjal seedlings. Data were collected on percent seedling emergence, seedling mortality, height and weight of shoot, length and weight of root of brinjal seedlings. The data were analyzed statistically by using the MSTATC program. The treatment effects were compared by applying the least significant different (LSD) test at p=0.05 level.

Results and Discussions

A. Efficacy of different substrate based *T. harzianum* bio-fungicides in the plastic tray experiment

The efficacy of *T. harzianum* bio-fungicides against *F. oxysporum* causing germination failure and mortality of brinjal seedlings was tested in a plastic tray experiment where the soil was artificially inoculated with the pathogen *F. oxysporum* and then treated with the different substrates based *T. harzianum* bio-fungicides. Percent seedling emergence as well as seedling mortality was varied among the substrate based *T. harzianum* bio-fungicides (Table1). The seedling emergence of brinjal was ranged from 82% (wheat bran) to 98% (rice bran + grasspea bran) due to *T. harzianum* bio-fungicide treatments whereas untreated control tray gave 75% seedling emergence. The amount of both pre-emergence as well as post-emergence seedling mortality of brinjal varied among the *T. harzianum* bio-fungicides resulting total seedling mortality of 10% to 31%. The highest total seedling mortality (51%) was recorded from the untreated control tray. Thus soil treatment with *T. harzianum* reduced seedling mortality up to 20% as compared to untreated control (Table 1). The result showed that *T. harzianum* treated tray soil gave higher amount of healthy seedlings (69- 90%) while untreated control tray soil produced only 49% healthy seedling. Considering the overall performance of bio-fungicides with respect to reduction of seedling disease and thereby producing healthy seedlings of brinjal under *F. oxysporum* sick soils, the rice bran + grasspea bran, rice bran + wheat bran, rice bran + wheat bran MOC, grasspea bran and rice bran based *T. harzianum* bio-fungicides were seemed to be better ones (Table 1).

B. Efficacy of different substrate based *T. harzianum* bio-fungicides in the seedbed experiment

a) Emergence and pre-emergence mortality of brinjal seedling

Seedling emergence of brinjal was enhanced in all the three years due to application of different substrate material based *T. harzianum* bio-fungicide in the *F. oxysporum* sick soils of seed bed. The seedling emergence varied from 53-62% among the bio-fungicide treated beds whereas untreated control bed gave comparatively low emergence (52%) of brinjal seedling in 1st year trial (Table 2). Similarly, the bio-fungicides gave higher seedling emergence in 2nd year (85-89%) and 3rd year (58-70%) while control seed beds showed 70% and 50% seedling emergence, respectively.

The *T. harzianum* bio-fungicide treated seed beds showed lower pre-emergence mortality of brinjal seedlings over untreated control beds in all the years (Table 2). The percentage of pre-emergence seedling mortality was lower among the bio-fungicide treated beds in 1st year (38-47%), 2nd year (11-15%) and also in 3rd year (30-42%). Besides, the pre-emergence seedling mortality of brinjal in control beds was 48, 30, and 50 percent, respectively in the consecutive three years. The results indicated that the effect of single and mixed substrate based *T. harzianum* bio-fungicides were almost similar among themselves in respect of seedling emergence as well as pre-emergence seedling mortality of brinjal in *F. oxysporum* sick soils in seed bed.

b) Post-emergence mortality of brinjal seedling

Post-emergence mortality of brinjal seedling in *F. oxysporum* sick soils in seed bed was sharply reduced in all the years by the different substrate based *T. harzianum* bio-fungicides and Provax as compared to the untreated control. The seedling mortality in 1st year trial was 18% in the untreated control bed while it ranged from 6 to 8% among the bio-fungicides treated seed beds (Table 3). As compared to untreated control bed, the reduction of seedling mortality was ranged from 57 to 68% among the bio-fungicide treated beds. In the 2nd year trial, 11 to 14% seedling mortality was observed in the bio-fungicides treated seed beds where control bed showed 35% seedling mortality. Thus the seedling mortality was reduced from 65 to 69% by the application of different bio-fungicides. The reduction of seedling mortality in the bio-fungicides treated seed beds and untreated control bed in the 3rd year trial were also showed similar trend of results. The Provax treated seed bed showed similar reduction of seedling mortality as observed in the bio-fungicides treated beds in all the years. The individual and mixed substrate material based *T. harzianum* bio-fungicides were equally effective on the post-emergence mortality of brinjal seedling in *F. oxysporum* sick soils of seed beds.

