Project ID CRG-599

Competitive Research Grant

Sub-Project Completion Report on

Collection and molecular characterization of resurged phytopathogen *Sclerotinia sclerotiorum* causing white mold disease of different crops and its integrated management

Project Duration

May 2017 to September 2018

Plant Pathology Division
Bangladesh Agricultural research Institute
Joydebpur, Gazipur

Submitted to

Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

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Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
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Abbreviation and Acronyms

RARS	:	Regional Agricultural Research Station
SS	:	*Sclerotinia sclerotiorum*
PDA	:	Potato Dextrose Agar
BARI	:	Bangladesh Agricultural Research Institute
ITS	:	Internal transcribed spacer
WP	:	Wettable Powder
AEZ	:	Agro-Ecological Zone
HQ	:	Head Quarter
DNA	:	Deoxyribonucleic acid
PCR	:	Polymerase Chain Reaction
EDTA	:	Ethylene Diamine Tetra-acetic Acid
TBE	:	Tris/Borate/EDTA
BLAST	:	Basic Local Alignment Search Tool
NCBI	:	National Center for Biotechnology Information
RCB	:	Randomized Complete Block
BBS	:	Bangladesh Bureau of Statistics
CD	:	Cow Dung

Executive Summary

Sclerotinia sclerotiorum (Lib.) de Bary is a soil-borne and cosmopolitan plant pathogenic fungus that attack more than 400 plant species at all stages of growth and harvested products in the world. In Bangladesh, *S. sclerotiorum* becoming as resurged phytopathogen for various crop including vegetables, fruits and field crops especially in cooler region of the country. First *S. sclerotiorum* was recorded on mustard in 2008 and then chilli, brinjal and cabbage, country bean in 2011, marigold in 2011, jackfruit in 2012 and lentil in 2014. Considering as a new phytopathogen for the country and recent outbreak of the disease, it is necessary to know the details about the fungus especially molecular characterization and genetic diversity along with pathogenic potentiality. So, the present study entitled "Collection and molecular characterization of resurged phytopathogen *Sclerotinia sclerotiorum* causing white mold disease of different crops and its integrated management" have been designed for country-wide survey to the disease along with morphological and molecular characterization for determination of genetic diversity of *S. sclerotiorum*, and finally, development of integrated management package against white mold disease of some selected crops.

During survey, a total of one hundred and eighty isolates of *S. sclerotiorum* were collected and isolated from different districts namely Rangpur, Dinajpur, Lalmonirhat, Jessore, Sirajgonj, Jamalpur, Mymensingh, Tangail, Pabna, Natore, Chittagang, and Bogra during 2016-17 and 2017-18 cropping seasons and maintained as pure culture or sclerotia stock at Plant Pathology Laboratory, BARI, Gazipur. Morphological characterization of the isolates was done by analyzing vegetative growth of the fungus. The average mycial growth range from 2.65 cm to 8.10 cm at 72 hrs after inoculation was recorded and the isolates SS67, SS44, SS178, SS136 and SS171 showed lower average mycial growth rate range from 2.65-3.40 cm where the isolates SS9, SS25, SS31, SS8, SS10, SS51 and SS36 showed higher average mycial growth range from 8.00-8.67 cm. Sclerotia were formed within ten days on PDA plate and size shape and number of sclerotia of different isolates varied considerable and a range of 9 to 64 sclerotia per Petri dish. The isolates SS136, SS43, SS44, SS14, SS118 and SS25 showed lower number of sclerotia per plate with 9.00 to 15.00 where isolates SS1, SS2, SS6, SS8, SS28, SS31, SS35, SS54, SS97, SS100, SS125, SS151, SS155 and SS175 showed higher number of Sclerotia/plate range from 50.00 to 64.00.

Molecular characterization of the collected 14 isolates (out of 180 isolates) was determined by the partial sequencing of ITS region followed by phylogenetic analysis. Molecular characterization of the 14 isolates by ITS sequencing indicated all the tested isolates were identified as publicly available *S. sclerotiorum*. Phylogenetic analysis of the isolates based on ITS sequences revealed the isolates belonged to a similar group of publicly available *S. sclerotiorum* and dissimilar with the group of *S. minor*, *S. trifolium* and disticnly differ from *S. nivalis* group

For development an integrated management package of white mold disease, three field experiments were carried out at the experimental field of three different locations viz. Plant Pathology Division, BARI, Gazipur, RARS, Burirhat, Rangpur and RARS, Ishurdi, Pabna with three different crops viz. bush bean, mustard and garden pea, respectively during Rabi season of 2017-18. Results from all the experiments revealed that integration of saw dust burning + soil amendments with *Trichoderma* based bio-fungicide + bacillus based bio-control agents + spray fungicide Rovral 50 WP is the best treatment which reduced 97.49%, 77.72% and 72.26% disease incidence and 84.61%, 81.14% and 71.01% disease severity of white mold of mustard, bush bean and garden pea, respectively. This treatment also increasing plant growth parameters as well as increasing 52.16%, 27.74% and 39.97% yield of mustard, bush bean and garden pea, respectively. Application of only fungicide Rovral 50 WP is also the most effective treatment which reduced 90.55%, 72.59% and 71.13% disease incidence and 73.07%, 73.58% and 70.00% disease severity also gave 42.63%, 20.41% and 35.01% higher yield of mustard, bush bean and garden pea, respectively compared to control. Soil amendments with *Trichoderma* based bio-fungicide, bacillus based bio-control agents and saw dust burning either singly or in integration is also reduced white mold disease incidences and severity and increasing yield of mustard, bush bean and garden pea but less effective. For recommendation it is necessary to trial this experiments for next year and further validation trials in different AEZ in the country and to calculate BCR of this technology.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. **Title of the CRG sub-project: Collection and molecular characterization of resurged phytopathogen *Sclerotinia sclerotiorum* causing white mold disease of different crops and its integrated management**
2. **Implementing organization:** Plant Pathology Division, Bangladesh Agricultural Research Institute
3. **Name and full address with phone, cell and E-mail of PI/Co-PI (s):**

3.1 Principal Investigator (Full address with phone and e-mail):

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4. **Sub-project budget (Tk):**

4.1 Total: 15,38,785.00
(Fifteen Lakh Thirty Eight Thousand Seven Hundred Eighty Five Taka Only)
4.2 Revised (if any): N/A

5. **Duration of the sub-project**: 17 months

5.1 Start date (based on LoA signed): May 2017
5.2 End date : 30 September 2018

6. **Justification of undertaking the sub-project**:

Sclerotinia sclerotiorum (Lib.) de Bary is a soil-borne, and cosmopolitan plant pathogenic fungus that attack more than 400 plant species in the world at all stages of growth and harvested products (Bolton *et al.* 2006).

The fungus *S. sclerotiorum* can cause a disease called white mold if conditions are conducive. *S. sclerotiorum* can also be known as cottony rot, watery soft rot, stem rot, drop, crown rot and blossom blight. A key characteristic of this pathogen is its ability to produce black resting structures known as sclerotia and white fuzzy growths of mycelium on the plant it infects. These sclerotia serve as infections propagules in soil and survival structures that may remain viable for several years in field even in adverse environmental situation. Moreover, depending on various environmental factors sclerotia can infect plants after producing airborne ascospores those can disperse over wider areas (Adams and Ayers, 1979).

In Bangladesh, *S. sclerotiorum* becoming as resurged phytopathogen for various crop including vegetables, fruits and field crops especially in cooler region of the country. First *S. sclerotiorum* was recorded on mustard in 2008 (Hossain *et al.* 2008) then on chilli, brinjal, cabbage and country bean in 2011, marigold in 2011, jackfruit in 2012 and lentil in 2014. High infection was observed on mustard, brinjal, cauliflower, garden pea and bush bean. Number of new host range of the fungus is increasing day by day (Dey *et al.* 2008, Rahman *et al.* 2015).

Considering the aggressiveness of the new phytopathogen in the country and outbreak of the disease caused by the pathogen *S. sclerotiorum*, it is necessary to undertaken research to know the details about the fungus especially molecular characterization and genetic diversity along with pathogenic potentiality. As a new disease it is important to control the disease before causing havoc by the pathogen. So, as a newly emerging threat for crop production in the country by the phytopathogen *S. sclerotiorum*, the present study have been designed for country-wide survey and assessment of crop loss due to the disease along with molecular characterization and determination of genetic diversity of *S. sclerotiorum*, and finally, development of integrated management package to control white mold disease of mustard, brinjal, cauliflower, garden pea and bush bean.

