EFFICACY OF SUBSTRATES USED TO FORMULATE *TRICHODERMA HARZIANUM* BASED BIO-FUNGICIDE TO CONTROL FOOT AND ROOT ROT DISEASE (*SCLEROTIUM ROLFSII*) OF CABBAGE

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ABSTRACT

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Efficacy of three organic substrates viz. rice bran, wheat bran, grasspea bran and their combinations with or without supplement with mustard oilcake (MOC) to formulate *Trichoderma harzianum* based bio-fungicides was studied to control foot and root rot disease of cabbage caused by *Sclerotium rolfsii* in the seedbed soil. The seedbed soil was inoculated with the foot and root rot causing fungal pathogen *S. rolfsii* colonized on barley grain before treatment with *T. harzianum* based bio-fungicides. The results from a series of experiments revealed that *T. harzianum* based bio-fungicides effectively reduced pre-emergence and post-emergence mortality of cabbage seedling. Besides, vegetative growth of cabbage seedlings viz. shoot length, shoot weight, root length and root weight were enhanced significantly by the different substrates based *T. harzianum* bio-fungicides in *S. rolfsii* sick seedbed condition. The individual use of different substrates viz. rice bran, wheat bran, grass pea bran and combination of different substrates viz. rice bran + wheat bran, rice bran + mustard oilcake, rice bran + wheat bran + MOC and wheat bran + grass pea bran + MOC were equally suitable for effective formulation of *T. harzianum* bio-fungicides against foot and root rot disease of cabbage in seedbed condition.

Key words: *Trichoderma harzianum*, *Sclerotium rolfsii*, cabbage seedling

INTRODUCTION

Among the vegetables grown and consumed in Bangladesh, cabbage (*Brassica oleracea*) is the most popular one. The crop is attacked by several soil borne diseases mostly caused by fungi. Among the diseases germination failure, seedling mortality, foot and root-rot caused by the most common soil borne fungal pathogen, *S. rolfsii* is the major constraints for seedling production of vegetable crops especially in seedbed (Najar *et al.* 2011). The soil borne pathogen can survive in soil under adverse environmental conditions for long time and it is very difficult to control this soil borne pathogen through conventional method such as application of fungicides or cultural methods (Mondal *et al.* 1996). Resistant variety of cabbage against the soil borne pathogen has not yet been developed and released in Bangladesh. Soil solarization, application of organic soil amendments and chemical fungicides have been used to control the disease but the success is not considerable (Brown and Hendrix 1980, Punja *et al.* 1982). Biological control methods, on the other hand may be considered as economical, sustainable and a potentially powerful alternative methods for the management of soil borne pathogens (Kulkarni *et al.* 2007, Anand and Reddy 2009).

About 90% of the beneficial microbes used as biological agents and effectively control soil borne plant pathogens are different strains of *T. harzianum*, *T. virens*, *T. viride* (Elad *et al.* 1983, Roy *et al.* 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). Moreover, *Trichoderma* have a stimulatory effect on plant growth as a result of modification of soil conditions (Naseby *et al.* 2000). 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 like *S. rolfsii* in 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). Available research reports reveal that *T. harzianum* has been formulated as bio-fungicides on various substrates including 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, 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 *T. harzianum* based bio-fungicides against foot and root rot disease of cabbage seedling in seedbed caused by *S. rolfsii*.

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 to control foot and root rot disease of cabbage seedlings in seedbed caused by *S. rolfsii*. The experiment was conducted in the seedbed of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARl), Gazipur during three consecutive years from 2011-12 to 2013-14 under pothouse conditions. The seedbed soils were inoculated with the fungal isolate *S. rolfsii* 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 bio-fungicides. The substrates were rice bran, wheat bran, grasspea bran, Rice bran + wheat bran (1:1), rice bran + grasspea bran (1:1), rice bran + mustard oilcake (1:1), rice bran + wheat bran + MOC (1:1:1), rice bran + grasspea bran + MOC (1:1:1), rice bran + wheat bran + grasspea bran (1:1:1) and rice bran + wheat bran + grasspea bran+ MOC(1:1:1:1). Six hundred gram of individual or combination of substrate materials were poured into 1000 ml Erlenmeyer flask, sterilized in an autoclave at 121C for 15 min and cooled down to make it ready for inoculation. The sterilized substrates were inoculated individually with 5 mm diameter mycelial disc of five-day old culture of *T. harzianum* grown on PDA and incubated at room temperature (25±2C) for 15 days. After incubation the colonized substrates were removed from the flasks, air dried and finally preserved in refrigerator at 10C. Each of the colonized substrate was considered as a treatment. Seed treatment with Provax-200WP (Carboxin+Thiram) (2.5%) and control were considered additional treatments for comparison.

