EFFICACY OF *Trichodarma harzianum* TO CONTROL *Sclerotium rolfsii* CAUSING DAMPING-OFF AND FOOT ROT OF VEGETABLES

A Thesis

BY

Shamima Naznin

Student No. 1405029

Session: 2014-2015

MASTER OF SCIENCE (M.S.)

IN

PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

June, 2015

EFFICACY OF *Trichodarma harzianum* TO CONTROL *Sclerotium rolfsii* CAUSING DAMPING-OFF AND FOOT ROT OF VEGETABLES

A Thesis

BY

Shamima Naznin

Student No. 1405029

Session: 2014-2015

Submitted to
The Department of Plant Pathology
Hajee Mohammad Danesh Science and Technology University, Dinajpur
InPartial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE (M.S.)

IN

PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

June, 2015

EFFICACY OF *Trichodarma harzianum* TO CONTROL *Sclerotium rolfsii* CAUSING DAMPING-OFF AND FOOT ROT OF VEGETABLES

A Thesis

By

Shamima Naznin

Student No. 1405029

Session: 2014-2015

Approved as to the style and content by:

_____

Professor Dr. A. T. M. Shafiqul Islam
Supervisor

_____

Professor Dr. M Mamunur Rashid
Co-supervisor

_____

Professor Dr. S. M. Mobarak Hossain

Chairman, Examination Committee
&
Chairman
Department of Plant Pathology
Hajee Mohammad Danesh Science and TechnologyUniversity, Dinajpur

June, 2015

ACKNOWLEDGEMENT

All praises are due to 'Almighty Allah' whose blessings enabled the author for successful completion of this research work.

The author wishes her profound sense of appreciation, heartiest gratitude and indebtedness to his Supervisor. Dr. A. T. M. Shafiqul Islam, Professor, Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for his help, keen interest, continuous encouragement, scholastic supervision, constructive suggestions and useful criticism during the entire period of the research work and in preparing the thesis.

The author is ever grateful and immensely indebted to his co-supervisor Dr. Md. Mamunur Rashid, Professor, Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for his valuable suggestions, encouragement during the research period.

The author would like to express his deepest gratitude to Prof. Dr. S. M. Mobarak Hossain, Chairman, Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for his sympathic and constant encouragement, constructive suggestions and advice during the study period.

The author would like to express his grateful thanks to Professor Dr. S. M. Emdadul Hassan, Dr. Md. Mohidul Hasan, Associate Professor and Md. Shamim Hasan, Assistant Professor, Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for their valuable advice and inspiration during the study period.

The author desires to express his cordial thanks to all staff members, Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for their sincere co-operation during the study period.

The author extremely thankful to Sheuli, Nur, Purobi and Mobin for their fellow feelings and continuous inspiration during the research period.

Finally, the author is highly indebted to her parents, brother, sister and relatives for their everlasting blessings, inspiration, co-operation, encouragement, sacrifices and dedicated efforts to educate her up to this level.