7. **Sub-project goal:** Increasing productivity of different crops through study of nature of the pathogen and development of integrated management technology of white mold disease

8. Sub-project objective (s):

a. Characterization and study of the nature of *S. sclerotiorum* collected from different hosts through cultural, morpho-physiology and pathogenicity test.

b. Molecular characterization and determination of genetic variability of the collected isolates of *S. sclerotiorum*.

c. Development of eco-friendly package for controlling the white mold disease through integrated approaches for safe production of selected crops.

9. **Implementing location (s):** BARI HQ, Gazipur; RARS, Burirhat, Rangpur and RARS, Ishurdi

10. Methodology in brief:

Brief description of different activities follows

10.1. Collection and characterization of isolates of *S. sclerotiorum*

A minimum scale survey for white mold disease was conducted in the country during 2016-17 and 2017-18 cropping seasons. Symptoms of white mold disease on different crops were studied. Diseased plant parts and sclerotia were collected and isolates were purified by single hyphal tip method using potato dextrose agar (PDA) followed by cultural and morphological characterization. Then sclerotia from pure cultures of isolates were preserved in small tube (screw cap eppendorf tube) for further studies. Morphological characterization of the isolates was done by analyzing growth of the fungus. Morphological and cultural characteristics (e.g., texture, aerial mycelium and color of mycelia; number and size of sclerotia) of fresh isolate cultures were analyzed. Mycelial characteristics were examined at 3 days after incubation, while sclerotial characteristics were recorded 15 d after inoculation.

A total of 180 isolates of *S. sclerotiorum* were isolated from infected samples collected from districts of Rangpur, Dinajpur, Lalmonirhat, Jessore, Sirajgonj, Jamalpur, Mymensingh, Tangail, Pabna, Natore, Chittagang, and Bogra during 2016-17 and 2017-18 cropping seasons. Symptoms from different isolates collected from different hosts were studied (Figure 1). After collection of samples, fungi were grown on PDA and preparation of pure cultures was done following hyphal tip technique.

10.2. Molecular characterization of *S. sclerotiorum*

Molecular characterization of the collected some selected isolates was determined by the partial sequencing of internal transcribed spacer (ITS) region followed by phylogenetic analysis. Total DNA was extracted from the isolates separately using Wizard Genomic DNA extraction/purification kit. A part of ITS was amplified in a 25 µl reaction with primers ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCGTAACAAGG) using commercial Mastermix kit (Promega)

following manufacturer's instructions following programs for polymerase chain reaction (PCR): initial denaturation at 94°C for 2 min followed by 30 cycles of denaturation of 98°C for 10 s, annealing at 62°C for 30s, polymerization at 68°C for 1 min, and final elongation at 68°C for 7 min. Five microliters of each amplification mixture was verified by agarose (1% w/v) gel electrophoresis in 0.5X Tris-borate-EDTA (TBE) buffer. The partial sequences were generated using following ITS4 and ITS5 primers from a company (1st BASE Company, Malaysia).

10.3. Phylogenetic analysis

The PCR amplified products were purified using commercial kit, and then incubated at 37 °C for 60 min followed by 80 °C for 20 min. The nucleotide sequences were determined using dideoxy sequencing techniques at 1st BASE Company, Malaysia (taken as commercial service). Partial sequences were generated using the ITS4 and ITS5 primers. The ITS sequences were combined using the Bioedit software, checked manually, corrected, and then analyzed using the Basic Local Alignment Search Tool (BLAST) available on the National Center for Biotechnology Information (NCBI) website (http://blast.ncbi.nlm.nih.gov/) to identify the isolates. Phylogenetic analyses were conducted using the MEGA 6 program, and a neighbor-joining tree was constructed using the Kimura two-parameter model. The phylogenetic tree was generated using the most identical fungal sequences available in the GenBank database. Confidence values were assessed from 1,000 bootstrap replicates of the original data.

10.4. Integrated management of white mold disease

For development an integrated management package of white mold disease, three field experiments were carried out in the experimental fields of Plant Pathology Division, BARI, Gazipur, RARS, Burirhat, Rangpur and RARS, Ishurdi, Pabna with three crops viz. bush bean, mustard and garden pea, respectively during Rabi season of 2017-18. The experiments were laid out in a RCBD with three replications. Eight treatments viz. T_1= Saw dust burning of soil, T_2= Stable bleaching powder (20 kg/ha) in soil, T_3= *Trichoderma* based bio-fungicide in soil, T_4= *Bacillus* based biocontrol agent (BCA) in soil, T_5= Fungicidal spray three times with Rovral 50 WP, T_6= T_1 + T_2 + T_3 + T_4, T_7= T_1 + T_2 + T_3 +

T_4 + T_5 and T_8= Control were tested in these experiments. Recommended dose fertilizers were applied to raise a good crop. Intercultural operations were done in order to maintain normal hygienic condition of the crop. Weeding was done two times during the period of crop growth. The experiment was monitored regularly to observe the onset of white mold disease. In case of bush bean and mustard data were recorded on disease incidence (%), disease severity (%), plant height, plant weight and yield. Whereas the disease incidence (%), disease severity (%), plant height, number of pods per plant, weight of pods per plant and pod yield were recorded for garden pea. The Data were analyzed statistically by using the MSTATC program. The treatment effects were compared following least significant difference (LSD) test (P=0.05).

11. Results and discussion:

11.1. Collection and characterization of isolates of *S. sclerotiorum*

All the isolates exhibited white to off-white mycelium growth with loose to dense velvety texture, and low, medium or high aerial growth (Figure 2). The mycelial growth rate differed considerably among the isolates (Table-1). The average mycial growth range from 2.65 to 8.10 cm at 72 hrs after inoculation. The isolates SS67, SS44, SS178, SS136 and SS171 showed lower average mycial growth of 2.65, 2.85, 2.85, 2.95 and 3.40 cm where as the isolates SS9, SS25, SS31, SS8, SS10, SS51 and SS36 showed higher average mycial growth of 8.00, 8.00, 8.00, 8.10, 8.15 8.30 and 8.67 cm at 72 hrs after inoculation. Sclerotia were formed within ten days of inoculation on PDA plate. After initiation of sclerotia, they formed within three to four days. Sclerotia were round to irregular in shape. Size shape and number of sclerotia of different isolates varied considerable. A range of 9 to 64 sclerotia were found to produce per Petri dish (Figure 2) and (Table-1). The isolates SS136, SS43, SS44, SS14, SS118 and SS25 showed lower average number of sclerotia formation per plate with 9.00, 10.00, 10.00, 11.50, 14.00 and 15.00 sclerotia per plate where as isolates SS1, SS2, SS6, SS8, SS28, SS31, SS35, SS54, SS97, SS100, SS125, SS151, SS155 and SS175 showed higher average number of Sclerotia ranging from 50.00 to 64.00 Sclerotia/plate.

