Page 660 to 667

Volume 3 • Issue 3 • 2019

Research Article

Innovative Techniques in Agriculture

ISSN: 2575-5196

Formulation of *Trichoderma harzianum* Based Bio fungicide against Seedling Disease of Cabbage Using Different Substrates

Iqbal Faruk[1]* and Lutfur Rahman[2]

[1]*Senior Scientific Officer, Plant Pathology Division, BARI*

[2]*Chief Scientific Officer, Training and Communication Wing, BARI*

***Corresponding Author:** Dr. Md. Iqbal Faruk, Senior Scientific Officer, Plant Pathology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur-1701, Bangladesh.

Received: December 11, 2017; **Published:** February 06, 2019

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 seedling disease (Fusarium oxysporum) of cabbage in seedbed. All combinations substrates were equally suitable for mass culturing and preparing of *T. harzianum* bio-fungicides and all the *T. harzianum* based bio-fungicides were effective in increasing seedling emergence and reducing pre-emergence and post-emergence mortality of cabbage seedling under *F. oxysporum* inoculated seed bed 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 seed bed conditions. The individual (rice bran, wheat bran, grass pea bran) and combination of substrates (rice bran + wheat bran, rice bran + grass pea bran, rice bran + mustard oilcake, rice bran + wheat bran + MOC and wheat bran + grass pea bran + MOC) were equally suitable for formulation of 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; Grass pea ban; Mustard oilcake; Trichoderma harzianum; Fusarium oxysporum; Cabbage*

Volume 3 Issue 3 February 2019

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 achieves 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 production of vegetable crops in seed bed (Najar., *et al*. 2011). Control measures like host resistance has not yet become a viable measure. No resistant variety of cabbage has yet been developed and released against this soil borne pathogen *F. oxysporum* of vegetable crops in Bangladesh. Soil solarization and organic

soil amendment have been used to control the disease but 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 powerful 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 have 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 is necessary for successful management of soil borne pathogens in cabbage 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). 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, 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 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 seed bed condition.

Materials and Methods

Efficacy of three organic substrates viz. rice bran, wheat bran, grass pea 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 the 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 bio-fungicides. The treatments 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), T7 = 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 flask. The flask with substrate materials were sterilized in an autoclave at 121°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°C) for 15 days. After incubation the colonized substrates were removed from the flasks, air dried and finally preserved in refrigerator at 10°C. The inoculum of *T. harzianum*, colonized on different substrates, were incorporated to the previously F. oxysporum inoculated seedbed soils @ 100 g/m2 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 of the seeds was 99% as per blotter test result. 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 due to treatment of *F. oxysporum* inoculated seedbed soil with *T harzianum* bio-fungicides. Under control the emergence was 49.33, 64.00 and 66.00% in 1st, 2nd and 3rd year, respectively. The seedling emergence increased range from 58.00-67.00, 80.33-86.67 and 79.00-91.00% due 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 not significantly different (Table 1).

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

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 tested in the present investigation (Table 1). However, efficacy of all bio-fungicides was more or less similar (Table 1).

b) Post-emergence mortality

Post-emergence mortality of cabbage in F. oxysporum inoculated seedbed soil was 21.33, 33.67 and 24.67% under control treatment in 1st, 2nd and 3rd year of study, respectively. Treatment of seedbed soil with different substrates based *T. harzianum* bio-fungicides reduced the disease incidence to 64.04-73.42, 60.41-66.35 and 60.80-72.96% in 1st, 2nd and 3rd year, respectively compared to untreated control. The reduction was significant under every bio-fungicide. Efficacy of all treatments with the bio-fungicides to reduce the disease incidence was not significantly different (Table 2).

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

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, 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 multiplied on rice bran, wheat bran, grasspea bran alone or in different combinations increased the shoot length to 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 was 3.75, 3.97 and 6.57 gplant-1

in first, second and third year, respectively. T. harzianum based bio-fungicidal treatments of seedbed soils increased the parameter to 5.53-5.81, 7.11-8.53 and 8.88-12.40 gplant^{-1}, respectively. Every year, the increase in length and weight of shoot of cabbage seedling due to bio-fungicidal seedbed soil treatment was significant compared to control. Effect of all the treatments on shoot growth was more or less similar (Table 3).