June 2015 *The author*

ABSTRACT

Experiments were conducted in the Plant Pathology laboratory, HSTU to find out the effectiveness of local isolate of *Trichoderma* to control *Sclerotium rolfsii* causing damping-off and foot rot of some selected vegetable crops during the period of June-April 2014/15. Soil samples were collected from rhizosphere zone of different vegetable crops at different locations of Dinajpur district. *Trichoderma* isolated from the rhizosphere soil of Indian spinach was identified as *Trichoderma harzianum*. Another isolate of *T. harzianum* was purified from the commercially formulated product by Rural Development Academy (RDA), Bogra. *Sclerotium rolfsii* was isolated and purified from infected eggplant. The two isolates of *T. harzianum* were tested for their antagonistic activity against *S. rolfsii* following the dual culture method on petridish. It was found that local isolate of *T. harzianum* showed better inhibition (92%) than RDA isolate (85%). The local isolate of *T. harzianum* was formulated with blackgram based substrate to test its efficacy as soil treatment. In pot experiments, three treatments viz. T_1 (formulated *Trichoderma* @ 10 g/kg soil), T_2 (formulated *Trichoderma* @ 15 g/kg soil) and T_3 (formulated *Trichoderma* @ 20 g/kg soil) along with a sterilized soil as control were tested against *S. rolfsii* causing the damping off and foot rot of bean, eggplant, tomato, cabbage and Indian spinach. Among the treatments, soil application with T_3 (formulated *Trichoderma* @ 20 g/kg soil) showed the highest effect followed by T_2 and T_1 in controlling pre-emergence death, damping-off and foot rot (71.87-100%) increasing germination percentage (34.37-48.46%) in all the crops over control. Seed treatment with spore suspension of *Trichoderma* @ 5.02×10^6 CFU/ml was also found effective against different seed-borne pathogens resulting higher germination (23.26-49.62%) and less incidence of foot rot and damping-off of seedlings (140.0-322.33%) over control. Finally it may be concluded that formulated *Trichoderma* as soil treatment (@ 20 g/kg soil) and spore suspension as seed treatment (@ 5.02×10^6 CFU/ml) resulted statistically significant in controlling the *S. rolfsii* causing damping-off and foot rot diseases of vegetable with higher germination.

Key words: *Trichoderma*, *S. rolfsii*, Formulation, Damping-off, Foot rot, Control.

CONTENTS

CHAPTER	TITLE	PAGE
	ACKNOWLEDGEMENT	**i**
	ABSTRACT	**ii**
	CONTENTS	**iii-vi**
	LIST OF TABLES	**vii**
	LIST OF PLATES	**viii-ix**
CHAPTER 1	**INTRODUCTION**	**1-3**
CHAPTER 2	**REVIEW OF LITERATURE**	**4-18**
CHAPTER 3	**MATERIALS AND METHODS**	**19-32**
	3.1 Experimental Site	19
	3.2 Experimental Period	19
	3.3 Experiments Conducted	19
	3.3.1 Study of local *Trichoderma* isolate	19
	3.3.1.1 Collection of soil samples	19
	3.3.1.2 Isolation, purification and preservation of *Trichoderma* isolate	19
	3.3.1.3 Identification of *Trichoderma* isolate by morphological characters	20
	3.3.2 Study of *Sclerotium rolfsii* isolate	21
	3.3.2.1 Collection, isolation and purification of *Sclerotium rolfsii*	21
	3.3.2.2 Identification of *Sclerotium rolfsii* by morphological characters	23
	3.3.3 Comparative bioassay of the local *Trichoderma* isolate and RDA (Rural Development Academy, Bogra) *Trichoderma* isolate against *S. rolfsii* by dual culture technique	23

CONTENTS (CONTD.)

CHAPTER	TITLE	PAGE
	3.3.4 Determination of effective dose of *Sclerotium rolfsii* on seed germination and seedling diseases of eggplant, tomato, bean, cabbage and indian spinach in tray soil	24
	3.3.4.1 Preparation of *Sclerotium rolfsii* inocula	24
	3.3.4.2 Sterilization of soil and preparation of trays	25
	3.3.4.3 Experimental Design and treatments	26
	3.3.4.4 Inoculation of tray soil with *S. rolfsii*	26
	3.3.4.5 Sowing of seeds	27
	3.3.4.6 Recording of data	27
	3.3.5 Determination of effective dose of formulated *Trichoderma* on seed germination and seedling diseases of eggplant, tomato, bean, cabbage and indian spinach in tray soil	27
	3.3.5.1 Formulation of *Trichoderma* isolate	27
	3.3.5.2 Sterilization of soil and preparation of trays	29
	3.3.5.3 Experimental Design and treatments	29
	3.3.5.4 Inoculation of tray soil with *S. rolfsii*	29
	3.3.5.5 Treatment of tray soil with formulated *Trichoderma* isolate	29
	3.3.5.6 Sowing of seeds	30
	3.3.5.7 Recording of data	30
	3.3.6 Determination of effective dose of *Trichoderma* suspension on seed germination and seedling diseases of eggplant, tomato, bean, cabbage and Indian spinach in tray soil	30
	3.3.6.1 Sterilization of soil and preparation of trays	30
	3.3.6.2 Experimental Design and treatments	30

CONTENTS (CONTD.)

