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EFFICACY OF DIFFERENT SUBSTRATES TO FORMULATE *Trichoderma harzianum* AGAINST SEEDLING DISEASE OF CABBAGE

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Abstract

Efficacy of three different substrates viz. rice bran, wheat bran, grass pea bran and their combination along with or without Mustard oilcake (MOC) were tested to formulate *Trichoderma harzianum* based bio-fungicides for the management of seedling disease (*Fusarium oxysporum*) of cabbage in seedbed. All combinations of substrates were equally suitable for mass culturing and preparing of *T. harzianum* bio-fungicides and all the substrates based *T. harzianum* bio-fungicides were effective in increasing seedling emergence and reducing pre-emergence and post-emergence mortality of cabbage seedling under *F. oxysporum* inoculated seedbed soils. The shoot length, shoot weight, root length and root weight of cabbage seedling were enhanced significantly by the application of different substrates based *T. harzianum* bio-fungicides under *F. oxysporum* inoculated soil under seedbed conditions. The individual (rice bran, wheat bran, grasspea bran) and combination of substrates (rice bran + wheat bran, rice bran + grasspea bran, rice bran + Mustard oilcake, rice bran + wheat bran + MOC and wheat bran + grass pea bran + MOC) were equally suitable to formulate effective *T. harzianum* based bio-fungicides for the management of foot and root rot disease of cabbage seedling in seed bed condition.

Keywords: Rice bran, wheat bran, grasspea bran, mustard oilcake, *Trichoderma harzianum*, *Fusarium oxysporum*, cabbage.

Introduction

Availability of quality food and nutrition are the major challenges to achieve healthy and prosperous Bangladesh like other developing countries of the world. Bangladesh is yet to be self-sufficient in quality food and nutrition. In the country vegetables play a vital role in everyday diet to a huge population in general. Among the vegetables, cabbage (*Brassica oleracea*) is one of the popular herbaceous annual or biennial vegetable that has achieved tremendous popularity over the last century. The crop attacked by several diseases mostly caused by fungi and bacteria leading to severe crop losses. Among the diseases germination failure, seedling mortality, foot and root-rot disease caused by the soil borne pathogen *Fusarium oxysporum*, is one of the major constraints for seedling

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production of vegetable crops in seedbed (Najar *et al.*, 2011). Control measures like host resistance is not yet become a viable measure. No resistant variety of cabbage has yet been developed and released against this soil borne pathogen *F. oxysporum* in Bangladesh. Soil solarization and organic soil amendment have been found less effective. 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). Biological methods, on the other hand can be economical, sustainable and free from residual effects and also consider as a potentially effective alternative method (Kulkarni *et al*., 2007; Anand and Reddy, 2009). The beneficial microbes are used as biological agents that effectively control soil borne plant pathogens, and about 90% of such bio-control agents are different strains of *T. harzianum*, *T. virens*, *T. viride* (Elad *et al*., 1983; Roy, 1989; Benítez *et al*., 2004). The fungus *Trichoderma* possesses different mechanisms to combat the targeted pathogen such as mycoparasitism, competition for space and nutrients, secretion of antibiotics and fungal cell wall degrading enzymes for the inhibition of growth and reproduction of phytopathogens (Kubicek *et al.*, 2001; Howell, 2003; Benítez *et al.*, 2004; Harman *et al*., 2004). In addition, *Trichoderma* has a stimulatory effect on plant growth (Naseby *et al*., 2000) as a result of modification of soil conditions. 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 with effective inoculum is necessary for successful management of soil borne pathogens in cabbage seedbed. 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). Several research report revealed that *T. harzianum* has been formulated as bio-fungicides in various substrates including wheat bran, rice bran, maize bran, sawdust (Das *et al*., 1997); rice straw, chickpea bran, grasspea bran, rice course powder, black gram bran (Shamsuzzaman *et al*., 2003); cow dung, poultry manure, groundnut shell, black ash, coir waste, spent straw from mushroom bed, talc, vermiculite (Rettinassababady and Ramadoss, 2000); and jaggery, groundnut cake, neem cake, niger cake, pongamia (Shamarao *et al*., 1998). All 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 seedling disease of cabbage under seedbed condition.

