PREVALENCE OF WHITE MOLD DISEASE IN DIFFERENT CROPS IN BANGLADESH AND ITS MANAGEMENT USING BIO-CONTROL AGENTS (*IN VITRO*)

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Abstract

A total of 115 fields in ten districts *viz*. Pabna, Bogra, Joypurhat, Rangpur, Jhenidah, Jessore, Moulvibazar, Sylhet, Hobiganj and Gazipur (latitude between 22° 56′ to 24° 52′ N and longitude 89° 01′ to 92° 11′ E) were surveyed to examine the white mold disease incidence in some vegetables, oil seed crops and flowers during 2016-17 in Bangladesh. The highest disease incidence (90%) was recorded in country bean at Bogra district and in silvia flower at Gazipur district. A total of 28 isolates of Sclerotinia sclerotiorum were collected from disease plant parts and soil. The colony colors of the isolates were white to ash in country bean, grey to blackish in silvia& marigold, and white in mustard, sunflower & eggplant on potato dextrose agar media. After five days of incubation, the subsurface mycelial cells swelled, pigmented and formed the dark black sclerotia. The size of sclerotia was 1.5- 6 x 2-17mm. Sclerotia germinated and brown round to globose shape apothecia was developed at the end of long stripe on wet sand media. Asci were hyaline and cylindrical in shape, while the ascospores were elliptical. The size of ascus and ascospore were 68-155 x 4.5-6.01 µm and 4.7-6.94 x 4-6.2 µm, respectively. Isolates collected from different host were found pathogenic. Fungal bio-control agents *viz. Trichodermharzianum* and*T. viride*, and bacterial bio-control agents *viz. Bacillus subtilis* BVC38, *Pseudomonas* sp. (N) and*Pseudomonas* sp. (S) were effectively control white mold pathogen *in vitro*. Interaction between pathogen and *Trichoderma* isolates was noted as Grade 3 type, while it was varied from 2-5 types in case of bacterial isolates. The radial growth of *Sclerotinia sclerotiorum* reduced remarkable in presence of *Trichoderma viride*, *T. harzinum*, *Pseudomonas* sp. (S), *Pseudomonas* sp. (N) and *Bacillus subtilis* BVC38. Mycelium growth inhibition was 95.33, 94.96, 93.67, 93.42 and 92.25%in*Trichodermharzianum*, *T. viride*, *Pseudomonas* sp. (S), *Pseudomonas* sp. (N), and *Bacillus subtilis* BVC38, respectively.

***Key words*:** White mold disease, Crop, Management, Bio-Control, Bangladesh

Introduction

White mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a major disease of country bean (*Lablab purpureus* L.) and main yield limiting factor, especially in the northwest part of Bangladesh. The disease varies in incidence and severity from year to year and region to region because of its sensitivity to weather conditions. The pathogen is a necrotrophic and non-host-specific fungal pathogen that infects more than 400 species of plants and 278 genera of plants approximately (Boland and Hall 1994, Purdy 1979). In Bangladesh, *S. sclerotiorum* was first recorded on mustard in 2008 (Hossain *et al.* 2008), then on chilli, auber-gine, and cabbage (Dey*et al.* 2008). Recently the disease is attacking almost all the vegetables, flowers (Mary-gourd, gerbera) and fruit crops (jackfruit). Pathogen causes serious yield losses by premature plant death through lower stem infection and/or infection of beans (Natti 1971).

