MANAGEMENT OF CAULIFLOWER SEEDLING DISEASE (*FUSARIUM OXYSPORUM*) WITH *TRICHODERMA HARZIANUM* BASE COMPOST

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

[1]Senior Scientific Officer, Plant Pathology Division, [2]Chief Scientific Officer, Training and Communication Wing, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur-1701

Email of first author: mifaruk2012@yahoo.com

ABSTRACT

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The efficacy of *Trichoderma harzianum* based bio-fungicides multiplied on individual and mixed substrates of rice bran, wheat bran, grass pea bran, and mustard oilcake (MOC) was assayed against seedling disease (*Fusarium oxysporum*) of cauliflower in seedbed during three consecutive growing seasons from 2011 through 2014 in a nethouse. The results indicated that both individual as well as mixed substrate based *T. harzianum* bio-fungicides were effective against seedling disease caused by the soil borne pathogen *F. oxysporum* in seedbed. Among the treated plots, pre-emergence seedling mortality of cauliflower ranged from 10.00-44.33% and post-emergence mortality from 5.67-13.33% while control plot showed up to 53.00% seedling mortality. However, four combinations viz. (1) rice bran, (2) rice bran + wheat bran, (3) rice bran + wheat bran + mustard oil cake (MOC), and (4) rice bran + wheat bran + grasspea bran +MOC were seemed better substrates for preparing *T. harzianum* bio-fungicides to suppress the soil borne disease (*F. oxysporum*) and thereby enhancing the shoot and root growth of cauliflower seedling in seedbed.

Key words: Cauliflower, seedling disease, *Fusarium oxysporum*, bio-fungicide, *Trichoderma harzianum*.

INTRODUCTION

Vegetables play a vital role in everyday diet of human. It contains fiber, vitamin C, vitamin B, anti-cancer compounds like glucosinolates, sulforaphane, etc., which help to prevent overweight, diabetes and offer protection from prostate, ovarian, and cervical cancers (Kirsh *et al.* 2007, Liu *et al*. 2012).

In Bangladesh, the vegetable is cultivated in about 13 thousand hectares of land with a production of about 166 thousand tons (Anon. 2013). The productivity of cauliflower in the country is low as compared to that of other countries (Anon. 2012). Germination failure and seedling mortality caused by the soil borne pathogens are the major constraints of cauliflower cultivation especially in seed bed. Among the soil borne pathogens *Fusarium oxysporum* is ubiquitous and tedious to manage even with chemical means that eradicates various beneficial organisms in soil due to their toxicity (Hayes and Laws 1991). Biological control of soil borne plant pathogens by the accumulation of antagonistic microorganisms to the soil is a potential nonchemical means for plant disease control. Many researchers described the scenario of biological control of soil-borne plant pathogens using *Trichoderma* spp. (Morsy *et al*. 2009, Sabalpara *et al*. 2009) including *Fusarium* wilt in many crops (Bell *et al*. 1982, Ramezani 2009). The *Trichoderma harzianum* has been reported as a bio-control agent against soil borne pathogens of many crops (Elad *et al*. 1983, Roy *et al*. 1989). *Trichoderma* have been used in lettuce, onion, cotton, grapes, peas, apples, sweet corn, carrots and others to control different pathogens such as *Phytophthora*, *Pythium*, *Sclerotinia*, *Botrytis*, *Rhizoctonia* and *Fusarium* (Benítez *et al*. 2004).

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 cauliflower 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 *Trichoderma harzianum* is grown on various substrates to formulate as bio-fungicides. Commonly used substrates are 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 (Rettinassabababy 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 is yet to be studied in the country.

The present experiment was undertaken to evaluate suitability of locally available substrates for mass multiplication of *T. harzianum* to formulate bio-fungicides effective to control seedling mortality disease of cauliflower caused by *F. oxysporum*.

MATERIALS AND METHODS

Preparation of *Trichoderma harzianum*

A total of 72 isolates of *T. harzianum* collected from different locations of Bangladesh were tested against soil-borne plant pathogens including a highly virulent isolate *F. oxysporum* in preliminary pot experiments under net house conditions. Among those isolate showed highly suppressive effect on the growth of *F. oxysporum*. The antagonistic isolate of *T. harzianum* was designated as TM7 and selected for the preparation of *T. harzianum* based bio-fertilizers.