Materials and Methods

Efficacy of three organic substrates viz. rice bran, wheat bran, grasspea bran and their combinations mixed with or without Mustard oilcake (MOC) was

evaluated to formulate *T. harzianum* based bio-fungicides for the management of foot and root rot disease of cabbage caused by *F. oxysporum*. The experiment was conducted in seedbed of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARl), Gazipur during three consecutive years from 2011-12 to 2013-14. The seedbed was inoculated with the fungal isolate *F. oxysporum* multiplied on the barley grains @ 100g/m^2 soil. The pathogen was allowed to colonize the soil in seedbed for 10 days. A pure culture of *T. harzianum* (TM7) isolated from the native soil was grown in potato dextrose agar (PDA) medium which was used as inocula for preparation of bio-fungicides. The treatments substrate in 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 + Grasspea 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 flask. The flask with substrate materials were sterilized in an autoclave at 121^{0}C 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, air dried and finally preserved in refrigerator at 10 ^{0}C. The inoculum of *T. harzianum,* colonized on different substrates, were incorporated to the previously *F. oxysporum* inoculated seedbed soils @ 100 g/m^2 soil and kept for 7 days maintaining proper soil moisture to establish *T. harzianum* in the soils. The control bed did not receive any colonized substrate of *T. harzianum* except the inoculum of *F. oxysporum*. The seeds of cabbage variety Atlas were sown in the seedbed @ 200 seeds per treatment. The initial germination in blotter test of the seeds was 99%. The percent emergence of the seedling was calculated on the basis of initial germination status of the seeds. The experiment was laid out in completely randomized design (CRD) with four replications. Proper weeding, irrigation and intercultural operations were done to raise cabbage seedlings in the seedbed. Data were collected on seedling emergence after 15 days of seed sowing. Similarly seedling mortality was recorded at an interval of 7 days starting from seedling emergence and it was continued up to 35 days of seedling age. The height and weight of shoot and length and weight of root of tomato seedlings were recorded at 35 days of seedling age. The percent data were converted into arcsine transformation values before statistical analysis. 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 Discussion

a) Seedling emergence and pre-emergence mortality

Every year, the seedling emergence of cabbage was significantly increased over control in the *F. oxysporum* inoculated seedbed due to *T harzianum* bio-fungicides. Under control treatment the emergence was 49.33, 64.00 and 66.00% in 1st, 2nd and 3rd year, respectively. The seedling emergence ranged from 58.00-
67.00, 80.33-86.67 and 79.00-91.00% due to treatment of seedbed soil with the bio-fungicides in 1st, 2nd and 3rd year, respectively. Seedling emergence under various treatments with the bio-fungicides was statistically similar (Table 1).

On the contrary, the pre-emergence seedling mortality of cabbage under control was 50.67, 36.00 and 34.00% in 1st, 2nd and 3rd year, respectively. The corresponding mortality under treatment of seedbed soils with the bio-fungicides was 33.00-42.00% in first year, 13.33-19.67% in second year and 09.00-21.00% in third year. It was reduced to 17.11-34.87, 45.36-62.97 and 38.23-73.53% in first, second and third year, respectively due to treatments of seedbed soils with various bio-fungicides (Table 1). However, efficacy of all bio-fungicides was more or less similar.

b) Post-emergence mortality

Post-emergence mortality of cabbage in *F. oxysporum* inoculated seedbed soil was
21.33, 33.67 and 24.67% under treatment control in 1st, 2nd and 3rd year of study, respectively. The substrates based *T. harzianum* bio-fungicides reduced the disease incidence from 64.04-73.42, 60.41-66.35 and 60.80-72.96% in 1st, 2nd and 3rd year, respectively compared to untreated control. Efficacy of all bio-fungicides to reduce the disease incidence was statistically similar (Table 2).