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 3: *Effect of different carrier material based T. harzianum bio-fungicides on the shoot growth of cabbage seedling in F. oxysporum inoculated seedbed soil.*

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. In first, second and third year, the root length of cabbage seedlings ranged 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).

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.

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

In first, second and third year, the ranges of root weight were 0.58-0.62, 0.52-0.61 and 0.65-0.91 gplant^{-1}, respectively in seedbed treated with bio-fungicides multiplied on various substrate materials and 0.43, 0.32 and 0.60 gplant^{-1} in control seedbeds, respectively (Table 4). The root weight was significantly higher compared to seedbeds received no bio-fungicide or Provax. Effect of the treatments on root growth was more or less similar (Table 4).

e) Effect of Provax

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

Results of the present experiment revealed that biological control agent *T harzianum* multiplied on different substrates materials, rice bran, wheat bran, grasspea bran used alone or in different combinations mixed with or without MOC are effective against foot and root disease of cabbage caused by *F oxysporum* under seedbed condition. The bio-fungicides achieved satisfactory increase in seed germination, pre- and post-emergence seedling mortality and also growth of cabbage seedling. Similar findings have been reported by other researchers (Bentez., *et al.* 2004; Mausam., *et al*. 2007; Prasad and Anes, 2008; John., *et al*. 2010). Enhanced seed germination due to Trichoderma species was also reported by Mukhtar (2008). The well-known antagonistic fungus Trichoderma spp. are directly parasitizing a diversity of fungi as they are capable of detecting other fungi in the soil and its 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 and 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). It was reported that *T. harzianum* remarkably proliferated the root system and accelerated biological nitrogen fixation in addition to the reduction of diseases caused by *F. oxysporum* and Pythium spp. in legume crops (John., *et al*. 2010).

This findings are in accordance with the observation of the present study where soil is 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 is significant increase in emergence, shoot and root length and also shoot and root weights of tomato seedling due to *T. harzianum* bio-fungicides which is supported by the findings of many investigators (Prasad and Anes, 2008; Mishra and Sinha, 2000; Chaur-Tsuen and Chien-Yih, 2002. It has been 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 has been noticed by Tjamos., *et al*. (1992) that *T. harzianum controls F. oxysporum* by competing for both rhizosphere colonization and nutrients. The study confirm 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.