c) Shoot growth of brinjal seedling

The shoot growth of brinjal seedling was significantly accelerated by the application of various substrate based *T. harzianum* bio-fungicides and Provax in *F. oxysporum* sick soils of seed bed in all the years. In the 1st year trial, the shoot length of brinjal seedling was 15.9 to 17.3 cm among the *T. harzianum* bio-fungicide treated beds whereas it was 13 cm in the untreated control seed bed (Table 4). Higher shoot length of brinjal seedling in 2nd year trial, ranging from 8.07 to 10.1 cm, was recorded from the bio-fungicides treatments and lowest length of 5.67 cm was observed in control. Similarly, longer shoots (7.93 to 10.87 cm) was recorded from the bio-fungicides treated seed beds in the 3rd trial, shortest shoot (6.27 cm) was in the control bed.

The shoot weight of brinjal seedling was significantly increased by the *T. harzianum* bio-fungicides over untreated control bed. The individual shoot weight was increased up to 4.63 g by the application of *T. harzianum* bio-fungicide while shoot weight was 2.99 g in control bed of the 1st year trial (Table 4). The bio-fungicide treated beds gave accelerated shoot weights of 6.17 to 8.8 g in the 2nd year and 6.37 to 7.8 g in the 3rd year trial when the untreated beds gave 3.87 and 3.6 g shoot weights, respectively. The inferior shoot growth was noticed in the Provax treated beds as compared to untreated control beds in all the years. The results indicated that different substrate based *T. harzianum* bio-fungicides possessed the ability to enhance shoot growth of brinjal seedling in addition to seedling disease reduction caused by *F. oxysporum* under seed bed conditions.

d) Root growth of brinjal seedling

The root growth of brinjal seedling was accelerated significantly by different substrate based *T. harzianum* bio-fungicides as compared to the untreated control in *F. oxysporum* sick soils of seed beds. In the 1st year experiment, the root length of brinjal seedling was ranged from 6.27 cm to 6.97 cm among the bio-fungicides treated beds which was minimum (4.63 cm) in the untreated control bed (Table 5). Similarly, the root length varied from 6.50 cm to 8.60 cm in the 2nd year and 6.33 cm to 8.80 cm in the 3rd year experiment due *T. harzianum* bio-fungicides. Minimum root lengths of 4.20 cm and 3.73 cm were recorded from the untreated control beds in the 2nd and 3rd year experiments, respectively. Seed treatment with Provax also gave comparatively inferior root length in all the years.

The root weight of individual brinjal seedling was enhanced significantly by different substrate based *T. harzianum* bio-fungicides while inferior root weights were recorded from the Provax treated as well as untreated control seed beds in all the years (Table 5). The root weights of individual brinjal seedling grown in bio-fungicide treated seed beds was varied from 385 to 410 mg, 410 to 480 mg and 550 to 700 mg during 1st year, 2nd year and 3rd year trials, respectively. Inferior root weights of brinjal seedling were recorded from the Provax (370-510 mg) and

untreated control beds (330-450 mg) in all the years.

The results of consecutive three years experiments revealed that both individual as well as mixed substrate based *T. harzianum* bio-fungicides effectively reduced the seedling mortality caused by *F. oxysporum* and also enhanced shoot and root growth of brinjal seedling under seed bed conditions.

The results of the present study revealed that rice bran and wheat bran based *T. harzianum* bio-fungicide gave better seed germination, reduced seedling mortality and increased vegetative growth of brinjal seedling in seed bed. Similar observation with wheat and rice bran for the formulation of *T. harzianum* bio-fungicide was reported by Sangeetha *et at.* (1993). Enhanced seed germination due to *Trichoderma* species was also reported by Mukhtar (2008). It was noticed by Tjamos *et al*. (1992) that *T. harzianum* controls *F. oxysporum* by competing for both rhizosphere colonization and nutrients. Disease incidence of brinjal, water melon and cotton was reported to be reduced considerably by the application of *T harzianum* (Sivan and Chet, 1986). Shoresh *et al.* (2005) stated that *Trichoderma* spp. were effective bio-control agents for a number of soil borne plant pathogens.