c) Shoot growth

Shoot length and shoot weight of cabbage seedlings were increased significantly by different substrates based *T. harzianum* bio-fungicides over Provax and untreated control in the *F. oxysporum* inoculated seed bed soil (Table 3). Under control seedbed, shoot length was 13.03 cm in first year, 4.90 cm in second year and 10.87 cm in third year. Treatment of seedbed soils with *T harzianum* based bio-fungicides resulted increase in shoot length ranging by 17.07-18.13, 8.30-10.13 and 16.33-22.07 cm in 1st, 2nd and 3rd year, respectively. The shoot weight under control treatment was 3.75, 3.97 and 6.57 g plant^{-1} in first, second and third year, respectively. Treatments of seedbed soils by *T. harzianum* based bio-fungicides increased the shoot weight ranging 5.53- 5.81, 7.11-8.53 and 8.88-12.40 g plant^{-1} in 1st, 2nd and 3rd year, respectively. Effect of all bio-fungicides treatments on shoot growth was more or less similar (Table 3).

Table 1. Effect of substrate based *T. harzianum* bio-fungicides on the emergence and mortality of cabbage seedling in *F. oxysporum* inoculated soils in seedbed

Name of substrates	Emergence (%) of cabbage seedling in seedbed soil			Pre-emergence mortality (%) of cabbage seedling in seedbed		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
T_1=Rice bran	63.00	85.33	86.00	37.00 (26.98)	14.67 (59.25)	14.00 (58.82)
T_2=Wheat bran	62.67	82.33	85.00	37.33 (26.33)	17.67 (50.92)	15.00 (55.88)
T_3=Grasspea bran	61.67	83.33	90.00	38.33 (24.35)	16.67 (53.69)	10.00 (70.58)
T_4=Rice bran + Wheat bran	63.33	84.33	89.00	36.67 (27.63)	15.67 (56.47)	11.00 (67.65)
T_5=Rice bran + Grass pea bran	66.00	82.00	91.00	34.00 (32.89)	18.00 (50.00)	09.00 (73.53)
T_6=Rice bran + Mustard oilcake	59.67	82.00	83.00	40.33 (20.41)	18.00 (50.00)	17.00 (50.00)
T_7=Rice bran + Wheat bran + MOC	61.00	86.00	85.00	39.00 (23.03)	14.00 (61.11)	15.00 (55.88)
T_8=Rice bran + Grasspea bran + MOC	67.00	80.67	86.00	33.00 (34.87)	19.33 (46.30)	14.00 (58.82)
T_9=Wheat bran + Grass pea bran + MOC	59.33	80.33	88.00	40.67 (19.74)	19.67 (45.36)	12.00 (64.70)
T_{10}=Wheat bran + Grass pea bran+ Rice bran + MOC	58.00	86.67	79.00	42.00 (17.11)	13.33 (62.97)	21.00 (38.23)
T_{11}=Seed treatment with Provax	57.67	79.00	83.00	42.33 (16.46)	21.00 (41.67)	17.00 (50.00)
T_{12}=Control	49.33	64.00	66.00	50.67	36.00	34.00

Values with in the parenthesis are percentage of reduction in pre-emergence mortality due to treatments over control.

Table 2. Reduction of cabbage seedling mortality by different substrate based *T. harzianum* bio-fungicides in *F. oxysporum* inoculated soils in seedbed

Name of substrates	Post-emergence cabbage seedling mortality (%) in seedbed soil			Reduction of cabbage seedling mortality (%) over control in seedbed soil		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
T_1=Rice bran	7.33 b	13.33 b	7.67 b	65.64	60.41	68.91
T_2=Wheat bran	7.67 b	11.67 b	7.67 b	64.04	65.34	68.91
T_3=Grasspea bran	7.00 b	11.67 b	9.33 b	67.18	65.34	62.18
T_4=Rice bran + Wheat bran	5.67 b	12.00 b	6.67 b	73.42	64.36	72.96
T_5=Rice bran + Grass pea bran	7.00 b	12.00 b	7.67 b	67.18	64.36	68.91
T_6=Rice bran + Mustard oilcake	7.00 b	11.33 b	7.33 b	67.18	66.35	70.29
T_7=Rice bran + Wheat bran + MOC	7.00 b	11.67 b	8.33 b	67.18	65.34	66.23
T_8=Rice bran + Grasspea bran + MOC	7.67 b	11.67 b	7.67 b	64.04	65.34	68.91
T_9=Wheat bran + Grass pea bran + MOC	7.33 b	12.67 b	9.67 b	65.64	62.37	60.80
T_{10}=Wheat bran + Grass pea bran+ Rice bran + MOC	6.33 b	12.67 b	8.33 b	70.32	62.37	66.23
T_{11}=Seed treatment with Provax	5.67 b	13.00 b	9.67 b	73.42	61.39	60.80
T_{12}=Control	21.33 a	33.67 a	24.67 a	-	-	-