References

1. Anand S and Reddy J. "Biocontrol potential of Trichoderma sp. against plant pathogens". *International Journal of Agriculture Sciences* 1.2 (2009): 30-39.
2. Benítez T., *et al*. "Biocontrol mechanisms of Trichoderma strains". *International Microbiology* 7.4 (2004): 249-260.
3. Brown EA and Hendrix FF. "Distribution and control of S. rolfsii on apple". *Plant Disease* 64 (1980): 205-206.
4. Chaur-Tsuen L and Chien-Yih L. "Screening strains of Trichoderma spp. for plant growth enhancement in Taiwan". *Plant Pathology Bulletin* 11 (2002): 215-220.
5. Chet I. "Trichoderma -application, mode of action and potential as a biocontrol agent of soil borne plant pathogenic fungi. In: Chet I (ed.), Innovative approaches to plant disease control". Wiley, New York (1987): 137-160.
6. Das BC., *et al*. "Mass multiplication of Trichoderma species on different media". *Journal of Agricultural Science Society of North-East India* 10.1 (1997): 95-100.
7. Dubey SC., *et al*. "Evaluation of Trichoderma species against Fusarium oxysporum f. sp. ciceris for integrated management of chickpea wilt". Biological Control 40.1 (2007): 118-127.
8. Elad Y., *et al*. "Trichoderma harzianum: A biocontrol agent effective against Sclerotium rolfsii and Rhizoctonia solani". *Phytopathology* 70 (1983): 119-121.
9. Harman GE. "Overview of mechanisms and uses of Trichoderma spp". Phytopathology 96.2 (2006): 190-194.
10. Harman GE., *et al*. "Trichoderma species opportunistic, avirulent plant symbionts". *Nature Reviews Microbiology* 2.1 (2004): 43-56.
11. Harman GE., *et al*. "Development of an effective biological seed treatment system". *Biological Control of Soil-borne Plant Pathogens* (1991): 415-426.
12. Howell CR. "Mechanisms employed by Trichoderma species in the biological control of plant diseases: The history and evolution of current concepts". *Plant Disease* 87.1 (2003): 4-10.
13. John RP., *et al*. "Mycoparasitic Trichoderma viride as a biocontrol agent against Fusarium oxysporum f. sp. adzuki and Pythium arrhenomanes and as a growth promoter of soybean". *Crop Protection* 29.12 (2010): 1452-1459.
14. Kubicek CP., *et al*. "Trichoderma: from genes to biocontrol". *Journal of Plant Pathology* 83.2 (2001): 11-23.
15. Kulkarni M., *et al*. "Novel tensio-active microbial compounds for biocontrol applicatins. In: General Concepts in Integrated Pest and Disease Management (eds. A. Ciancio and K.G. Mukerji)". *Springer* 1 (2007): 295-304.
16. Manju S and Mall TP. "Efficacy of Trichoderma species on Phytopthora dresceleri f.sp. cajani of Pigeon pea". *Annals of Plant Protection Sciences* 16.1 (2008): 162-164.
17. Mausam V., *et al*. "Antagonistic fungi, Trichoderma spp.: Panoply of biological control". *Biochemical Engineering Journal* 37.1 (2007): 1-20.
18. Mishra DS and Sinha AP. "Plant growth promoting activity of some fungal and bacteria agents on rice seed germination and seedling growth". *Tropical Agriculture* 77.3 (2000): 188-191.
19. Mukhtar I. "Influence of Trichoderma species on seed germination in okra. Mycopath 6.1 (2008): 47-50.
20. Najar AG., *et al*. "Evaluation of native biocontrol agents against Fusarium solani f. sp. melongenae causing wilt disease of brinjal in Kashmir". *Journal of Phytology* 3.6 (2011): 31-34.
21. Naseby DC., *et al*. "Effect of biocontrol strains of Trichoderma on plant growth, Pythium ultimum populations, soil microbial communities and soil enzyme activities". *Journal of Applied Microbiology* 88.1 (2000): 161-169.
22. Prasad D and Anes KM. "Effect of metabolites of Trichoderma harzianum and T. viride on plant growth and Meloidogyne incognita on okra". *Annals of Plant Protection Sciences* 16 (2008): 461-465.

23. Punja ZK., *et al*. "Comparative control of S. rolfsii on golf grass in Northern Calfornia with fungicides, inorganic salt and Trichoderma spp". *Plant Disease* 66 (1982): 1125-1128.
24. Rettinassabababy C and Ramadoss N. "Effect of different substrates on the growth and sporulation of Trichoderma viride native isolates". *Agricultural Science Digest* 20.3 (2000): 150-152.
25. Rojo FG., *et al*. "Biological control by Trichoderma species of Fusarium solani causing peanut brown root rot under field conditions". *Crop protection* 26.4 (2007): 549-555.
26. Roy SJ., *et al*. "Non pestcidal management of damping-off of cabbage caused by Rhizoctonia solani kuehn". *Journal of Agricultural Science Society of North-East India* 11.2 (1989): 127-130.
27. Shamarao J., *et al*. "Screening of substrates of mass multiplication of Trichoderma viride". *Karnataka Journal of Agricultural Sciences* 11.1 (1998): 233-236.
28. Shamsuzzaman Islam., *et al*. "Trichoderma culture and germination of sweet gourd seed". *Bangladesh Society of Seed Technology* 7.1 (2003): 91-95.
29. Shoresh M., *et al*. "Involvement of Jasmonic Acid/Ethylene Signaling Pathway in the Systemic Resistance Induced in Cucumber by Trichoderma asperellum T203". *Phytopathology* 95.1 (2005): 76-84.
30. Tjamos EC., *et al*. "Biological control of plant diseases. Progress and challenges for the future". *Plenum Press,* New York (1992):