The *T. harzianum* based Bio-fungicides were incorporated to the previously *S. rolfsii* 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 *S. rolfsii*. The seeds of cabbage variety Atlas were sown in the seedbed @ 200 seeds per treatment. The initial germination of the seeds was 99% as per blotter test. The emergence of seedling was calculated on the basis of initial germination status of the seeds. The experiment was laid out in completely randomized design with four replications. Proper weeding, irrigation and intercultural operations were done for proper growth of cabbage seedlings in the seedbed.

Data were collected on seedling emergence after 15 days of seed sowing. Data on 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 length and weight of shoot and root of cabbage 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 following least significant difference (LSD) test (P=0.05).

RESULTS AND DISCUSSION

Seedling emergence

Seedling emergence of cabbage was 45.67, 62.33 and 67.00% under control during first, second and third year, respectively. It was increased to 53.00-69.00, 74.00-81.33 and 74.00-92.00% in first, second and third year due to treatment of seedbed soils with *T. harzianum* based bio-fungicides multiplied on different organic substrates materials and Provax-200 WP. The differences in seedling emergence under various treatments were not considerable in every year's trials (Table 1).

Pre-emergence and Post-emergence mortality

In control treatment, the pre-mergence seedling mortality was 54.33, 37.67 and 33.00% in first, second and third year, respectively. The pre-emergence seedling mortality was reduced to 13.49-50.91, 30.98-50.44 and 21.21-75.76% in first, second and third year trials, respectively due to treatments of seed with Provax-200 WP or seedbed soils with various *T. harzianum* based bio-fungicides. However, efficacy of all bio-fungicides was more or less similar to improve seedling emergence and to reduce pre-emergence mortality (Table 1).

Application of Provax-200 WP or various *T. harzianum* based bio-fungicides significantly reduced post emergence seedling mortality of cabbage in *S. rolfsii* inoculated seedbed soil under pothouse conditions. In control treatment the post emergence mortality was 14.67, 21.33 and 19.33% in first, second and third year, respectively. Treatment of inoculated seedbed soils with *T. harzianum* based bio-fungicides or seed treatment with Provax-200 WP reduced the disease incidence of cabbage seedling by 50.02-69.63, 66.94-72.16 and 65.90-75.01%, respectively in three consecutive years. The efficacy of different treatments with bio-fungicides and Provax-200 WP was not significantly different in all years (Table 2).

Length and weight of shoot

Under control, shoot length was 13.43, 4.50 and 14.20 cm, and shoot weight was 5.74, 3.38 and 7.67 g plant^{-1} in first, second and third year, respectively. Treatment of *S. rolfsii* inoculated seedbed soils with *T. harzianum* based bio-fungicides multiplied on different substrates or seed treatment with Provax-200 WP increased the shoot length to 17.47-20.13, 5.53-10.57 and 15.80-22.90 cm, and shoot weight to 9.44-9.95, 4.82-8.58 and 10.70-13.63 g plant^{-1} in first, second and third year, respectively. Every year, the increase in shoot growth was significant compared to control and rice bran along with wheat bran, grasspea bran and MOC for formulation of *T. harzianum* based bio-fungicides gave the maximum shoot growth of cabbage than other treatments (Table 3).

Table 1. Effect of soil treatment with *Trichoderma harzianum* based bio-fungicides multiplied on different substrates on emergence and pre-emergence mortality of cabbage seedling grown in *Sclerotium rolfsii* inoculated seedbed