In this trial there was significant increase in emergence, shoot and root growth of brinjal seedling due to *T. harzianum* bio-fungicides which was supported by the findings of many investigators (Prasad and Anes, 2008; Mishra and Sinha, 2000; Chaur-Tsuen and Chien-Yih, 2002). Harman, (2006) and Manju and Mall, (2008) also reported positive role of *Trichoderma* species in increasing plant growth and productivity. Enhanced the root growth of brinjal seedling in *F. oxysporum* sick soils was observed in the present experiment which was supported by the findings of (John *et al*., 2010). The study confirmed the reports of other researchers regarding the role of *T. harzianum* to enhanced seedling emergence as well as root and shoot growth of seedlings (Dubey *et al*., 2007) and thereby increasing the frequency of healthy plants (Rojoa *et al*., 2007).

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Table 1. Effect of different substrate based *T. harzianum* bio-fungicides on the emergence and mortality of brinjal seedling in *Fusarium oxysporum* sick soils in plastic tray trial

Substrate based *T. harzianum* bio-fungicides	Percent seedling emergence (%)	Pre-emergence seedling mortality (%)	Post-emergence seedling mortality (%)	Total seedling mortality (%)	Total healthy seedling (%)	Seedling mortality reduction (%)
Rice bran	93	7	11	18	82	33
Wheat bran	82	18	11	29	71	22
Grasspea bran	97	3	14	17	83	34
Rice bran + Wheat bran	96	4	9	13	87	38
Rice bran + Grasspea bran	98	2	8	10	90	41
Rice bran + MOC	93	7	13	20	80	31
Rice bran + Wheat bran + MOC	95	5	9	14	86	37
Rice bran +Grasspea bran +MOC	94	6	13	19	81	32
Wheat bran + Grasspea bran + MOC	84	16	15	31	69	20
Rice bran + Wheat bran + Grasspea bran+ MOC	83	17	14	31	69	20
Seed treatment with Provax	78	22	11	33	67	18
Untreated Control	75	25	26	51	49	-

Table 2. Effect of substrate based *T. harzianum* bio-fungicides on emergence and pre-emergence mortality of brinjal seedling in *F. oxysporum* sick soils in seed bed experiment

Substrate based *T. harzianum* bio-fungicides	Emergence (%) of brinjal seedling in three years trial			Pre-emergence mortality (%) of brinjal seedling in three years trial		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran	59	88	62	41	12	38
Wheat bran	55	88	60	45	12	40
Grasspea bran	56	87	59	44	13	41
Rice bran + Wheat bran	62	87	70	38	13	30
Rice bran + Grass pea bran	59	87	58	42	13	42
Rice bran + Mustard oilcake	56	85	58	44	15	42
Rice bran + Wheat bran + MOC	58	87	65	42	13	35
Rice bran + Grasspea bran + MOC	57	87	63	43	14	37
Wheat bran + Grass pea bran + MOC	53	85	66	47	15	34
Wheat bran + Grass pea bran+ Rice bran + MOC	54	89	63	46	11	37
Seed treatment with Provax	60	83	56	40	17	44
Control	52	70	50	48	30	50

Table 3. Effect of substrate based *T. harzianum* bio-fungicides on mortality of brinjal seedling in *F. oxysporum* sick soils in seed bed experiment