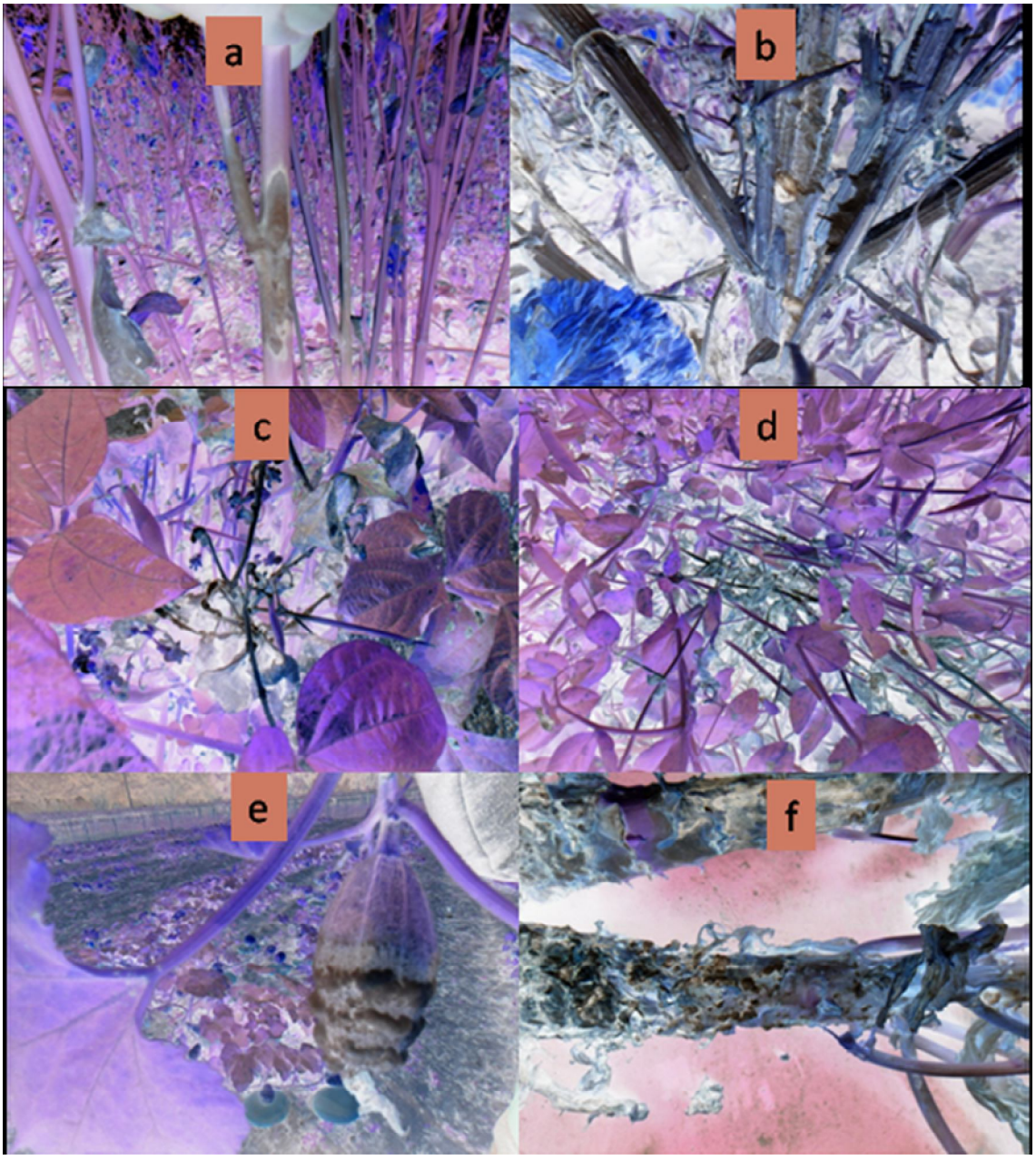

Figure 1. Common symptoms of white mold disease (a) mustard (b) marigold (c) bush bean (d) garden pea (e) ornamental gourd and (f) broccoli caused by *Sclerotinia sclerotiorum;* observing sclerotia inside marigold plant and infected plants of bush bean and broccoli

Figure 2. Mycelial growth of *Sclerotinia sclerotiorum* on PDA plate and formation of sclerotia by *Sclerotinia sclerotiorum*

Table 1. Morphological characteristics of isolates of *Sclerotinia sclerotiorum*

Accessions name	Av. radial growth at 72hrs(cm)	Average number of sclerotia/plate	Accessions name	Av. radial growth at 72hrs(cm)	Average number of sclerotia/plate
SS1	7.50	53.50	SS31	8.00	52.00
SS2	7.80	52.00	SS32	5.50	19.00
SS3	5.60	36.50	SS33	5.30	40.00
SS4	4.60	22.00	SS34	4.10	42.50
SS5	5.30	24.00	SS35	6.27	56.00
SS6	6.30	50.00	SS36	6.67	35.00
SS7	5.70	31.00	SS37	7.25	18.50
SS8	8.10	56.50	SS38	7.15	42.00
SS9	8.00	47.50	SS39	4.16	19.00
SS10	8.30	47.50	SS40	4.35	27.00
SS11	6.40	22.00	SS41	4.72	15.00
SS12	4.80	24.50	SS42	3.70	32.00
SS13	3.90	20.00	SS43	3.65	10.00
SS14	3.80	11.50	SS44	2.85	10.00
SS15	4.60	23.50	SS45	7.05	25.00
SS16	4.30	20.50	SS46	6.85	35.00
SS17	3.80	20.50	SS47	6.63	28.00
SS18	4.30	21.50	SS48	6.02	43.00
SS19	4.10	18.50	SS49	6.85	35.50
SS20	4.10	28.00	SS50	4.85	17.00
SS21	6.10	31.00	SS51	8.15	34.50
SS22	6.30	43.50	SS52	6.50	23.50
SS23	4.65	20.00	SS53	5.62	30.00
SS24	5.35	28.00	SS54	6.82	53.50
SS25	8.00	25.50	SS55	5.45	40.00
SS26	4.60	15.00	SS56	6.25	30.00
SS27	7.60	42.00	SS57	5.15	19.50
SS28	3.85	56.50	SS58	6.15	36.00
SS29	5.72	41.00	SS59	5.25	30.00
SS30	5.00	35.00	SS60	5.40	37.50

Table -1 continued

Accessions name	Av. radial growth at 72hrs(cm)	Average number of sclerotia/plate	Accessions name	Av. radial growth at 72hrs(cm)	Average number of sclerotia/plate
SS61	4.15	25.00	SS91	5.37	35.00
SS62	6.77	23.00	SS92	5.15	31.00
SS63	5.15	26.00	SS93	5.22	25.00
SS64	6.15	27.00	SS94	5.10	21.00
SS65	7.15	41.00	SS95	6.15	32.00
SS66	5.15	35.00	SS96	6.20	30.00
SS67	2.65	18.50	SS97	6.22	58.00
SS68	6.15	32.50	SS98	4.53	34.50
SS69	5.12	28.50	SS99	7.17	48.00
SS70	7.50	60.00	SS100	7.47	64.00
SS71	7.15	43.00	SS101	7.60	35.00
SS72	6.22	29.50	SS102	7.75	59.00
SS73	6.17	20.50	SS103	7.35	40.00
SS74	5.20	22.50	SS104	5.72	33.00
SS75	4.25	21.00	SS105	7.30	41.00
SS76	7.10	42.50	SS106	6.97	57.50
SS77	6.10	18.00	SS107	5.67	58.50
SS78	6.05	25.00	SS108	6.85	38.50
SS79	5.18	30.50	SS109	5.60	49.50
SS80	6.20	45.00	SS110	7.47	42.50
SS81	6.12	51.00	SS111	6.90	35.00
SS82	4.17	24.00	SS112	4.95	43.50
SS83	6.25	47.50	SS113	5.40	41.50
SS84	4.82	40.00	SS114	6.27	44.00
SS85	3.95	24.00	SS115	6.10	56.00
SS86	6.18	33.00	SS116	4.07	51.00
SS87	5.42	27.00	SS117	6.65	21.50
SS88	5.40	46.00	SS118	4.15	14.00
SS89	6.25	28.00	SS119	5.90	22.50
SS90	6.36	32.50	SS120	6.75	20.00

Table -1 continued

Accessions name	Av. radial growth at 72hrs(cm)	Average number of sclerotia/plate	Accessions name	Av. radial growth at 72hrs(cm)	Average number of sclerotia/plate
SS121	7.35	18.00	SS151	7.10	52.00
SS122	6.40	29.50	SS152	7.15	19.00
SS123	4.80	20.00	SS153	4.93	40.00
SS124	7.15	35.50	SS154	5.70	42.50
SS125	6.35	55.00	SS155	6.85	56.00
SS126	4.85	28.50	SS156	6.62	35.00
SS127	5.25	29.50	SS157	7.50	18.50
SS128	6.20	20.00	SS158	6.85	42.00
SS129	4.85	29.00	SS159	5.72	19.00
SS130	6.25	42.50	SS160	5.80	27.00
SS131	5.15	24.00	SS161	4.85	15.00
SS132	3.95	18.00	SS162	5.15	32.00
SS133	5.15	26.00	SS163	4.10	10.00
SS134	5.18	17.50	SS164	4.18	10.00
SS135	4.85	30.00	SS165	6.26	25.00
SS136	2.95	9.00	SS166	4.80	35.00
SS137	6.16	34.00	SS167	4.95	28.00
SS138	4.85	16.00	SS168	6.12	43.00
SS139	5.45	28.50	SS169	5.00	35.50
SS140	4.16	16.00	SS170	4.67	17.00
SS141	5.15	30.00	SS171	3.80	34.50
SS142	6.85	38.00	SS172	6.35	23.50
SS143	7.02	45.00	SS173	3.40	30.00
SS144	7.72	17.00	SS174	6.42	53.50
SS145	4.95	17.50	SS175	4.85	40.00
SS146	4.85	22.50	SS176	6.65	30.00
SS147	6.15	35.00	SS177	6.90	19.50
SS148	4.95	29.00	SS178	2.85	36.00
SS149	6.25	30.00	SS179	7.35	30.00
SS150	6.16	16.50	SS180	7.80	37.50