Four commonly available organic substrates viz. rice bran, wheat bran, grass pea bran and mustard oilcake (MOC) were used alone or in different combinations to prepare *T. harzianum* based bio-fungicides. Ten bio-fungicides were prepared using the selected substrates. Each of the bio-fungicide represented a treatment. Altogether 12 treatments (ten substrates and a seed treating fungicide) including a control were tested in present experiment. These 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 + mustard oilcake (1:1:1), Rice bran + grasspea bran + mustard oilcake (1:1:1), Wheat bran + grasspea bran + mustard oilcake (1:1:1), Rice bran + wheat bran + grass pea bran+ mustard oilcake (1:1:1:1), Seed treatment with Provax-200 WP (Carboxin+Thiram) and Control. According to the treatment combinations, 600 g of individual or mixture of substrates were taken separately in 1000 ml Erlenmeyer flask and autoclaved at 121C for 15 minutes under 1.1kg/cm^2 pressure. The sterilized substrates were allowed to cool and were inoculated separately with single 5 mm mycelia disc cut from 5 days old PDA (potato dextrose agar medium) culture of *T. harzianum* TM7. The inoculated flasks were incubated at 25C for 15 days for the colonization of substrates with the antagonist. Colonized substrates were removed from the flasks and air dried. The colonized and air dried substrates were considered as bio-fungicides.

Evaluation of the bio-fungicides

The bio-fungicides were evaluated for their efficacy to control seedling mortality disease of cauliflower caused by *F. oxysporum* in the seedbed under a net house of the Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur during 2011-2012, 2012-2013 and 2013-2014. Seedbed soils were inoculated with barley grains completely colonized with the isolates of *F. oxysporum.* For inoculation colonized barley grains were thoroughly incorporated with the seedbed soils @ 100 g/m^2 and were allowed to grow in seedbed soil for 7 days maintaining proper soil moisture.

After 7 days of *T. harzianum* bio-fungicide incorporation in the soil, 200 seeds of cauliflower were planted in each seedbed. Recommended quantity of fertilizers and manure were applied and proper weeding, irrigation and intercultural operations were done until the seedlings appeared at the optimum stage for transplanting. Data were recorded on percent emergence and mortality of seedlings, height and weight of shoots, and length and weight of roots of seedlings. The experiment was laid out following completely randomized design with four replications. The collected data on disease severity and seedling growth were analyzed statistically with MSTATC program and the treatment means were compared following least significant difference (LSD) test (P=0.05).

RESULTS AND DISCUSSIONS

Pre- emergence mortality

In control seedbed where soil was inoculated with *F. oxysporum* but *T. harzianum* based bio-fungicides were not applied, pre-emergence mortality of cauliflower seedling was 43.67, 53.30 and 37.00% in first, second and third year, respectively. Amendment of seedbed soils with ten *T. harzianum* based bio-fungicides and a chemical fungicide reduced pre-emergence mortality to 19.67-43.00, 37.00-44.33 and 11.00-28.00% during first, second and third year, respectively. Every year, the reduction in pre-emergence mortality was not significant compared to control. Efficacy of different substrates used to multiply *T. harzianum* for the preparation bio-fungicides was almost similar among themselves in respect of pre-emergence seedling mortality of cauliflower in seedbed inoculated with *F. oxysporum* (Table 1).

Post-emergence mortality of cauliflower seedling

Post-emergence mortality of cauliflower seedling in seedbed inoculated with *F. oxysporum* but not amended with *T. harzianum* based bio-fungicides (control) was 17.33, 35.33 and 25.33% respectively in first, second and third year. Pre-sowing amendment of seedbed soils with *T. harzianum* based bio-fungicides multiplied on various substrates caused 5.67 to 8.33, 10.00 to 13.33 and 7.00 to 9.33% in first, second and third year, respectively. Every year, the rate of reduction obtained with various treatments was not significant compared to control (Table 1).

Findings of the present experiment reveal that the effect of seed treatment with Provax 200 WP on seedling mortality is almost similar to bio-fungicides in reducing seedling mortality in each year. The individual and mixed substrates used to formulate *T. harzianum* based bio-fungicides are equally effective to reduce both pre- as well as post-emergence seedling

mortality of cauliflower. The antagonistic fungus, *Trichoderma* may directly kill soil borne plant pathogens by parasitizing a diversity of fungi as they are capable of detecting other fungi growing towards them. It also destroys fungi through increasing the expression of cell wall degrading enzymes, mostly chitinases, glucanases and proteases (Harman *et al*. 2004). Most *Trichoderma* strains produce volatile and nonvolatile toxic metabolites that obstruct colonization by antagonized microorganisms. Some of these metabolites such as harzianic acid, alamethicins, tricholin, peptaibols, 6-penthyl-alpha-pyrone, massoi-lactone, viridin, gliovirin, glisoprenins and heptelidic acid have been described by Vey *et al*. (2001).