Table 3. Effect of different substrate based *T. harzianum* bio-fungicides on the shoot growth of cabbage seedling in *F. oxysporum* inoculated seedbed 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
T_1=Rice bran	17.07 b	8.53 cd	16.97 c	5.78 ab	7.18 b	8.88 cd
T_2=Wheat bran	17.70 ab	8.50 cd	16.33 cd	5.60 ab	7.40 b	9.58 bcd
T_3=Grasspea bran	17.60 ab	8.30 d	16.33 cd	5.67 ab	7.11 b	9.28 bcd
T_4=Rice bran + Wheat bran	18.17 ab	8.67 cd	20.00 ab	5.56 ab	8.57 a	10.88 abc
T_5=Rice bran + Grass pea bran	17.80 ab	8.93 bcd	22.07 a	5.73 ab	8.47 a	12.40 a
T_6=Rice bran + Mustard oilcake	18.13 ab	9.63 abc	20.67 ab	5.74 ab	8.85 a	11.08 ab
T_7=Rice bran + Wheat bran + MOC	17.83 ab	9.67 abc	20.73 ab	5.81 a	8.73 a	11.90 ab
T_8=Rice bran + Grasspea bran + MOC	18.13 ab	9.87 ab	20.13 ab	5.60 ab	8.73 a	10.77 abc
T_9=Wheat bran + Grass pea bran + MOC	17.13 b	10.07 ab	19.73 b	5.67 ab	7.45 b	10.38 abc
T_{10}=Wheat bran + Grass pea bran+ Rice bran + MOC	19.13 a	10.13 a	21.10 ab	5.53 b	7.57 b	10.50 abc
T_{11}=Seed treatment with Provax	14.97 c	5.83 e	14.27 d	4.74 c	4.88 c	7.70 de
T_{12}=Control	13.03 d	4.90 e	10.87 e	3.75 d	3.97 d	6.57 e

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

Table 4. Effect of different substrate based *T. harzianum* bio-fungicides on the root growth of cabbage seedling in *F. oxysporum* inoculated seedbed soil

Name of substrates	Root length of cabbage seedling in consecutive three years (cm)			Root weight of cabbage seedling in consecutive three years (mgplant^{-1})		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
T_1=Rice bran	7.13 a	7.33 ab	6.07 bcd	610 a	600 ab	660 b
T_2=Wheat bran	7.13 a	7.20 ab	5.67 d	620 a	540 ab	680 b
T_3=Grasspea bran	7.07 a	7.17 b	5.93 cd	590 ab	560 ab	650 b
T_4=Rice bran + Wheat bran	7.37 a	7.17 b	7.33 a	590 ab	610 a	830 a
T_5=Rice bran + Grass pea bran	7.76 a	7.60 ab	8.00 a	610 a	550 ab	870 a
T_6=Rice bran + Mustard oilcake	7.40 a	8.50 ab	7.07 abc	570 b	570 ab	860 a
T_7=Rice bran + Wheat bran + MOC	7.53 a	8.73 a	7.20 ab	610 a	580 ab	830 a
T_8=Rice bran + Grasspea bran + MOC	7.47 a	8.47 ab	7.53 a	580 ab	610 a	890 a
T_9=Wheat bran + Grass pea bran + MOC	7.87 a	8.27 ab	7.07 abc	610 a	600 ab	910 a
T_{10}=Wheat bran + Grass pea bran+ Rice bran + MOC	7.53 a	8.40 ab	7.33 a	610 a	520 b	830 a
T_{11}=Seed treatment with Provax	6.25 b	5.23 c	5.13 de	480 c	400 c	650 b
T_{12}=Control	5.46 b	4.47 c	4.27 e	430 d	320 d	600 b