A survey carried out in 1982 showed that the average incidence of white mold in bean fields was 29.2% (Howard and Huang 1983)**.** The disease is incited by infection from airborne ascospores released from the apothecia produced on sclerotia in the soil and secondary spread of the disease is due to direct contact between diseased and healthy tissue (Abawi and Grogan 1979). The disease is generally serious only if apothecia of the fungus occur in the field during or shortly before flowering. Apothecia occur only if soil moisture is sufficiently high for about two weeks (Boland and Hall 1987). Infected plants develop pale white or tan-colored lesions on leaves, petioles, stems, and/or pods, sometimes with white mycelial mats or black sclerotia on the infected tissue. Severe infection often results in the premature death of plants. Kim and Cho (1998) also reported some mycological and pathological characteristics of *S. sclerotiorum* and *S. minor* causing sclerotinia rot of vegetable crops. In Bangladesh *Sclerotinia* infection on stem and pod in country bean was first report by Prova*et al.* (2014). However the information on detail study and characteristics of white mold pathogen of different crops bean is not available.

Chemical fungicide usage has negative ecological impact due to toxic residues (Rocha and Oliveira, 1998). Prominent practice of management of plant diseases by fungi is the use of biocontrol. Several species of *Trichoderma* have been reported as potential biocontrol agents of phytopathogenic fungi on soil (Chet 1987), including *S. sclerotiorum* (Trutmann and Keane 1990, Pereira *et al.* 1996, Gracia-Garza *et al.* 1997, IllipróntiJúnior and Machado 1998) Although a number of fungicides and bio-control agent (*Conothyriumminitans)* areavailable to combat the disease in US, Europe and some other countries (Bolland 1997), the information to control the disease in Bangladesh is not available. This life cycle pattern, and its wide host range, makes *Sclerotinia*stem rot in country bean difficult to manage through cultural or chemical practices. Therefore the research was undertaken (i) to survey the disease occurrence of different crops, and the detail study of white mold pathogen *S. sclerotiorum* and (ii) to screen fungal and bacterial bio-control agents for providing resources for further studies on bio-control in field conditions.

Materials and Methods

Disease survey, samples collection, pathogen isolation and preservation

A survey was conducted to identify the occurrence of white mold disease in different crop of Bangladesh during winter sessions 2016-17. Ten districts *viz*. Pabna, Bogra, Joypurhat, Rangpur, Jhenidah, Jessore, Moulvibazar, Sylhet, Hobiganj and Gazipur were selected in different agro-ecological zones of Bangladesh. One upazila from each of the districts of were selected for the survey. A total of 115 fields were randomly surveyed at early vegetative to end of harvesting stage of different winter crops *viz*. Mustard, Eggplant, Country bean, Sunflower, Marigold, Silvia and Weed during 2016 to 2017. During survey, each of five sampling points of the fields was selected and *Sclerotinia* rot disease incidence was examined by following CIAT (1987). From each of the disease infected fields, plant parts, twig, raceme, pod andsoil were collected for further study.

Sclerotia and disease infected plant parts were sterilized using 70% ethanol for a minute and rinsed in sterile distilled water and dried with blotted paper towels. Large plant parts were aseptically cut into 1 cm pieces and sclerotia were in halves before plating. After cutting sclerotia and infected plant parts were plated onto potato dextrose agar (PDA) media containing 0.1% lactic acid (added after cooling) and incubated at 25 ± 1°C in the dark for 5 days. Collected soil samples were place onto autoclaved carrot discs and incubated at 25 ± 1°C for 5 days. Isolates were purified by hyphal tip isolation and cultured into new PDA plates. A pure culture of white mycelial growth developed after three days of inoculation and preserved it at 4 °C for further study.

Morphological characteristics of *S. sclerotinia*

Each of the isolates was cultured on PDA in 9-cm-diameter petri dishes at 25°C in the dark for 12 days for the production of sclerotia. Sclerotia produced on the medium were examined for morphological characteristics, and were preserved in refrigerator for apothecia formation. Then the sclerotia were placed in a water jar for conditioning. After two weeks sclerotia were placed on sterile wet sand media in the Petri plate and incubated at 15°C for 2- 5 weeks in alternating cycles of light and darkness to induce the formation of apothecia. Apothecia also collected from sclerotinia infected country bean fields from Bogra district. Apothecia were cut into thin slices and stained with cotton blue to observe the morphology of asci and ascospores under microscope.