CHAPTER	TITLE	PAGE
	3.3.6.3 Inoculation of tray soil with *S. rolfsii*	30
	3.3.6.4 Preparation of *Trichoderma* suspension	31
	3.3.6.5 Seed treatment with different concentration of conidial suspension of *Trichoderma*	31
	3.3.6.6 Sowing of seeds	32
	3.3.6.7 Recording of data	32
	3.7 Statistical analysis	32
CHAPTER 4	**RESULTS**	**33-56**
	4.1 Study of local *Trichoderma* isolate	33
	4.1.1 Collection, isolation and purification of isolate of *Trichoderma* spp.	33
	4.1.2 Identification of *Trichoderma* isolate by morphological characters	33
	4.2 Study of *Sclerotium rolfsii* isolate	35
	4.2.1 Collection, isolation, purification of isolate of *Sclerotium rolfsii*	35
	4.2.2 Identification of *Sclerotium rolfsii* isolate by morphological characters	35
	4.3 Comparative bioassay of the local *Trichoderma* isolate and RDA (Rural Development Academy, Bogra) *Trichoderma* isolate against *S. rolfsii* by dual culture technique	37
	4.4 Determination of effective dose of *Sclerotium rolfsii* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach in tray soil	38
	4.4.1 Effect of different doses of *Sclerotium rolfsii* on germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach seedlings at 7 DAS	38

CONTENTS (CONTD.)

CHAPTER	TITLE	PAGE
	4.4.2 Effect of different doses of *Sclerotium rolfsii* on germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach seedlings at 14 DAS	44
	4.5 Determination of effective dose of formulated *Trichoderma* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach in tray soil	47
	4.5.1 Effect of different doses of formulated *Trichoderma* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach in tray soil at 7 DAS	47
	4.5.2 Effect of different doses of formulated *Trichoderma* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach in tray soil at 14 DAS	50
	4.6 Determination of effective dose of *Trichoderma* suspension on seed germination and seedling diseases of eggplant, tomato, bean, cabbage and indian spinach in tray soil	52
	4.6.1 Effect of different doses of *Trichoderma* suspension on seed germination and seedling diseases of eggplant, tomato, bean, cabbage and indian spinach in tray soil at 7 DAS	52
	4.6.2 Effect of different doses of *Trichoderma* suspension on seed germination and seedling diseases of eggplant, tomato, bean, cabbage and indian spinach in tray soil at 14 DAS	55
CHAPTER 5	**DISCUSSION**	**57-60**
CHAPTER 6	**SUMMARY AND CONCLUSION**	**61-63**
	REFERENCES	**64-75**
	APPENDICES	**76**

LIST OF TABLES

NO.	TITLE	PAGE
Table 1.	Effect of different doses of *S. rolfsii* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach at 7 DAS	41
Table 2.	Effect of different doses of *S. rolfsii* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and viindian spinach at 14 DAS	46
Table 3.	Effect of different doses of formulated *Trichoderma* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach in tray soil at 7 DAS	48
Table 4.	Effect of different doses of formulated *Trichoderma* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach at 14 DAS	51
Table 5.	Effect of different doses of *Trichoderma* suspension on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach at 7 DAS	53
Table 6.	Effect of different doses of *Trichoderma* suspension on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach at 14 DAS	56