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

d) Root growth

Every year, the root length of cabbage seedling was significantly lower in non-treated
seedbed (control) compared to bio-fungicide and Provax treated beds. The root length of cabbage seedlings during consecutive three years ranged from 7.07-7.87, 7.17-8.37 and 5.67-8.00 cm under different treatments and 5.46, 4.47 and 4.27 cm in control seedbeds, respectively (Table 4).

The root weights were also higher having 0.58-0.62, 0.52-0.61 and 0.65-0.91 g plant^{-1}, in the 1st, 2nd and 3rd year respectively in the seedbed treated with *T. harzianum* based bio-fungicides and the lowest root weight 0.43, 0.32 and 0.60 g plant^{-1} in 1st, 2nd and 3rd year, respectively in the control seedbeds (Table 4). The root weight was significantly higher compared to seedbeds received neither bio-fungicide nor Provax. Effect of bio-fungicide treatments on root growth was more or less similar (Table 4).

Seed treatment with Provax was also effective to reduce the incidence of foot and root rot disease and to increase shoot and root growth of cabbage seedlings over control in seedbed soil infested with *F. oxysporum*. However, its efficacy was lower compared to all bio-fungicides (Tables 1- 4).

Results of the present experiment revealed that biological control agent *T. harzianum* multiplied on different substrates materials viz. rice bran, wheat bran, grasspea bran used alone or in different combinations mixed with or without MOC were effective against foot and root disease of cabbage caused by *F. oxysporum* under seedbed condition. The bio-fungicides achieved satisfactory increase in seed germination, decrease pre- and post-emergence seedling mortality and also enhance growth of cabbage seedling. Similar findings were reported by other researchers (Bentez *et al.*, 2004; Mausam *et al.*, 2007; Prasad and Anes, 2008; Mukhtar, 2008; John *et al.*, 2010). The well-known antagonistic fungus *Trichoderma* spp. could directly parasitize a diversity of fungi as they were capable of detecting other fungi in the soil and destroyed other plant pathogenic fungi through expression of cell wall degrading enzymes, mostly chitinases, glucanases and proteases (Harman *et al*., 2004). The fungus *T. harzianum* prevailing in the soil was being used in many crops, like lettuce, tomato, onion, cotton, grapes, peas, apples, sweet corn and carrots to control various diseases caused by *Phytophthora, Pythium, Sclerotinia, Botrytis, Rhizoctonia* and *Fusarium* (Benítez *et al*., 2004; Mausam *et al.*, 2007). These findings were in accordance with the observation of the present study where soil was treated with different substrates based *T. harzianum* bio-fungicides that enhanced the growth of cabbage seedling in *F. oxysporum* inoculated seedbed soils though the degree of shoot and root growth varied among the treatments. Harman (2006) and Manju and Mall (2008) also reported positive role of *Trichoderma* species in increasing plant growth and productivity. In present experiment there was significant increase in emergence, shoot and root length

and also shoot and root weights of tomato 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). It was reported that *Trichoderma* isolates possesses the ability to compete for key exudates from seeds that stimulate germination of propagules of plant pathogenic fungi in the soil as they compete with microorganisms for nutrient and space. The three well known mechanisms associated with pathogen control by *Trichoderma* were competition for nutrients, antibiosis, and myco-parasitism (Chet, 1987). It was noticed by Tjamos *et al*. (1992) that *T. harzianum* controls *F. oxysporum* by competing for both rhizosphere colonization and nutrients. The study confirmrd the reports of other researchers regarding the role of *T. harzianum* to enhance seed germination and root and shoot growth of seedlings (Dubey *et al*., 2007) as well as increasing the frequency of healthy plants (Rojo *et al*., 2007). Shoresh *et al.* (2005) stated that *Trichoderma* spp. were 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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