Pathogenicity test of *Sclerotinia sclerotiorum* isolates

Isolates were examined their pathogenicity by inoculating them on Btbrinjal and country bean leaves separately following Detached-leaf method. A 17 cm petri-plate was sterilized and host leaves were placed on paper towel. Purified isolates were cultured on potato dextrose agar media for three days and mycelia dices were cut from edges of petri-plate and placed on leaves. Mycelial growth and symptom development was observed periodically.

Evaluation of antagonistic potential of some fungi and bacteria against *S. sclerotiorum*.

The experiment was conducted in the laboratory with pathogen and biocontrol agents. Isolates of pathogen and fungal antagonistic *viz,Trichodermharzianum*, *T. viride* and *T. virens* were grown on potato dextrose agar media, and the isolates of bacterial antagonistic viz,*Bacillus subtilis* B20, *B. subtilis* B18, *B. subtilis* BVC38, *B. amyloliqufaciens* Egg25, *Pseudomonas* sp. (N) and *Pseudomonas* sp. (N) were grown on nutrient agar media at 25±1°C. Pathogen and all biocontrol agents were incubated for 3 to 5 days in order to obtain juvenile colonies (Riker and Riker 1936). Each of 5 mm mycelial discs of pathogen and fungal antagonistic (Trichoderma isolates) were transferred on PDA media with 30 mm apart from each other following Dual Culture Technique (Skidmore and Dickinson 1976). Similarly pathogen and bacterial

antagonistic (Bacillus and Pseudomonas isolates) were transferred on PDA media where the 5 mm mycelial disc of pathogen was placed 30 mm apart from bacterial colony. Patri-plates of fungal and bacterial isolates were arranged separates following completely randomized design with four replications. Experiment with fungal antagonistic was incubated at 25±1°C temperatures and experiment with bacterial antagonistic was incubated at 27±1°C. The colony growth of the pathogen was measured in presence (treatments) and in absence (control) of antagonistic bio-control agents. The radial growth was measured from central loci of pathogen when it touches at edge of petri-plate in control treatment. At the same time intermingled and inhibition zone development was observed between pathogen and antagonistic bio-control agents in treatment plate and the radial growth pathogen towards bio-control agents were also recorded. For both groups of antagonistic agents the assessment was made after 7 days of incubation.

Assessments of antagonistic interaction between the test pathogen and bio-control agents were done in terms of grades which were determined by the model of Skidmore and Dickinson (1976). The grades are as follows:

Grade 1: Mutually intermingling growth were both pathogen & bio-control agent grew into one another without any microscopic sign of interaction.

Grade 2: Intermingling growth where the pathogen being observed into the opposed bio-control agent either above or below its colony.

Grade 3: Intermingling growth where the pathogen under observation has ceased the growth and is being overgrown by bio-control colony.

Grade 4: Slight inhibition with a narrow demarcation line (1-2 mm).

Grade 5: Mutual inhibition at a distance more than 2 mm.

The percent inhibition of radial growth was calculated by the formula of Behzad*et al.* (2008) and Rini and Sulochana (2007).

Percentage inhibition of growth=$\frac{R1-R2}{R2} \times 100$

Where R1 is radial growth of pathogen in absence of antagonistic agents (control) and R2 is the radial growth of pathogen in absence of antagonistic agents.