LIST OF PLATES

NO.	TITLE	PAGE
Plate 1.	Pure culture of *Trichoderma* isolate (7 days old)	20
Plate 2.	Symptom showing foot rot caused by *S. rolfsii*on eggplant	21
Plate 3.	Pure culture of *Sclerotium rolfsii* in PDA media (7 days old)	22
Plate 4.	Mature culture of *Sclerotium rolfsii* with Sclerotia in PDA media (14 days old)	22
Plate 5.	Inocula of *Sclerotium rolfsii* (7 days old culture)	25
Plate 6.	Inocula of *Sclerotium rolfsii* after air drying	25
Plate 7.	Growth of *Sclerotium rolfsii* in inoculated tray soil	26
Plate 8.	Formulated *Trichoderma* inocula used as bio-fungicides	28
Plate 9.	Air drying of formulated *Trichoderma* spp.	28
Plate 10.	Formulated *Trichoderma* as packing form	28
Plate 11.	Soil treated with formulated *Trichoderma* after inoculation of *Sclerotium rolfsii*	29
Plate 12.	Conidial suspension of *Trichoderma* in beaker	31
Plate 13.	*Trichoderma* isolate of dark green colour (7 days old culture)	34
Plate 14.	Conidiophores, phialides and conidia of *Trichoderma harzianum* observed under microscope	34
Plate 15.	Mycelial growth of *Sclerotium rolfsii* in PDA media (7 days old culture)	36
Plate 16.	Mycelial growth of *Sclerotium rolfsii* with Sclerotia in PDA media (14 days old culture)	36
Plate 17.	Effect of local *Trichoderma* isolate on *Sclerotium rolfsii* in dual culture (7 days old culture)	37

LIST OF PLATES (CONTD.)

NO.	TITLE	PAGE
Plate 18.	Effect of RDA *Trichoderma* isolate on *Sclerotium rolfsii* in dual culture (7 days old culture)	38
Plate 19.	Bean seedlings in tray soil (A) *Sclerotium rolfsii* inoculated soil (B) control (only sterilized soil)	42
Plate 20.	Eggplant seedlings in tray soil (A) *Sclerotium rolfsii* inoculated soil (B) control (only sterilized soil)	42
Plate 21.	Cabbage seedlings in tray soil (A) *Sclerotium rolfsii* inoculated soil (B) control (only sterilized soil)	43
Plate 22.	Indian spinach seedlings in tray soil (A) *Sclerotium rolfsii* inoculated soil (B) control (only sterilized soil)	43
Plate 23.	Bean seedlings in tray soil (A) control (*S. rolfsii* inoculated soil) (B) treatment of formulated *Trichoderma* after inoculation of *S. rolfsii*	49
Plate 24.	Cabbage seedlings in tray soil (A) control (*S. rolfsii* inoculated soil) (B) treatment of formulated *Trichoderma* after inoculation of *S. rolfsii*	49
Plate 25.	Been seedlings in tray soil (A) control (*S. rolfsii* inoculated soil) (B) seeds treated with *Trichoderma* suspension after inoculation of *S. rolfsii*	54
Plate 26.	Cabbage seedlings in tray soil (A) control (*S. rolfsii* inoculated soil) (B) seeds treated with *Trichoderma* suspension after inoculation of *S. rolfsii*	54

CHAPTER 1

INTRODUCTION

Sclerotium rolfsii is an important soil-borne plant pathogen and a major constrain for vegetables production in Bangladesh (Ahmed and Hossain, 1985). It mainly causes nursery diseases like damping-off and foot rot of vegetables. It has a very extensive host range; at least 500 species in 100 families are susceptible, the most common hosts are legumes, crucifers, and cucurbits (Chupp and Sherf, 1960). It significantly reduces vegetable yield.

Sclerotium rolfsii is a facultative parasite and is found in a wide range of soil. The fungus survives in soil mainly as sclerotia, which is the main source of inocula and remain viable in soil for several months. *S. rolfsii* attacks the plants at any growth stage but more devastating at seedling stage (Bag and Sinha, 1997). It causes both pre and post emergence mortality of vegetables crops (Elad *et al*., 1990).