11.2. Molecular characterization and genetic diversity of *S. sclerotiorum*

A total of 14 samples (samples were collected from four different district viz. collected from different districts namely Rangpur, Dinazpur, Lalmonirhat and Bogra of four different host plants viz. mustard. marigold, country bean and lettuce) were selected for DAN extraction. After DNA extraction, the DNA was used for PCR using ITS primers ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCGTAACAAGG) for amplification ITS regions. During PCR all the DNA samples of the isolates were amplified properly and those were verified by agarose gel electrophoresis (Figure 3). Amplified DNA was sent for sequencing for molecular characterization.

Molecular characterization of the 14 isolates by ITS sequencing indicated all the tested isolates were identified as *Sclerotinia sclerotiorum*. The ITS sequences of the 14 isolates were identical to many publicly available *S. sclerotiorum* sequences (eg. KY750530). Phylogenetic analysis of the isolates based on ITS sequences revealed the isolates belonged to a similar group of publicly available *S. sclerotiorum* and dissimilar with the group of *S. minor*, *S. trifolium* and disticnly differ from *S. nivalis* group (Fig. 4).

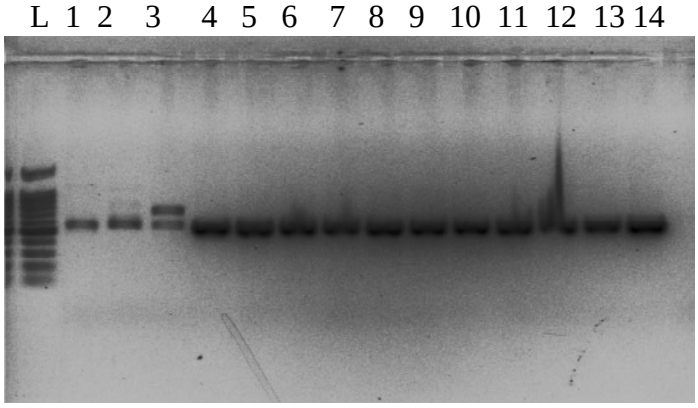

Figure 3: Gel documentation for fourteen isolates of *Sclerotinia sclerotiorum* after PCR amplification

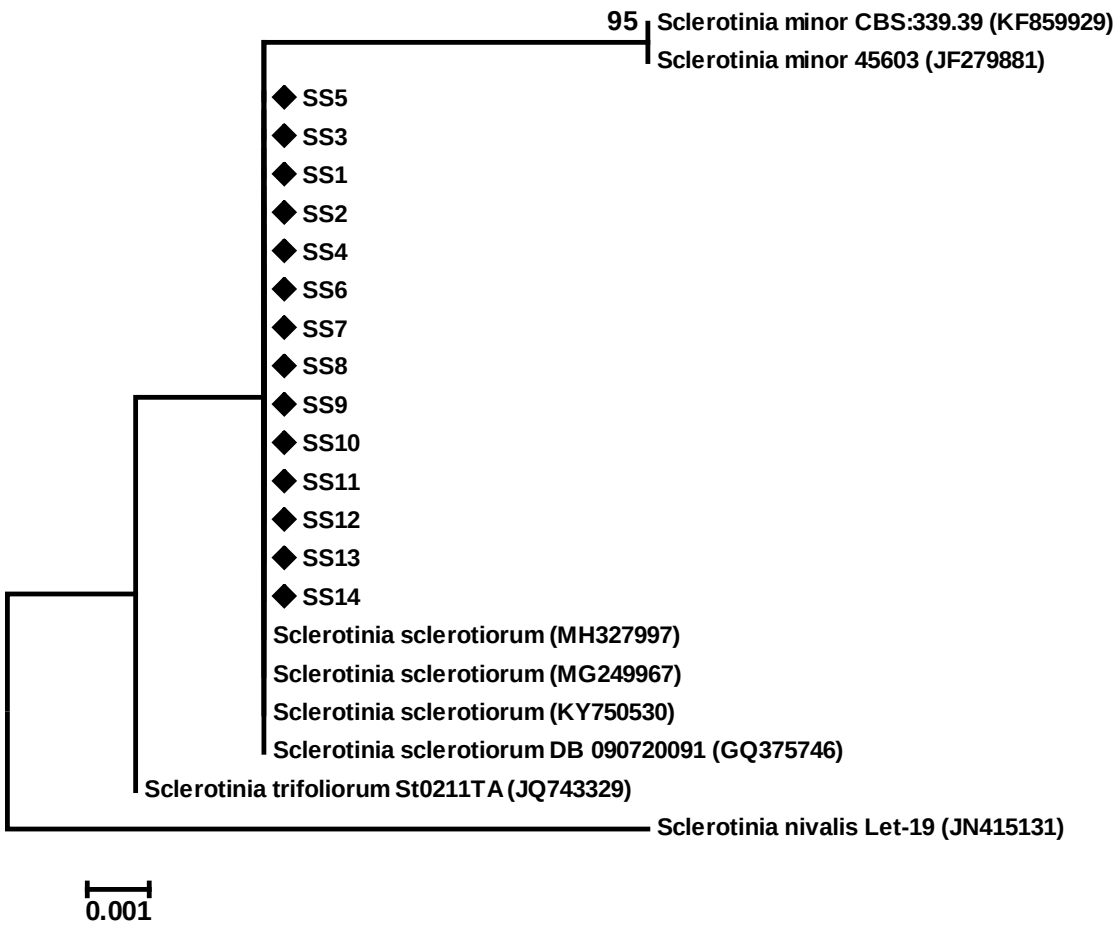

Figure 4. Phylogenetic tree based on internal transcribed spacer sequences revealing the phylogenetic relationships among the *Sclerotinia sclerotiorum* isolates and related species. The 14 isolates from this study are indicated in the tree with a black diamond.

11.3. Integrated management of white mold disease

Mustard: Effects of different treatments against white mold disease caused by soil-borne pathogen *S. sclerotiorum* of mustard are presented in tables 2 and 3 and Figure 5. The highest disease incidence 62.56% and disease severity of 69.33% was recorded from control and the lowest disease incidence 1.57% and disease severity 10.67% was recorded from T7 treatment. Application of only fungicide Rovral 50 WP (T5) also gave lower disease incidence 5.91% and disease severity 18.67%. Integration of different treatments viz. saw dust burning + soil amendments with Trichoderma + bacillus based bio-control agents + application fungicide Rovral 50 WP gave the highest reduction of disease incidence and severity by 97.49% and 84.61%, respectively followed by application only fungicide Rovral 50 WP where the reduction

of disease incidence and severity was 90.55% and 73.07%, respectively. Saw dust burning and soil amendments with *Trichoderma* and bacillus based bio-control agents also gave significant reduction of disease incidence range from 37.88 to 56.63% and disease severity range from 36.54 to 46.16% but less effective than T5 and T7 treatments. Integration of different treatments viz. saw dust burning + soil amendments with *Trichoderma* + bacillus based bio-control agents + application fungicide Rovral 50 WP gave the highest plant height and plant weight followed by Rovral 50 WP, *Trichoderma* based bio-fungicide, bacillus based bio-control agents and saw dust burning treatments. The lowest plant height and plant weight was recorded from control.