Antagonist was also rated for inhibitory effects using a scale (slightly modified) by Sangoyomi (2004) as:≤ 10% inhibition (not effective),>10-20% inhibition (slightly effective), >20-50% inhibition (moderately effective), >50-<80% inhibition (effective) and >80% and above inhibition (highly effective)

Results and Discussion

Disease survey, samples collection, pathogen isolation and preservation

The survey areas were located into different Agro-ecological zones of Bangladesh which covered latitude between 22° 56′ to 24° 52′ N and longitude 89° 01′ to 92° 11′ E (Table 1.). White mold disease of country

bean crop was found in several locations in Bangladesh. Under field condition *Sclerotinia* rot was identified by observing the symptoms of the disease (Fig.1). The symptom appeared in initial stage as water- soaked lesions on the stem and pod. When severely infected white mycelial mat covered the whole raceme and was not produced any pod. In mature stage of country bean, whole pod was rotten and covered with white mycelium. Occurrence of the disease was observed in 78 out of 115 surveyed fields of ten districts during winter. Highest disease incidence was recorded (90%) in country bean and Silvia fields of Shahjahanpur in Bograand Gazipurdistrict,respectively whichfollowed by marigold at 80% in Gazipur district. Lowest disease incidence (8%) was observed in country bean fields of Pabna district. A small number of fields (20%) were disease infected in Monirampur of Jessoreand Shailkupa, Jhenidah district. Disease incidence was recorded 10- 15% in both locations. In Gazipur district, no white mold pathogen infection was observed in country bean during the growing season.Disease incidence was recorded by 60, 55, 60- 75, 55 and 45-50% in Pirganj, Rangpur, Joypurhatsadar, Joypurhat, Bahubal, Hobiganj, Borolekha, Moulvibazar and Sylhet district, respectively in country bean fields. After five days of incubation *Sclerotinia sclerotiorum* isolates were existence in maximum disease infected field soil samples (Fig.2).

Morphological characteristics and pathogenic potentiality of *Sclerotiniasclerotinia*

A total 28 isolates of *Sclerotinia sclerotiorum* was collected from diseased plant parts and disease infected soil samples (Table 1 and Fig.1). Myceliogenic and sporogenic characteristic was examined. The fungus produced aerial mycelium, which was hyaline, branched, well developed and appeared cottony and septate. The subsurface mycelial cells swelled, became pigmented and formed the dark bulbous rind cells which darkened with age (Colotelo 1974) called sclerotia. After five days of incubation, fungus started to produce large black color sclerotia. The sclerotia were found round to irregular in shape and measured 1.5- 6 mm in width and 2-17 mm in length. Sclerotia formed on host surface were usually loaf shaped or globose while those formed in the pith of the stem were elongated, which might be due to the space available for growth. Sclerotia started germination and giving rise to several columnar structures (stripes). At the tip of long stripe later developed apothecium in wet sand media. Apothecia were brown in colour and round or globose in shape. The length and diameter of apothecia was measured which was 4- 21 and 2- 8 mm, respectively. The asci were hyaline and cylindrical in shape, which measured 68 -155 x 4.5- 6.01 µm in size. Each asci contained eight ascospores which were found to be released in clouds. Ascospores were elliptical and ranged from 4.7-6.94 x 4- 6.2 µm in size (Table 2). Isolates collected from different host were found pathogenic and produced disease symptom after 3 days of inoculation. The morphological characters of the fungus were in accordance with the taxonomic keys given by Willetts and Wong (1980). Thus confirming the identity of the fungus was *Sclerotinia sclerotiorum* (Lib.) de Bary.

Table 1. Soil sample collection from different areas and culturing on carrot media for its mycelia growth