Table 1. Effect of *Trichoderma harzianum* based bio-fungicides multiplied on different substrates on cauliflower seedling mortality in seedbed inoculated with *Fusarium oxysporum*

Substrates used to prepare bio-fungicide	Pre-emergence seedling mortality (%)			Post-emergence seedling mortality (%)		
	1stt year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran (RB)	19.67a	37.00a	14.00a	7.33a	12.33a	9.33a
Wheat bran (WB)	36.33a	39.33a	15.00a	7.67a	10.00a	9.00a
Grasspea bran (GP)	43.00a	40.67a	10.00a	7.33a	11.33a	8.33a
Rice bran + Wheat bran	40.00a	40.67a	20.00a	6.67a	12.33a	7.33a
Rice bran + Grasspea bran	34.00a	44.00a	14.00a	5.67a	13.00a	8.33a
Rice bran + Mustard oilcake	41.00a	39.67a	15.00a	7.67a	11.00a	8.67a
Rice bran+ WB + MOC	41.33a	38.67a	16.00a	6.33a	10.33a	7.00a
Rice bran+ GB +MOC	41.67a	42.33a	28.00a	7.33a	12.00a	9.00a
Wheat bran +GB + MOC	40.00a	42.67a	20.00	8.33a	13.00a	8.33a
WB +GB+ RB + MOC	31.67a	44.33a	11.00	7.00a	13.33a	8.00a
Seed treatment with Provax 200 WP	23.00a	41.67a	20.00a	5.67a	11.33a	9.33a
Control	43.67a	53.00a	37.00a	17.33a	35.33a	25.33a

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

Shoot length and shoot weight

The lowest seedling shoot length of 18.60, 5.17 and 14.80 cm were recorded from control seedbeds in first, second and third year. Every year, all treatments with *T. harzianum* based bio fungicides multiplied on different substrates increased shoot length signi-ficantly over control. Shoot length of cauliflower seedling varied from 24.20 to 28.80 cm in first year, 8.76 to 9.93 cm in the second year and 18.47 to 22.47 cm in third year in seedbed treated with T. *harzianum* based ten bio-fungicides treated seedbeds (Table 2).

The shoot weight of cauliflower seedling was 2.90, 5.17 and 3.48 g in first, second and third year, respectively under control. All bio- fungicides significantly increased the shoot weight compared to control every year. In first year, the highest shoot weight of 4.81 g was obtained with the application of *T. harzianum* bio-fungicide multiplied on only rice bran and the lowest of 4.58 g where the antagonist was multiplied on mixture of rice and wheat brans. In second year, the shoot weight under the substrates rice bran, wheat bran, grasspea bran, rice + wheat bran, rice + grasspea brans was statistically similar but significantly lower compared to the substrates mixed with MOC which also gave statistically similar shoot weight. In third year, all bio-fungicides except grasspea bran alone gave 11.00 to 14.93 g shoot weigh, which was statistically similar. The lowest increase in shoot weight and length was obtained with seed treatment with Provax 200 WP. The lowest increase in shoot growth was noticed in seedbed treated with Provax 200 WP treated beds as compared to bio-fungicide treated seedbeds in every year (Table 2).

The results of the present experiment indicate that rice alone, wheat + grasspea + mustard oilcake, and wheat + rice + grasspea + mustard oilcake based *T. harzianum* bio-fungicides possess prospective potentiality to improve shoot growth of cauliflower seedling in addition to seedling disease reduction caused by *F. oxysporum* under seedbed conditions. Function of *Trichoderma* was reported to play positive role in plant growth promotion and enhanced germination, rapid flowering and increase in height and fresh weigh of plants (Chang *et al*. 1986).

Root growth of cauliflower seedling

In the first year, the root length of cauliflower seedling was 6.67-7.67 cm among the bio-fungicides treated seedbeds whereas the minimum of 4.73 cm root length was recorded from control bed. The minimum root length of 3.60 and 3.47 cm was recorded from the untreated control seedbeds in the second and third

years, respectively. The root length increased to 6.27-8.03 cm in the second year and 4.33-6.47 cm in the third year due to treatment of seedbed soils with bio-fungicides. The root weight was 490, 320 and 540 mg under control during first, second and third year, respectively. Amendment of seedbed with bio-fungicides increased the root weight to 570- 610, 490-570 and 760-970 mg in first, second and third year, respectively. Every year, the increase in both length and weight of roots of cauliflower seedlings due to amendment of *F. oxysporum* inoculated seedbed soils with *T. harzianum* based bio-fungicides was significant as compared to control where inoculated soil was not treated with bio-fungicides. Each year, root growth was better where MOC was added to the substrates compared to other *T. harzianum* based bio-fungicides where the substrates did not receive MOC. Improvement of root growth due to seedbed treatment with Provax 200 WP was not considerable compared to bio-fungicides (Table 3).