Farmers try to overcome this problem through different cultural practices and use of chemical fungicides. But the control of *S. rolfsii* with chemicals is not cost effective. In addition, non-judicial use of chemicals in agriculture causes environment pollution and health hazards, destroying the natural balance and beneficial micro-flora of the soil. Moreover, consumers are becoming concerned about chemical pollution of the environment and pesticide residues in food, and farmers more often face with development of pathogen's resistance to chemical fungicides. Therefore, there is a need for development of efficient alternative of chemicals to combat the disease and inocula buildup in soil.

As alternative of chemical method, bio-fungicides are included in the concept of biological control. Biological control represents a natural and ecological approach in controlling diseases that reduces chemical inputs and their hazardous effects (Papavizas and Lumsden, 1980; Mukhopadhyay, 1994). In biological control,

living microorganisms act as antagonist, parasites and predators (Kwok *et al*., 1987). The antagonism of a biological agent can reduce pathogen's ability to produce inoculums. In the absence of other sources of inocula, future disease levels will eventually be reduced by such biological control measures (Fokkema, 1995). *Trichoderma* played a considerable role as biocontrol agent (Papavizas, 1985). Several strains of *Trichoderma* have been found to be effective as bio-control agent against various soil borne plant pathogenic fungi like *Fusarium, Sclerotium*, *Rhizoctonia* etc. (Chet and Inbar, 1994). Previous reports revealed that some isolates of *T. harzianum* are effective biocontrol agent against those soil-borne pathogens (Hadar *et al.*, 1979; Elad *et al*., 1983 and Roy *et al.*, 1989). Mechanisms of antagonist *Trichoderma* include mycoparasitism by the action of cell-wall degrading enzymes, antibiosis by the production of antibiotics, competition for space and nutrients through rhizosphere competence, facilitation of seed germination and enhance growth of the plants releasing important minerals and trace elements from soil and also induction of the defense responses in plants (Herrera and Chet, 2003; Howell, 2003; Benytez *et al*., 2004).

Trichoderma are free living fungi that are present in substantial numbers in most of the agricultural soils and in the environments. *Trichoderma* spp. grow topically toward hyphae of other fungi, coil about them in a lectin-mediated reaction, and degrade cell walls of the target pathogenic fungi. *Trichoderma* produces chemicals called trichodermin which is responsible for its antagonistic properties (Tvetdyukov *et al*., 1994). *Trichoderma* significantly destroys the sclerotia of *S. rolfsii* (Susceelendra and Schlosser, 1999) and it is antagonistic to *S. rolfsii*, overlaps the pathogen and suppresses its growth (Iqbal *et al*., 1995).

Among the most widely commercialized biocontrol agents for soil borne diseases of crops, *Trichoderma* frequently present in plant root ecosystem (Harman *et al*., 2004). Isolates of *T. harzianum* have been reported as potential bio-control agents for use against *S. rolfsii* (Jinantana and Sariah, 1997)

Biological treatment of tomato, potato, chickpea, lentil and peanut seeds with *Trichoderma harzianum* and *Gliocladium virens* resulted in excellent potentials against a wide range of plant pathogens including *S. rolfsii* and the treatments were consistently as effective as or better than fungicidal seed treatment (Mukhopadhyay, 1989).

The high degree of ecological adaptability of *Trichoderma* isolates in the rhizosphere zones of plants (Mukhopadhyay, 1995) and their amenability of growing an inexpensive substrates, makes *Trichoderma* as potential candidates for bio-control application.

To obtain *T. harzianum* having a natural adaptability to function in adverse climatic conditions (low rainfall, drought, extreme temperatures, poor soil quality, etc.) against *S. rotfsii*, a novel biological formulation is necessary to use in the field condition. But still sufficient works on formulation of *Trichoderma* to control *S. rolfsii* in the vegetable field are not available in Bangladesh. In the present perspective, selection of indigenous isolate of *Trichoderma* and its proper formulation to control foot/collar rot of vegetables caused by *S. rolfsii* is the aim of this study.