Sl No.	Location	Geographical location	Host	Pathogen presence in soil	Pathogen presence in crops	Colony color	Melanin formation	Disease Incidence (%)	Disease scoring (0-5 scale)	Pathogenic potentiality
1	Shajahanpur, Bogra	24^0 45′ 35″ N, 89^0 24′45″ E	Country Bean	+	+	White to ash	+	90	5	Pathogenic
2	Shajahanpur, Bogra	24^0 44′ 55″ N, 89^0 25′ 13″ E	Country Bean	+	+	White to ash	+	80	4	Pathogenic
3	Shajahanpur, Bogra	24^0 45′ 40″ N , 89^0 25′ 03″ E	Country Bean	+	+	White to ash	+	75	4	Pathogenic
4	Shajahanpur, Bogra	24^0 45′ 30″ N , 89^0 23′ 47″ E	Country Bean, Weed	+	+	White to ash	+	80 20	4 2	Pathogenic
5	Shajahanpur, Bogra	24^0 45′ 03″ N, 89^0 23′ 56″ E	Country Bean	-	+	White to ash	+	70	4	Pathogenic
6	Shajahanpur, Bogra	24^0 75′ 44″ N, 89^0 40′ 01″ E	Country Bean	+	+	White to ash	+	80	3	Pathogenic
7	Pirganj, Rangpur	25^0 26′ 34″ N, 89^0 18′ 90″ E	Country Bean	+	+	White to ash	+	60	3	Pathogenic
8	Joypurhatsadar, Joypurhat	25^0 05′ 36″ N, 89^0 01′ 71″ E	Country Bean	+	+	White to ash	+	55	2	Pathogenic
9	Bahubal, Hobiganj	24^0 23′ 70″ N, 91^0 34′ 71″ E	Country Bean	+	+	White to ash	+	75	4	Pathogenic
10	Bahubal, Hobiganj	24^0 24′ 39″ N, 91^0 33′ 45″ E	Country Bean	-	+	White to ash	+	60	4	Pathogenic
11	Borolekha, Moulvibazar	24^0 42′ 55″ N, 92^0 11′ 41″ E	Country Bean	+	+	White to ash	+	55	4	Pathogenic
12	SylhetSadar, Sylhet	24^0 52′ 38″ N, 91^0 48′ 27″ E	Country Bean	+	+	White to ash	+	50	3	Pathogenic
13	Golapganj, Sylhet	24^0 51′ 03″ N, 92^0 00′ 27″ E	Country Bean	-	+	White to ash	+	45	4	Pathogenic

Table 1. Soil sample collection from different areas and culturing on carrot media for its mycelia growth

Sl No.	Location	Geographical location	Host	Pathogen presence in soil	Pathogen presence in crops	Colony color	Melanin formation	Disease Incidence	Disease scoring (0-5 scale)	Pathogenic potentiality
14	Monirampur, Jessore	22^0 56′ 37″ N, 89^0 08′ 25″ E	Country Bean	-	+	White to ash	+	10	2	Pathogenic
15	Monirampur, Jessore	22^0 56′ 23″ N , 89^0 10′ 21″ E	Country Bean	+	+	White to ash	+	15	2	Pathogenic
16	Monirampur, Jessore	22^0 94′ 39″ N , 89^0 11′ 14″ E	Country Bean	+	+	White to ash	+	15	2	Pathogenic
17	Jessoresador, Jessore	23^0 11′ 21″ N , 89^0 09′ 13″ E	Country Bean	-	+	White to ash	+	12	2	Pathogenic
18	Jessoresador, Jessore	23^0 10′ 52″ N , 89^0 11′ 17″ E	Country Bean	+	+	White to ash	+	10	2	Pathogenic
19	Jessoresador, Jessore	23^0 13′ 50″ N , 89^0 10′ 03″ E	Country Bean	+	+	White to ash	+	15	2	Pathogenic
20	Shailkupa, Jhenidah	23^0 41′ 16″ N , 89^0 10′ 03″ E	Country Bean	+	+	White to ash	+	10	3	Pathogenic
21	Ishurdi, Pubna	24^0 06′ 34″ N , 89^0 04′ 25″ E	Mustard,	+	+	White	-	35	3	Pathogenic
			Brinjal					15	2	
22	Ishurdi, Pubna	24^0 07′ 18″ N, 89^0 04′ 49″ E	Country Bean	+	-	White	-	8	3	Pathogenic
23	Ishurdi, Pabna	24^0 07′ 08″ N, 89^0 04′ 38″ E	Sunflower	+	+	White	-	10	2	Pathogenic
24	Ishurdi, Pabna	24^0 07′ 59″ N , 89^0 08′ 03″ E	Country Bean	+	-	White to ash	+	15	2	Pathogenic
25	Ishurdi, Pabna	24^0 10′ 01″ N , 89^0 08′ 59″ E	Country Bean	+	-	White to ash	+	10	3	Pathogenic
26	BARI, Joydebpur, Gazipur	230 59′ 37″ N, 900 24′ 40″ E	Marigold,	-	+	Gray to blackish	+	80	4	Pathogenic
			Silvia					90	4	