Integration of different treatments viz. saw dust burning + soil amendments with *Trichoderma* + bacillus based bio-control agents + application fungicide Rovral 50 WP gave the highest yield of mustard at 1566 $kgha^{-1}$ followed by Rovral 50 WP, *Trichoderma* based bio-fungicide, integration saw dust burning + stable bleaching powder + *Trichoderma* based bio-fungicide + Bacillus based bio-control agent, only Bacillus based bio-control agent and saw dust burning where the yield was 1306, 1178, 1156, 1086 and 1073 $kgha^{-1}$, respectively. The lower yield of mustard 749.2 and 825.4 $kgha^{-1}$ were recorded from the saw dust burning and control treatments, respectively. Yield was higher 52.16% over control due to integration of different treatments viz. saw dust burning + soil amendments with *Trichoderma* + bacillus based bio-control agents + Rovral 50 WP where as it was 42.63% due to application of Rovral 50 WP. Soil amendment with only *Trichoderma* based bio-fungicide gave higher yield 36.40% where it was 35.19%, 31.01% and 30.18 % by integration saw dust burning + stable bleaching powder + *Trichoderma* based bio-fungicide + Bacillus based bio-control agent, only Bacillus based bio-control agent and saw dust burning, respectively compared to control.

Table 2. Effect of different treatments and its integration against white mold disease of mustard

Treatments	Disease incidence (%)	Reduction of disease incidence then control (%)	Disease severity (PDI)	Reduction of disease severity then control (%)
T_1= Sawdust burning	25.47 e (30.31)	59.29	38.67 c (38.44)	44.22
T_2= Stable bleaching powder	38.87 b (38.55)	37.88	44.00 b (41.54)	36.54
T_3= *Trichoderma* based bio-fungicide	30.95 cd (33.79)	50.53	37.33 c (37.66)	46.16
T_4= Bacillus based bio-control agent	34.10 bc (35.73)	45.49	40.67 bc (39.62)	41.34
T_5= Rovral 50 WP	5.91 f (13.92)	90.55	18.67 d (25.47)	73.07
T_6= T_1 +T_2 +T_3 +T_4	27.13 de (31.37)	56.63	38.67 c (38.44)	44.22
T_7= T_1 +T_2 +T_3 +T_4+ T_5	1.57 g (7.12)	97.49	10.67 e (19.04)	84.61
T_8= Control	62.56 a (52.28)	-	69.33 a (56.38)	-

Values in a column having same letter did not differ significantly (P=0.05) by LSD; values within the parenthesis is the Arcsin Transformed value.

Table 3. Effect of different treatments and its integration on the growth and yield of mustard

Treatments	Plant Height (cm)	Plant weight ($gplant^{-1}$)	Yield ($kgha^{-1}$)	Yield higher then control (%)
T_1= Sawdust burning	74.00 de	7.20 d	1073.0 c	30.18
T_2= Stable bleaching powder	72.00 e	7.00 d	825.4 d	9.23
T_3= *Trichoderma* based bio-fungicide	78.93 bc	7.73 c	1178.0 c	36.40
T_4= Bacillus based bio-control agent	75.00 cde	7.27 cd	1086.0 c	31.01
T_5= Rovral 50 WP	82.07 ab	8.27 b	1306.0 b	42.63
T_6= T_1 +T_2 +T_3 +T_4	77.27 cd	7.40 cd	1156.0 c	35.19
T_7= T_1 +T_2 +T_3 +T_4+ T_5	86.20 a	10.73 a	1566.0 a	52.16
T_8= Control	64.27 f	5.60 c	749.2 d	-

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

Table 3. Cost, return and marginal analysis of different treatments

Treatment	Variable cost (Tk. ha^{-1})	Yield ($kgha^{-1}$)	Gross return (Tk. ha^{-1})	Gross margin (Tk. ha^{-1})	Marginal benefit (Tk. ha^{-1})	MBCR
T_1	21000	1073.0 c	85840	64840	4544	1:0.22
T_2	5400	825.4 d	66032	60632	696	1:0.13
T_3	17500	1178.0 c	94240	76740	16804	1:0.96
T_4	19500	1086.0 c	86880	67380	7444	1:0.38
T_5	30000	1306.0 b	104480	74480	14544	1:0.49
T_6	35400	1156.0 c	92480	57080	-2856	1:-0.08
T_7	50400	1566.0 a	125280	74880	24480	1:0.49
T_8	-	749.2 d	59936	59936	-	-

T_1= Sawdust burning, T_2= Stable bleaching powder, T_3= *Trichoderma* based bio-fungicide, T_4= Bacillus based bio-control agent, T_5= Rovral 50 WP, T_6= T_1 +T_2 +T_3 +T_4, T_7= T_1 +T_2 +T_3 +T_4+ T_5 and T_8= Control

Table 3. Cost, return and marginal analysis of different treatments

Treatment	Variable cost (Tk. ha^{-1})	Yield (tha^{-1})	Gross return (Tk. ha^{-1})	Gross margin (Tk. ha^{-1})	Marginal benefit (Tk. ha^{-1})	MBCR
T_1	21000	6.60 ab	198000	177000	33300	1:1.58
T_2	5400	5.72 bc	171600	166200	22500	1:4.16
T_3	17500	7.14 a	214200	196700	53000	1:3.02
T_4	19500	6.44 ab	193200	173700	30000	1:1.54
T_5	30000	7.37 a	221100	191100	47400	1:1.58
T_6	35400	6.42 ab	192600	157200	13500	1:0.38
T_7	50400	7.60 a	228000	177600	33900	1:0.67
T_8	-	4.79 c	143700	143700	-	-

T_1= Sawdust burning, T_2= Stable bleaching powder, T_3= *Trichoderma* based bio-fungicide, T_4= Bacillus based bio-control agent, T_5= Rovral 50 WP, T_6= T_1 +T_2 +T_3 +T_4, T_7= T_1 +T_2 +T_3 +T_4+ T_5 and T_8= Control

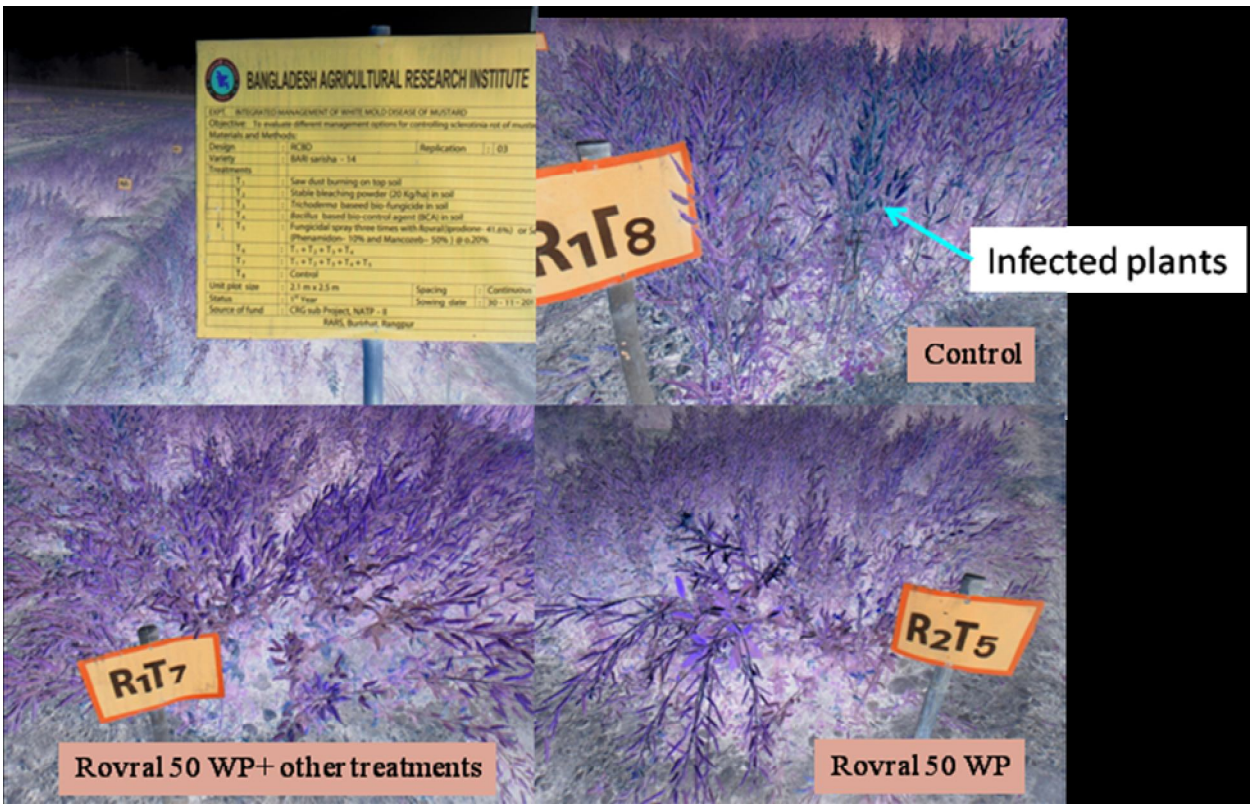

Figure 5 Experimental field view of integrated management of white mold disease of mustard at RARS, Burirhat, Rangpur and white mold disease symptom in control plot and disease free plot in the field