Name of substrates	Emergence of cabbage seedling in seedbed (%)			Pre-emergence mortality of cabbage seedling in seedbed (%)		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran	69.00	77.00	91.00	31.00 (42.94)	23.00 (38.94)	09.00 (72.73)
Wheat bran	53.00	74.00	81.00	47.00 (13.49)	26.00 (30.98)	19.00 (42.42)
Grasspea bran	59.00	81.33	74.00	41.00 (24.54)	18.67 (50.44)	26.00 (21.21)
Rice bran + Wheat bran	58.00	78.67	82.00	42.00 (22.69)	21.33 (43.38)	18.00 (45.45)
Rice bran + Grasspea bran	55.00	79.00	81.00	45.00 (17.17)	21.00 (44.25)	19.00 (42.42)
Rice bran + Mustard oilcake	58.00	77.00	86.00	42.00 (22.69)	23.00 (38.94)	14.00 (57.58)
Rice bran + Wheat bran + MOC	58.67	77.00	92.00	41.33 (23.93)	23.00 (38.94)	08.00 (75.76)
Rice bran + Grasspea bran + MOC	59.67	80.00	82.00	40.33 (25.77)	20.00 (46.91)	18.00 (45.45)
Rice bran + Wheat bran + Grasspea bran	55.00	79.33	80.00	45.00 (17.17)	20.67 (45.13)	20.00 (39.39)
Wheat bran + Grasspea bran+ Rice bran + MOC	63.67	74.00	77.00	26.67 (50.91)	26.00 (30.98)	23.00 (30.30)
Seed treatment with Provax-200 WP	65.33	76.33	76.00	34.67 (36.19)	23.67 (37.16)	24.00 (27.27)
Control	45.67	62.33	67.00	54.33	37.67	33.00

Values within the same column with a common letter(s) do not differ significantly (P=0.05).

Table 2. Effect of soil treatment with *Trichoderma harzianum* based bio-fungicides multiplied on different substrates on post emergence mortality of cabbage seedling grown in *Sclerotium rolfsii* inoculated seedbed

Name of substrates	Post-emergence cabbage seedling mortality in seedbed (%)			Reduction of cabbage seedling mortality over control in seedbed (%)		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran	8.33 bc	12.33 b	8.33 b	55.38	67.83	71.60
Wheat bran	6.67 cd	11.33 b	10.00 b	64.27	70.44	65.90
Grasspea bran	9.33 b	12.33 b	9.00 b	50.02	67.83	69.31
Rice bran + Wheat bran	6.67 cd	12.67 b	7.33 b	64.27	66.94	75.01
Rice bran + Grass pea bran	5.67 d	11.67 b	7.33 b	69.63	69.55	75.01
Rice bran + Mustard oilcake	7.00 cd	10.67 b	8.00 b	62.51	72.16	72.72
Rice bran + Wheat bran + MOC	6.00 d	11.00 b	7.67 b	67.86	71.30	73.85
Rice bran + Grasspea bran + MOC	6.33 cd	11.33 b	8.33 b	66.09	70.44	71.60
Rice bran + Wheat bran + Grasspea bran	7.33 bcd	11.67 b	9.00 b	60.74	69.55	69.31
Wheat bran + Grass pea bran+ Rice bran + MOC	6.67 cd	11.33 b	7.67 b	64.27	70.44	73.85
Seed treatment with Provax-200 WP	7.33 bcd	11.67 b	9.67 b	60.74	69.55	67.03
Control	14.67 a	21.33 a	19.33 a	-	-	-

Values within the same column with a common letter(s) do not differ significantly (P=0.05).

Length and weight of root

The range in root length was 6.37-7.60 cm in first year, 6.73-8.20 cm in second year and 5.00-6.10 cm in third year under treatments with *T. harzianum* based bio-fungicides. The corresponding root length under control was 5.30, 4.00 and 3.67 cm, respectively and 6.17, 4.70 and 4.53 cm, respectively in Provax-200 WP treatment. The increase of root length was significant compared to control in each year. Every year, significant increase in root weight was achieved with all treatments with bio-fungicides compared to control and efficacy of all bio-fungicides to increase root weight was statistically similar. Effectiveness of Provax-200 WP was lower than bio-fungicides (Table 4).

Results of the present investigation clearly show that treatment of *S. rolfsii* infested seedbed soils with *T. harzianum* based bio-fungicides multiplied on rice bran, wheat bran, grasspea bran and mustard oil cake in different combinations and alone are effective to control pre-emergence as well as post-emergence mortality of cabbage seedlings. The treatments increase seed germination and seedling growth effectively. Many other investigators reported similar results. The potentiality of *Trichoderma* species as bio-control agents for enhancing seed germination and seedling growth in addition to suppression of soil-borne plant pathogenic fungi like *Phytophthora, Pythium, Sclerotium, Botrytis, Rhizoctonia* and *Fusarium* of various crops have been recorded by Benitez *et al.* (2004), Celar and Valic (2005), Dubey *et al.* (2007) and Rojo *et al.* (2007). Significant increase in seedling emergence and suppression of pre-emergence mortality of cabbage seedling have also been reported by Mishra and Sinha (2000), Prasad and Anes (2008) and Mukhtar (2008). Podder *et al.* (2004) and Rojo *et al.* (2007) recorded the efficacy of *Trichoderma* spp. as bio-control agents to formulate bio-fungicides after colonization on

organic materials. Findings of the present investigation have also been in agreement with findings of other researchers (Begum *et al.* 1999, Chowdhury *et al.* 2000, Hossain and Samsuzzaman 2003, Yeasmin 2004, Hossain and Naznin 2005, Hermosa *et al.* 2012, Samolski *et al*. 2012). Based on findings of the present investigation, it may be concluded that treatment of seedbed soil with *T. harzianum* based bio-fungicides multiplied on rice bran, wheat bran and grasspea bran alone or in combinations may be effective to control foot and root rot of cabbage seedling caused by *S. rolfsii.*