” –“ = Absent, ” +“ = Present

Table 2. Morphological characteristics of *Sclerotinia sclerotiorum* collected from different host

Characteristics	Color	Number per unit	Shape	Size	
				Width/ diameter	Length
Sclerotia	Black	20-45 (Petri plate)	Round or irregular	1.5-6 mm	2- 17 mm
Apothecia	Brown	1-3 (Sclerotia)	Round or globate	2-8 mm	4-21mm
Asci	Hyaline	Numerous (Apothecia)	Cylindrical	4.5-6.01µm	68-155 µm
Ascospore	Hyaline	8 (Ascus)	Elliptical	4.0-6.2 µm	4.7-6.94µm

Effectiveness of bacterial bio-control agents against *Sclerotinia sclerotiorum*

Results revealed that the interaction between *Bacillus amyloliquefaciens* Egg 25 and pathogen on PDA media was noted as Grade 2 type (intermingling growth where the pathogen being observed into the opposed bio-control agents bio-control agents agent either above or below its colony) (Fig 3). While the interaction between *Bacillus subtilis* B18 and *S. sclerotiorum* on PDA media was Grade 3 type (intermingling growth where the pathogen under observation has ceased the growth and is being overgrown by bio-control colony). Similar trend observed between *Bacillus subtilis* B_{20} and *S. sclerotiorum.* Grade 4 type [slight inhibition with a narrow demarcation line (1-2 mm)] was noted between pathogen and two bacterial isolates [*Bacillussubtilis* BVC38 and *Pseudomonas* sp. (N)] and grade 5 type was noted betweenpathogen and *Pseudomonas* sp. (S). *Pseudomonas* sp. (N) and *Pseudomonas* sp. (S) both were showed high antagonistic reaction than *Bacillus subtilis* (Table 4).

The minimum radial growth of *S. sclerotiorum* towards antagonism was recorded in *Pseudomonas*sp (S) followed by *Bacillus subtilis* BVC38, *Pseudomonas* sp. (N), *Bacillus subtilis* B20, *Bacillus subtilis* B18 and *Bacillus amyloliquefaciens* Egg25. The radial growth of *S. sclerotiorum* was the maximum in control (in absence of antagonistic). Radial growth of mycelium of *S. sclerotiorum* was inhibited by 93.67, 93.42, 92.25, 67.50, 61.65 and 50.83% in *Pseudomonas* sp. (S), *Bacillus subtilis* BVC38, *Pseudomonas* sp., *Bacillussubtilis* B20, *Bacillus subtilis* B18 and *Bacillus amyloliquefaciens* Egg 25 treated dual culture petri-plate, respectively (Table 3).

Effectiveness of fungal bio-control agents against *Sclerotinia sclerotiorum*

Interaction between *Trichoderma* isolates and pathogen was noted as Grade 3 type (Fig 4&5). All the species of *Trichoderma* reduced the radial growth of *S. sclerotiorum*. Radial growth of *S. sclerotiorum* towards *Trichoderma viride*and *T. harzianum* was the minimum on PDA media. However, the radial growth of *S. sclerotiorum* was comparatively higher in presence of *Trichoderma virens*, although it was significantly lower than control treatment ($P<0.05$). Radial growth of mycelium of *S. sclerotiorum* was inhibited by 95.33, 94.96 and 92.00% in *Trichoderma viride*, *T. harzianum*and *T. virens*, respectively (Table 3.).