Bush bean: Results showed that all the treatments significantly reduced white mold disease incidence, disease severity and increasing plant growth as well as yield of bush bean (Table 4 and 5 and Figure 6). The highest disease incidence 11.13% and disease severity 70.67% was recorded in control and the lowest disease incidence 2.48% and disease severity 33.33% was observed in the treatment when integration of different treatments viz. saw dust burning + soil amendments with *Trichoderma* based bio-fungicide + bacillus based bio-control agents + Rovral 50 WP was used which is followed by the fungicide treatment Rovral 50 WP, integration of saw dust burning + soil amendments with *Trichoderma* based bio-fungicide + bacillus based bio-control agents and soil amendment with *Trichoderma* based bio-fungicide. Integration of saw dust burning + soil amendments with *Trichoderma* based bio-fungicide + bacillus based bio-control agents + Rovral 50 WP gave the highest reduction disease incidence 77.72% and disease severity 81.14% compared to control. Application of fungicide Rovral 50 WP reduced 75.74%

disease incidence and 73.58% disease severity followed by integration saw dust burning + soil amendments with *Trichoderma* based bio-fungicide + bacillus based bio-control agents, soil amendments with *Trichoderma* and bacillus based bio-control agent treatments where reduction of disease incidence 72.59%, 71.97% and 64.87% and disease severity 52.84%, 54.72% and 52.84%, respectively compared to control. Integration of saw dust burning + soil amendments with *Trichoderma* based bio-fungicide + bacillus based bio-control agents + Rovral 50 WP gave the highest plant height, plant weight and yield of bush bean followed by the application fungicide Rovral 50 WP, soil amendments with *Trichoderma* and integration of saw dust burning + soil amendments with *Trichoderma* + bacillus based bio-control agents. Integration of saw dust burning + soil amendments with *Trichoderma* + bacillus based bio-control agents + application fungicide Rovral 50 WP gave 27.74% higher yield of bush bean compared to control, Application of only fungicide Rovral 50 WP gave 20.41% higher yield compared to control followed by soil amendments with *Trichoderma* and integration of saw dust burning + soil amendments with *Trichoderma* + bacillus based bio-control agents where the yield was 20.25% and 17.73%, respectively higher than control.

Table 4. Effect of different treatments and its integration against white mold disease of bush bean

Treatments	Disease incidence (%)	Reduction of disease incidence then control (%)	Disease severity (PDI)	Reduction of disease severity then control (%)
T_1= Sawdust burning	4.08 bc (12.01)	63.34	36.00 bc (36.85)	49.06
T_2= Stable bleaching powder	3.91 c (11.38)	64.87	41.33 b (40.00)	41.52
T_3= Trichoderma based bio-fungicide	3.12 c (11.37)	71.97	32.00 c (34.45)	54.72
T_4= Bacillus based bio-control agent	4.95 b (12.15)	55.53	33.33 c (35.26)	52.84
T_5= Rovral 50 Wp	3.05 d (10.05)	72.59	18.67 d (25.50)	73.58
T_6= T_1 +T_2 +T_3 +T_4	2.70 de (9.45)	75.74	33.33 c (35.25)	52.84
T_7= T_1 +T_2 +T_3 +T_4+ T_5	2.48 e (9.05)	77.72	13.33 e (21.27)	81.14
T_8= Control	11.13 a (19.46)	-	70.67 a (57.28)	-

Values in a column having same letter did not differ significantly (P=0.05) by LSD; values within the parenthesis is the Arcsin Transformed value.

Table 5. Effect of different treatments and its integration on the growth and yield of bush bean

Treatments	Plant Height (cm)	Plant weight ($gplant^{-1}$)	Yield (t/ha)	Yield higher then control (%)
T_1= Sawdust burning	15.13 d	9.83 c	14.83 cd	15.85
T_2= Stable bleaching powder	16.07cd	9.43 c	14.22 e	12.24
T_3= Trichoderma based bio-fungicide	17.93 bc	9.67 c	15.65 b	20.25
T_4= Bacillus based bio-control agent	17.23 bcd	9.83 c	14.48 de	13.81
T_5= Rovral 50 WP	19.23 ab	12.17 b	15.68 b	20.41
T_6= T_1 +T_2 +T_3 +T_4	18.03 bc	12.17 b	15.17 c	17.73
T_7= T_1 +T_2 +T_3 +T_4+ T_5	20.93 a	13.67 a	17.27 a	27.74
T_8= Control	12.10 e	7.00 d	12.48 f	-

Values in a column having same letter did not differ significantly (P=0.05) by LSD; values within the parenthesis is the Arcsin Transformed value.

Table 7. Cost, return and marginal analysis of different treatments

Treatment	Variable cost ($Tk.\ ha^{-1}$)	Yield (tha^{-1})	Gross return ($Tk.\ ha^{-1}$)	Gross margin ($Tk.\ ha^{-1}$)	Marginal benefit ($Tk.\ ha^{-1}$)	MBCR
T_1	21000	14.83 cd	222450	201450	14250	1:0.68
T_2	5400	14.22 e	213300	207900	20700	1:3.83
T_3	17500	15.65 b	234750	217250	30050	1:1.72
T_4	19500	14.48 de	217200	197700	10500	1:0.54
T_5	30000	15.68 b	235200	205200	18000	1:0.60
T_6	35400	15.17 c	227550	192150	4950	1:0.14
T_7	50400	17.27 a	259050	208650	21450	1:0.42
T_8	-	12.48 f	187200	187200	-	-

T_1= Sawdust burning, T_2= Stable bleaching powder, T_3= *Trichoderma* based bio-fungicide, T_4= Bacillus based bio-control agent, T_5= Rovral 50 WP, T_6= T_1 +T_2 +T_3 +T_4, T_7= T_1 +T_2 +T_3 +T_4+ T_5 and T_8= Control