Substrate based *T. harzianum* bio-fungicides	Post-emergence (%) seedling mortality in three years trial			Seedling mortality reduced (%) over control in three years trial		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran	8	13	8	57	65	65
Wheat bran	7	12	8	59	65	65
Grasspea bran	7	11	9	59	69	60
Rice bran + Wheat bran	6	13	7	68	63	66
Rice bran + Grasspea bran	7	12	8	62	66	65
Rice bran + Mustard oilcake	7	11	8	60	68	63
Rice bran + Wheat bran + MOC	7	11	9	59	68	60
Rice bran +Grasspea bran +MOC	6	12	9	64	65	60
Wheat bran + Grasspea bran + MOC	7	14	9	59	61	60
Wheat bran + Grasspea bran+ Rice bran + MOC	6	13	8	64	64	63
Seed treatment with Provax	8	13	8	53	64	62
Control	18	35	22	-	-	-

Table 4. Role of substrate based *T. harzianum* bio-fungicides on the shoot growth of brinjal seedling in *F. oxysporum* sick soils in seed bed experiment

Substrate based *T. harzianum* bio-fungicides	Shoot length (cm) of brinjal seedlings in three years trial			Shoot weight (gplant^{-1}) of brinjal seedlings in three years trial		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran	16.57 ab	8.20 cd	8.67 c	4.23 a	6.37 b	6.53 b
Wheat bran	15.90 ab	8.67 cd	7.93 c	4.29 a	6.53 b	6.37 b
Grasspea bran	16.20 ab	8.07 d	8.13 c	4.47 a	6.17 b	6.87 ab
Rice bran + Wheat bran	16.33 ab	8.60 cd	10.33 ab	4.45 a	6.80 b	6.80 ab
Rice bran + Grasspea bran	16.40 ab	8.60 cd	10.60 a	4.61 a	6.53 b	7.43 ab
Rice bran + Mustard oilcake	16.20 ab	9.07 cd	10.53 ab	4.63 a	8.03 a	7.80 a
Rice bran + Wheat bran + MOC	16.83 ab	9.63 bc	10.87 a	4.30 a	8.27 a	7.16 ab
Rice bran+ Grasspea bran +MOC	17.30 a	9.67 ab	9.77 b	4.48 a	8.60 a	6.97 ab
Wheat bran + Grasspea bran + MOC	17.23 a	9.53 ab	10.50 ab	4.53 a	8.47 a	6.73 ab
Wheat bran + Grasspea bran+ Rice bran + MOC	17.03 ab	10.10 a	9.13 bc	4.43 a	8.80 a	6.53 b
Seed treatment with Provax	15.67 b	6.53 e	6.43 c	3.42 b	5.00 c	4.83 c
Control	13.00 c	5.67 f	6.27 d	2.99 b	3.87 d	3.60 d

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

Table 5. Role of substrate based *T. harzianum* bio-fungicides on root growth of brinjal seedling in *F. oxysporum* sick soils in seed bed experiment

Substrate based *T. harzianum* bio-fungicides	Root length (cm) of brinjal seedling in three years trial			Root weight (mgplant^{-1}) of brinjal seedling in three years trial		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran	6.67 ab	6.63 b	8.80 a	390 a	410 b	560 abc
Wheat bran	6.47 ab	6.80 b	6.53 ab	390 a	420 b	580 abc
Grasspea bran	6.40 b	6.50 b	6.33 ab	386 a	400 b	550 abc
Rice bran + Wheat bran	6.97 a	6.83 b	7.00 a	380 a	420 b	620 ab
Rice bran + Grasspea bran	6.27 b	6.76 b	8.07 a	395 a	410 b	690 a
Rice bran + Mustard oilcake	6.33 b	7.90 a	7.00 a	390 a	470 a	620 ab
Rice bran+ Wheat bran + MOC	6.77 ab	8.07 a	7.00 a	400 a	480 a	680 a
Rice bran+ Grasspea bran +MOC	6.70 ab	8.26 a	8.33 a	410 a	470 a	640 ab
Wheat bran + Grasspea bran + MOC	6.50 ab	7.73 a	8.27 a	390 a	480 a	700 a
Wheat bran + Grasspea bran+ Rice bran + MOC	6.47 ab	8.60 a	7.80 a	385 a	470 a	640 ab
Seed treatment with Provax	5.70 c	5.13 c	5.73 ab	390 a	370 c	510 bc
Control	4.63 d	4.20 d	3.73 b	350 b	330 d	450 c

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