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Table 3. Effect of soil treatment with *Trichoderma harzianum* based bio-fungicides multiplied on different substrates on shoot growth of cabbage seedling grown in *Sclerotium rolfsii* inoculated seedbed

Name of substrates	Shoot length in consecutive three years (cm)			Shoot weight in consecutive three years (g/plant)		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran	19.57 ab	6.73 bc	19.00 abc	9.44 ab	5.02 bc	11.07 cd
Wheat bran	17.07 c	6.63 bc	18.33 bc	9.55 ab	5.28 bc	11.17 cd
Grasspea bran	18.03 bc	6.83 bc	18.47 bc	9.45 ab	4.82 bc	10.70 d
Rice bran + Wheat bran	18.00 bc	9.20 ab	20.93 ab	9.67 a	7.27 b	12.03 bc
Rice bran + Grasspea bran	17.47 c	8.50 b	19.93 ab	9.73 a	7.02 b	13.63 a
Rice bran + Mustard oilcake	18.33 abc	9.50 ab	21.30 ab	9.75 a	8.13 a	11.93 bc
Rice bran + Wheat bran + MOC	18.23 abc	9.67 ab	20.63 ab	9.36 ab	8.23 a	12.53 b
Rice bran + Grasspea bran + MOC	17.80 bc	10.57 a	22.73 a	9.59 ab	8.22 a	13.47 a
Rice bran + Wheat bran + Grasspea bran	18.47 abc	9.77 ab	22.90 a	9.62 ab	8.58 a	13.63 a
Wheat bran + Grasspea bran+ Rice bran + MOC	20.13 a	10.23 ab	20.67 ab	9.95 a	8.15 a	11.77 bc
Seed treatment with Provax-200 WP	19.07 abc	5.53 c	15.80 cd	7.97 b	4.40 c	9.10 e
Control	13.43 d	4.50 c	14.20 d	5.74 c	3.38 d	7.67 f

Values within the same column with a common letter(s) do not differ significantly (P=0.05).

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Table 4. Effect of soil treatment with *Trichoderma harzianum* based bio-fungicides multiplied on different substrates on root growth of cabbage seedling grown in *Sclerotium rolfsii* inoculated seedbed

Name of substrates	Root length of cabbage seedling in consecutive three years (cm)			Root weight of cabbage seedling in consecutive three years (g/plant)		
	1st year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran	7.07 c	6.87 b	5.00 bc	5.90 bcd	5.50 abc	9.10
Wheat bran	7.46 abc	6.93 b	5.27 abc	6.00 abc	5.40 bc	8.70
Grasspea bran	7.07 c	6.73 b	5.43 abc	5.80 cd	5.20 cd	8.80
Rice bran + Wheat bran	7.47 abc	6.87 b	5.57 ab	5.70 d	5.10 cd	8.80
Rice bran + Grasspea bran	7.16 bc	6.93 b	5.83 ab	5.90 bcd	5.60 abc	9.20
Rice bran + Mustard oilcake	7.13 bc	8.20 a	5.76 ab	5.70 bcd	5.70 abc	8.70
Rice bran + Wheat bran + MOC	6.37 d	8.20 a	5.73 ab	5.90 ab	6.10 a	9.20
Rice bran + Grasspea bran + MOC	7.50 ab	7.83 ab	6.10 a	6.00 ab	5.90 ab	8.80
Rice bran + Wheat bran + Grasspea bran	7.37 abc	7.72 ab	6.00 a	5.70 d	6.00 ab	8.70
Wheat bran + Grass pea bran+ Rice bran + MOC	7.60 a	8.13 a	5.56 ab	5.70 d	5.90 ab	8.80
Seed treatment with Provax-200 WP	6.17 d	4.70 c	4.53 c	6.20 a	4.80 de	6.50
Control	5.30 e	4.00 c	3.67 d	4.60 e	4.30 e	5.60

Values within the same column with a common letter(s) do not differ significantly (P=0.05).

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