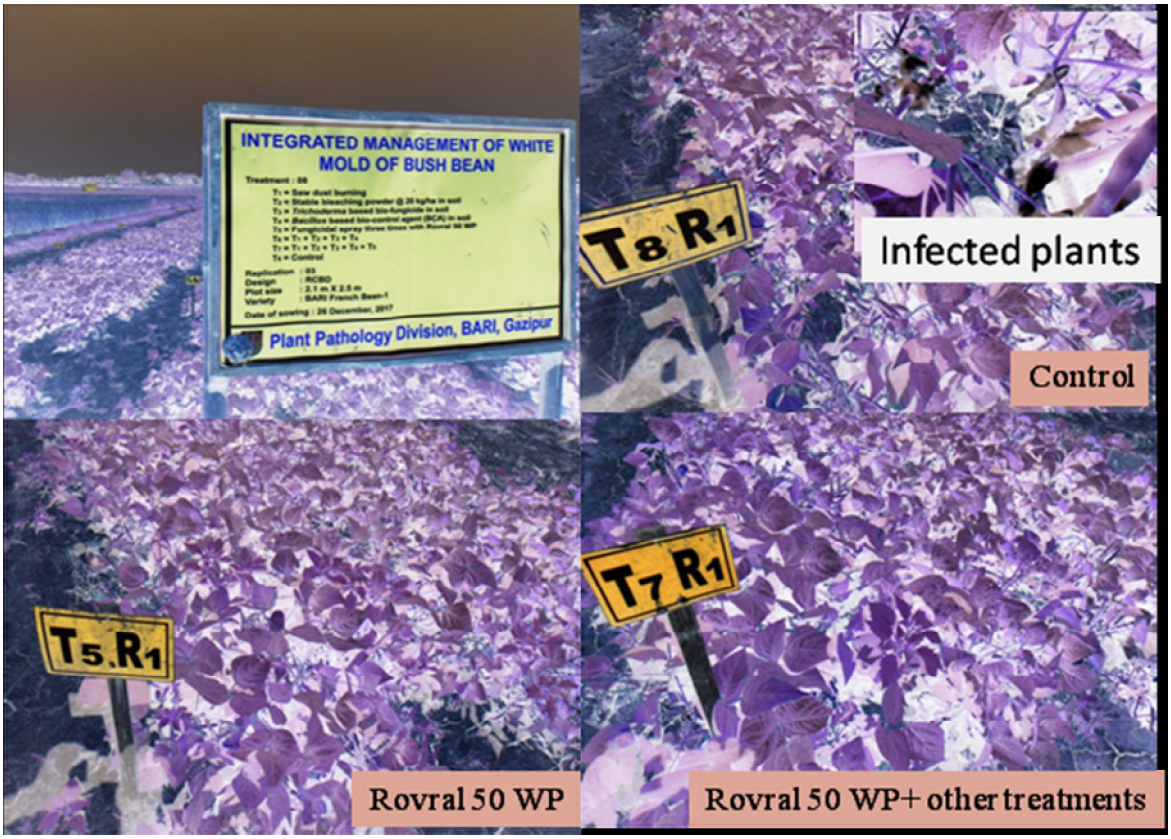

Figure 6 Experimental field view of integrated management of white mold disease of bush bean in Plant Pathology Division, BARI, Gazipur and white mold disease symptom in control plot and disease free plot in the field

Garden pea: Results showed that all the treatments significantly reduced the disease as compared to control (Table 6 and Figure 7). The incidence (%) and severity (%) of white mold disease of garden pea varied significantly among the treatments. The disease incidence ranged from 3.17%-11.43%. The lowest was found in treatment T_7 (T_1 + T_2 + T_3 + T_4 + T_5) which was statistically similar to treatment T_6 (3.30%) and T_5 (4.18) and the highest was recorded from control plots. The lowest disease severity (2.87%) was observed from treatment T_7 (T_1 + T_2 + T_3 + T_4 + T_5) which was significantly closed to T_6 (2.97%) and T_5 (3.20%) whereas, the highest was found in control plots. The highest reduction of disease incidence 72.26% and disease severity 71.01% compared to control was observed in T_7 (T_1 + T_2 + T_3 + T_4 + T_5) followed by T_5, T_6, T_1 and T_4 where the reduction of disease incidence 71.13%, 63.43%, 50.04% and 49.52% and disease severity 70.00%, 67.68%, 58.79% and 55.85%, respectively.

All the treatments showed significant effect on yield and yield contributing characters of garden pea except plant height and number of pods per plant (Table 7). The highest weight of pods per plant (16.28) was recorded in T_7 (T_1 + T_2 + T_3 + T_4 + T_5) which was statistically similar to treatment T_2 (13.27), T_5 (13.20) and T_6 (14.51) while the lowest (10.12) was found in control plots. The highest pod yield (7.60 t/ha) was obtained from T_7 (T_1 + T_2 + T_3 + T_4 + T_5) which was statistically similar to T_6 (7.37t/ha) and T_3 (7.14t/ha) whereas the lowest yield (4.79 t/ha) was recorded in control plots. Yield was 36.97% higher compared to control in the T_7 (T_1 + T_2 + T_3 + T_4 + T_5) followed by T_5, T_3, T_1, T_4 and T_6 treatment where the yield was 35.01%, 32.91%, 27.42%, 25.62% and 25.39%, respectively higher than control.

Figure 7 Experimental field view of integrated management of white mold disease garden pea at RARS, Ishurdi, Pabna and white mold disease symptom in the field

Table 6. Effect of treatments in controlling white mold disease of garden pea

Treatments	Disease incidence (%)	Reduction of disease incidence then control (%)	Disease severity (PDI)	Reduction of disease severity then control (%)
T_1= Sawdust burning	5.71 c (2.39)	50.04	4.37 c (2.08)	55.85
T_2= Stable bleaching powder	8.36 b (2.89)	26.86	6.60 b (2.57)	33.33
T_3= Trichoderma based bio-fungicide	7.66 b (2.76)	32.98	6.55 b (2.55)	33.83
T_4= Bacillus based bio-control agent	5.77 c (2.40)	49.52	4.08 cd (2.02)	58.79
T_5= Rovral 50 Wp	3.30 d (1.81)	71.13	2.97 de (1.72)	70.00
T_6= T_1 +T_2 +T_3 +T_4	4.18 d (2.04)	63.43	3.20 cde (1.78)	67.68
T_7= T_1 +T_2 +T_3 +T_4+ T_5	3.17 d (1.78)	72.26	2.87 e (1.69)	71.01
T_8= Control	11.43 a (3.38)	-	9.90 a (3.14)	-

Means in a column, having similar letter (s) do not differ significantly at 1% level of significance. Values in the parentheses indicated as square root transform value

Table 7. Effect of treatments on plant height, yield and yield contributing characters of garden pea

Treatments	Plant height (cm)	No. of pods/ plant	Weight of pods/ Plant (g)	Pod yield (t/ha)	Yield higher then control (%)
T_1= Saw dust burning of soil	59.20	3.46	12.27 bc	6.60 ab	27.42
T_2= Stable bleaching powder (20 kg/ha) in soil	58.93	3.80	13.27 abc	5.72 bc	16.26
T_3=*Trichoderma* based bio-fungicide in soil	62.80	3.35	11.49 bc	7.14 a	32.91
T_4= *Bacillus* based biocontrol agent (BCA) in soil	58.93	3.30	12.47 bc	6.44 ab	25.62
T_5= Fungicidal spray three times with Rovral 50 WP	59.80	3.44	13.20 abc	7.37 a	35.01
T_6= T_1 + T_2 + T_3 + T_4	57.40	4.42	14.51 ab	6.42 ab	25.39
T_7= T_1 + T_2 + T_3 + T4 + T_5	58.27	4.53	16.28 a	7.60 a	36.97
T_8= Control	57.07	2.96	10.12 c	4.79 c	-

Means in a column showing similar letter (s) do not differ significantly at 1% (**) and 5% (*) level of significance. NS = Not Significant

Table 3. Cost, return and marginal analysis of different treatments

Treatment	Variable cost (Tk. ha^{-1})	Yield (tha^{-1})	Gross return (Tk. ha^{-1})	Gross margin (Tk. ha^{-1})	Marginal benefit (Tk. ha^{-1})	MBCR
T_1	21000	6.60 ab	198000	177000	33300	1:1.58
T_2	5400	5.72 bc	171600	166200	22500	1:4.16
T_3	17500	7.14 a	214200	196700	53000	1:3.02
T_4	19500	6.44 ab	193200	173700	30000	1:1.54
T_5	30000	7.37 a	221100	191100	47400	1:1.58
T_6	35400	6.42 ab	192600	157200	13500	1:0.38
T_7	50400	7.60 a	228000	177600	33900	1:0.67
T_8	-	4.79 c	143700	143700	-	-

T_1= Sawdust burning, T_2= Stable bleaching powder, T_3= *Trichoderma* based bio-fungicide, T_4= Bacillus based bio-control agent, T_5= Rovral 50 WP, T_6= T_1 +T_2 +T_3 +T_4, T_7= T_1 +T_2 +T_3 +T_4+ T_5 and T_8= Control

Different workers reported the antagonistic activity of the mycoparasitic fungi viz. *Coniothyrium minitans*, *Trichoderma* spp., *Gliocladium* spp. *Sporidesmium sclerotivorum*, *Fusarium*, *Hormodendrum*, *Mucor*, *Penicillium*, *Aspergillus*, *Stachybotrys*, and bacterial bio-control agents viz. *Bacillus* species, *Pseudo-monas* spp. *P. chlororaphis* against *S. sclerotiorum* (Budge et. al. 1995, Huang and Kozub 1991, Huang et al. 2000, Nelson *et al.* 2001 and Savchuk and Fernando 2004). Shivpuri *et al.* (2001) observed that fungicides, carbendazim, thiophenate methyl andphenylpyrrole had completely inhibited the growth of *S. sclerotiorum* at all tested concentrations *in vitro*. Eisa *et al.,* (2013) recorded that, under field conditions combining the fungicide Folicur with compost has enhanced the control of white rot of onion and bulb yield compared with using alone. Abdel-Kader *et al*., (2013) found that, combination of (compost + *T. harzianum* + thyme) and (compost + *T. harzianum* + lemongrass) reduced the peanut crown rot disease incidence at both pre- and post-emergence growth stages, respectively compared with untreated control.