Table 2. Effect of different substrates to multiply *T. harzianum* based bio-fungicides on shoot growth of cauliflower seedling in *Fusarium oxysporum* infested seedbed in three consecutive years

Name of substrates	Shoot length (cm)			Shoot weight (g/plant)		
	1stt year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran (RB)	27.00 ab	8.76 b	18.53 bc	4.81 a	6.55 b	11.87 abc
Wheat bran (WB)	24.20 bc	9.50 ab	18.47 bc	4.69 a	7.07 b	11.97 abc
Grasspea bran (GP)	26.07 ab	8.80 b	18.69 bc	4.70 a	6.46 b	11.00 bc
Rice bran + Wheat bran	24.20 bc	9.40 ab	21.67 ab	4.58 a	6.75 b	14.67 a
Rice bran + Grasspea bran	26.33 ab	9.20 ab	20.47 ab	4.61 a	7.03 b	14.50 a
Rice bran + Mustard oilcake	26.33 ab	9.73 ab	21.53 ab	4.72 a	8.13 a	13.53 ab
Rice bran+ WB + MOC	27.07 ab	9.60 ab	20.80 ab	4.63 a	8.15 a	13.77 ab
Rice bran+ GB +MOC	28.00 a	9.93 a	20.60 ab	4.75 a	8.60 a	13.83 ab
Wheat bran +GB + MOC	27.60 a	9.63 ab	22.47 a	4.73 a	8.72 a	14.93 a
WB +GB+ RB + MOC	28.80 a	10.03 a	19.80 abc	4.60 a	8.42 a	13.77 ab
Seed treatment with Provax 200 WP	22.93 c	6.07 c	16.80 cd	3.99 b	4.55 c	9.90 cd
Control	18.60 d	5.17 c	14.80 d	2.90 c	3.48 d	7.30 d

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

The results of three consecutive years reveal that both individual as well as mixed substrates used for multiplication of *T. harzianum* to formulate bio-fungicides are almost equally effective in reducing seedling mortality caused by *F. oxysporum* and also to enhance shoot and root growth of cauliflower seedling in seedbed. However, efficacy of the substrates, rice bran + wheat bran, rice bran +grasspea bran, and wheat bran+ grasspea bran mixed with mustard oilcake was better than other substrates.

The results of the present experiment reveal that rice bran and wheat bran based *T. harzianum* bio-fungicide enhance seed germination, reduce seedling mortality and increase vegetative growth of cauliflower seedling in seedbed. Similar, observation with wheat and rice bran based *T. harzianum* bio-fungicide was reported by Sangeetha *et al.* (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 cauliflower, 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 *Trichoderma* spp. as effective bio-control agents for a number of soil borne plant pathogens. It was also reported that *Trichoderma* improved in root system and biological nitrogen fixation besides controlling pathogens like *Fusarium oxysporum* and *Pythium* sp (John *et al*. 2010, Dubey *et al*. 2007). The mechanisms related to pathogens control by *Trichoderma* were competition for nutrients, antibiosis and myco-parasitism (Chet 1987).

In the present experiment, there was significant increase in emergence, shoot and root growth of cauliflower seedling due to *T. harzianum* bio-fungicides which is in agreement with the findings of different investigators (Mishra and Sinha 2000, Chaur-Tsuen and Chien-Yih 2002, Prasad and Anes 2008, John *et al*. 2010).

Table 3. Effect of different substrates to multiply *T. harzianum* based bio-fungicides on root growth of cauliflower seedling in *Fusarium oxysporum* infested seedbed in three consecutive years

Name of substrates	Root length (cm)			Root weight (mg/plant)		
	1stt year	2nd year	3rd year	1st year	2nd year	3rd year
Rice bran (RB)	6.93 a	6.77 bc	4.80 bcd	590 c	500 ab	760 bc
Wheat bran (WB)	7.00 a	6.80 bc	4.33 cde	610 a	550 ab	790 abc
Grasspea bran (GP)	7.07 a	6.67 c	5.00 bc	580 d	490 ab	760 bc
Rice bran + Wheat bran	7.47 a	6.27 c	6.00 a	600 b	570 a	930 ab
Rice bran + Grasspea bran	7.67 a	6.80 bc	6.13 a	610 a	500 ab	880 ab
Rice bran + Mustard oilcake	6.67 ab	7.43 abc	5.93 a	580 d	490 ab	950 ab
Rice bran+ WB + MOC	7.47 a	6.73 bc	6.07 a	570 e	540 ab	850 ab
Rice bran+ GB +MOC	7.40 a	7.40 abc	6.10 a	610 a	560 ab	890 ab
Wheat bran +GB + MOC	6.80 ab	7.93 ab	6.47 a	590 c	500 ab	970 a
WB +GB+ RB + MOC	7.60 a	8.03 a	5.67 ab	610 a	530 ab	890 ab
Seed treatment with Provax 200 WP	5.77 bc	4.60 d	4.07 de	610 a	410 bc	620 cd
Control	4.73 c	3.60 d	3.47 e	490 f	320 c	540 d

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

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