Table 3. Inhibition of radial growth of *Sclerotinia sclerotiorum* against antagonistic bacteria and fungi in dual culture method

Name of antagonist	Radial growth of pathogen mycelium (cm)	Radial growth Inhibition (%)
Bacterial bio-control agents		
Bacillus amyloliquefaciens Egg 25	2.95 b	50.83 (7.18)* c
Bacillus subtilis PB18	2.30 c	61.67 (7.91) b
Bacillus subtilis B20	1.95 c	67.50 (8.28) b
Bacillussubtilis BVC38	0.40 d	93.42 (9.72) a
Pseudomonas sp. (N)	0.40 d	93.42 (9.72) a
Pseudomonas sp. (S)	0.38 d	93.67 (9.68) a
Control	6.00 a	00.00 (1.00) d
CV(%)	10.39	3.90
Fungal bio-control agents		
Trichoderma viride	0.28 c	95.33 (9.81)* a
Trichoderma harzianum	0.30 c	94.96 (9.80) a
Trichoderma virens	0.48 b	92.00 (9.64) b
Control	6.00 a	00.00 (1.00) c
CV(%)	2.07	0.42

* Data within parenthesis are square root transformed value

Table 4. Antagonistic level and grade of different bacterial and fungal antagonist against *Sclerotinia sclerotiorum*

Name of antagonist	Antagonistic level	Grade of interaction
Bacterial bio-control agents		
Bacillus amyloliquefaciens Egg 25	moderately effective	2
Bacillus subtilis PB18	effective	3
Bacillus subtilis B20	effective	3
Bacillussubtilis BVC38	highly effective	4
Pseudomonas sp. (N)	highly effective	4
Pseudomonas sp. (S)	highly effective	5
Fungal bio-control agents		
Trichoderma viride	highly effective	3
Trichoderma harzianum	highly effective	3
Trichoderma virens	highly effective	3

It is imperative to mention that *Trichoderma* species are renowned to produce a range of antibiotics suchas trichodernin, trichodermol and harzianolide (Dennis and Webster 1971a, Howell 2003, KucukandKivanc 2004) in addition to some cell wall degrading enzymes such as glucanase and chitinase which break down thepolysaccharides and chitins, therebyobliterating the cell wall integrity of pathogen (Elad*et al.* 1983, Elad 2000). The possible mechanism of antagonism employed by *Trichoderma* species include nutrient and niche competition, antibiosis by producing volatile components and non-volatile antibiotics (Harman and Hadar 1983, Behzad*et al.* 2008) that are

inhibitory against a range of soil borne fungi, over and above parasitism. *Trichoderma* spp. was capable of influencing thegrowth of most of the tested pathogens in dual culture and as a result may well be used as a broadspectrum bio-controlling agent for management various fungal diseases (Dennis and Webster 1971b, Lone *et al.* 2012).

Pseudomonas chlororaphis (PA-23), *Bacillus amyloliquefaciens* (BS6), *Pseudomonas* sp. (DF41) and *B. amyloliquefaciens* (E16) were found effective bio-control agents against *S. sclerotiorum*, the causal agent of stem rot of canola in vitro and in greenhouse efficacy test (Fernando *et al.* 2007) which was supporting the results.

Fig 1. Sclerotia separated from crop residues of country bean (Bogra area)

Fig. 2. Mycelial growth of *Sclerotinia* pathogen on carrot slice

Fig. 3. Radial growth of *S. sclerotinia* towards bacterial antagonistic on PDA media after 5 days of inoculation

Fig. 4. Radial growth of *Sclerotinia sclerotiorum* towards fungal antagonistic on PDA media after 7 days of inoculation

Fig. 5. Radial growth of *Sclerotinia sclerotiorum* towards fungal antagonistic on PDA media after 10 days of inoculation

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