Conclusion

From the present study it may be concluded that integration of integration of saw dust burning + soil amendments with *Trichoderma* based bio-fungicide + bacillus based bio-control agents + application fungicide Rovral 50 WP the effective for reducing white mold disease caused by *S.*

sclerotiarum and increasing plant growth as well as yield of mustard, bush bean and garden pea. Application of only fungicide Rovral 50 WP 3-4 times at 10-12 days interval started after white mold disease initiation also effective against white mold disease and also enhanced plant growth as well as yield of mustard, bush bean and garden pea.

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11. Research highlight/findings (Bullet point – max 10 nos.):

i. A total of one hundred and eighty isolates of *S. sclerotiorum* were isolated and maintained as pure culture or sclerotia stock at Plant Pathology Laboratory, BARI, Gazipur for future research

ii. Morphological characterization of all the isolates was done by analyzing mycelia characteristics and sclerotial characteristics of the fungus. The average mycial growth range from 2.65 cm to 8.10 cm at 72 hrs after inoculation. Sclerotia was formed within three to four days after inoculation and size shape and number of sclerotia of different isolates varied considerable and a range of 9 to 64 sclerotia were to produce per Petri dish

iii. Molecular characterization of the collected 14 isolates (out of 180 isolates) was determined by the partial sequencing of ITS region followed by phylogenetic analysis. Molecular characterization of the 14 isolates by ITS sequencing indicated that all the tested isolates were identified as publicly available *S. sclerotiorum*.

iv. Phylogenetic analysis of the 14 isolates based on ITS sequences revealed that the isolates belonged to a similar group of publicly available *S. sclerotiorum* and dissimilar with the group of *S. minor*, *S. trifolium* and distinctly differ from *S. nivalis* group

v. Results of experiments on integrated management packages revealed that integration of saw dust burning + soil amendments with *Trichoderma* based bio -fungicide + bacillus based bio-control agents + application fungicide Rovral 50 WP or application of only fungicide Rovral 50 WP are the best treatment in reduction of white mold disease incidence and disease severity and increasing plant growth as well as yield of mustard, bush bean and garden pea

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	02	70000/-	02	70000/-	
(b) Lab &field equipment	0	0	0	0	
(c) Other capital items	0	0	0	0	

2. Establishment/renovation facilities: N/A

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

3. Training/study tour/ seminar/workshop/conference organized: N/A

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training					
(b) Workshop					

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	256993	256993	256993	0	100	
B. Field research/lab expenses and supplies	718463	666856	666856	0	92.82	
C. Operating expenses	190494	187795	187795	0	98.58	
D. Vehicle hire and fuel, oil & maintenance	151240	146690	146690	0	96.99	
E. Training/workshop/ seminar etc.	0	0	0	0	0	
F. Publications and printing	40000	15000	15000	0	37.50	
G. Miscellaneous	111595	109997	107995	2002	96.77	
H. Capital expenses	70000	70000	70000	0	100	
	1538785	**1453331**	**1451329**	**2002**	**99.86**	

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output(i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
Characterization and study of the nature of *S. sclerotiorum* collected from different hosts through cultural, morpho-physiology and pathogenicity test	Collection and purification of isolates from previously collected samples.	A total of one hundred and eighty isolates of *S. sclerotiorum* were isolated and maintained as pure culture or sclerotia stock at Plant Pathology Laboratory, BARI, Gazipur.	
	Morphological characterization of collected isolates was done by analyzing mycelial characteristics and sclerotial characteristics of the fungus.	Morphological characterization of all the isolates showed the differential among the isolated. The average mycial growth range from 2.65 cm to 8.10 cm at 72 hrs after inoculation. Sclerotia was formed within three to four days after inoculation and size shape and number of sclerotia of different isolates varied considerable and a range of 9 to 64 sclerotia were to produce per Petri dish.	*Sclerotinia sclerotiorum* isolates in Bangladesh showed differential vegetative characteristics
Molecular characterization and determination of genetic variability of the collected isolates of *S. sclerotiorum*.	Molecular characterization of the collected 14 isolates (out of 180 isolates) was determined by the partial sequencing of ITS region followed by phylogenetic analysis.	Molecular characterization of the 14 isolates by ITS sequencing indicated all the tested isolates were identified as publicly available *S. sclerotiorum*. Phylogenetic analysis of the isolates based on ITS sequences revealed the isolates belonged to a similar group of publicly available *S. sclerotiorum* and dissimilar with the group of *S. minor*, *S. trifolium* and distinctly differ from *S. nivalis* group	White mold disease of different crops in Bangladesh caused by similar group of publicly available *S. sclerotiorum*
Development of eco-friendly package for controlling the white mold disease through integrated approaches for safe production of selected crops.	For development an integrated management package of white mold disease, three field experiments were carried out at the experimental field of three different locations viz. Plant Pathology Division, BARI, Gazipur, RARS, Burirhat, Rangpur and RARS, Ishurdi, Pabna with three different crops viz. bush bean, mustard and garden pea, respectively during Rabi season of 2017-18	. Results from all the experiments revealed that integration of saw dust burning + soil amendments with *Trichoderma* based bio-fungicide + bacillus based bio-control agents + application fungicide Rovral 50 WP or application of only fungicide Rovral 50 WP are the best treatment in reduction of white mold disease incidence and disease severity and increasing plant growth parameter as well as increasing yield of mustard, bush bean and garden pea.	Integration of different components or Fungicidal treatment is effective against the white mold disease

E. Materials Development/Publication made under the Sub-project: N/A

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.			
Journal publication			
Information development			
Other publications, if any			

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

- Integration of saw dust burning + soil amendments with *Trichoderma* based bio-fungicide + bacillus based bio-control agents + application fungicide Rovral 50 WP reduced the white mold disease caused by *S. sclerotiarum*
- Application of fungicide Rovral 50 WP 3-4 times at 10-12 days interval started just after white mold disease initiation successfully control the white mold disease incidence and disease severity and increasing plant growth parameter as well as increasing yield of mustard, bush bean and garden pea

ii. **Generation of new knowledge that help in developing more technology in future**

- Molecular characterization and Phylogenetic analysis of the collected 14 isolates (out of 180 isolates) by ITS sequencing indicated all the tested isolates were identified as publicly available *S. sclerotiorum* and dissimilar with the group of *S. minor*, *S. trifolium* and distinctly differ from *S. nivalis* group which help the development of disease management technology

iii. Technology transferred that help increased agricultural productivity and farmers' income

- The developed technology (i) integration of saw dust burning + soil amendments with *Trichoderma* based bio-fungicide + bacillus based bio-control agents + application fungicide Rovral 50 WP
- or application of fungicide Rovral 50 WP 3-4 times at 10-12 days interval started just after disease initiation will be helped the farmers for management of devastating white mold diseases caused by soil borne pathogen *S. sclerotiorum* which also helped the increased agricultural productivity and farmers' income

iv. Policy Support

- The information generated from this study will be helped for policy maker/researcher for further study for development of very effective management technology against the disease.

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring [description & output of consultation meeting, monitoring workshops/seminars etc.):

ii) Field Monitoring (time & No. of visit, Team visit and output):

Monitoring team	Date(s) of visit	Total visit till date	Remarks
Technical Division/Unit, BAEC	14/03/18	1	-
PIU-BARC, NATP-2	14/03/18	1	-
Internal Monitoring (BARI)	06/02/2018, 19/02/2018	2	-

I. Lesson Learned/Challenges (if any)

i) White mold disease of different crop becoming epidemic especially northern region in Bangladesh.
ii) Management of white mold disease depends on chemical fungicide without chemical fungicide the disease may not be control.
iii) Delay in allocation of fund by PIU-BARC. The process to get fund is lengthy.

J. Challenges (if any)

- Delay in allocation of fund by PIU-BARC made difficult to contract research timely.

Signature of the Principal Investigator
Date …….

Seal

Counter signature of the Head of the organization/authorized representative
Date ……..
Seal