Objectives of the research

l. To isolate *Trichoderma* spp. from rhizosphere soil of vegetables crops.
2. To study the efficacy of *Trichoderma* spp. as biocontrol agents against *Sclerotium rolfsii.*
3. To formulate *Trichoderma* based biofungicide for controlling foot rot of vegetables.

CHAPTER 2

REVIEW OF LITERATURE

Biological control of soil- borne diseases using *Trichoderma harzianum* is of great interest to the researches as an effective alternative to conventional chemical fungicide based disease management approach. Some important literatures to control *Sclerotium rolfsii* through biopesticide studies are reviewed here.

An experiment was conducted by Akrami *et al*., (2015). They evaluated that the effects of *Trichoderma harzianum*, *T. asperellum*, and *T. virens* on the wilt disease complex of tomato (*Solanum lycopersicum*) caused by *Fusarium oxysporum* f. sp. *Ciceri* and *Rhizoctonia solani* under greenhouse conditions and the disease control was highest with a combination of *T. harzianum, T. asperellum*, and *T. virens* (80-87%) followed by binary combination of *Trichoderma* spp. (79%-82%), while the lowest control was done with *T. viride* (65%).

Faruk *et al*. (2015) evaluated that individual and combine use of carrier materials based *T. harzianum* bio-fungicides were effective in increasing seedling emergence and reducing pre-emergence as well as post-emergence mortality of tomato seedling under *R. solani* inoculated seedbed condition.

An experiment was conducted by Faruk (2015). Effectiveness of three organic substrates rice bran, wheat bran, grass pea bran and their combinations mixed with or without mustard oilcake (MOC) were tested to formulate *Trichoderma harzianum* based bio-fungicides for the management of seedling rot disease of cabbage. *T. harzianum* based bio-fungicides were effective to reduce pre-emergence and post-emergence mortality of cabbage seedling under *R. solani* inoculated seedbed condition. The individual (rice bran, wheat bran, grass pea bran) and combination of substrates (rice bran + wheat bran, rice bran + mustard oilcake, rice bran + wheat bran + MOC and wheat bran + grass pea bran + MOC) were

equally suitable for mass production of effective *T. harzianum* bio-fungicides for the management of seedling rot disease of cabbage in seedbed condition.

Motesharrei *et al.* (2014) tested the isolates of *Trichoderma* spp. as biocontrol agent to control *Fusarium.* Experiment was done in a way that *Fusarium* at different times, (before, at the same and after inoculation of *Trichoderma*), added to the soil and results revealed that it is better to add *Trichoderma* to the soil before inoculation of *Fusarium*.

An experiment was conducted by Patel *et al*. (2014). They isolated different strains of *Trichoderma* for testing their antagonistic activity against *Fusarium* (soil borne pathogen) which is expressed as a zone of inhibition in the culture plates.

Afrin (2013) was conducted an experiment to evaluate the efficacy of fungicides and *Trichoderma harzianum* against seed borne pathogens (*Fusarium* oxysporum) of cotton. She observed that *Fusarium oxysporum* can be better controlled and germination (%) of different cotton varieties were increased by using Bavistin 50WP and Proud 250EC as fungicides and *Trichoderma harzianum* as bio control agent.

Sundaramoorthy *et al*., (2013) evaluated the efficacy of the native isolates of *Trichoderma* species to promote the growth and yield parameters of tomato and to manage *Fusarium* wilt disease. The results revealed that *Trichoderma harzianum* (ANR-1) isolate was found to effectively inhibit the radial mycelial growth of the pathogen (by 53%) when compared to all other isolates. Under greenhouse conditions, the application of *Trichoderma harzianum* (ANR-1) exhibited the least disease incidence (by 15.33%). Also tomato plants treated with *Trichoderma harzianum* (ANR-1) isolate showed a significant stimulatory effect on plant height (by 73.62 cm) and increased the dry weight (by 288.38 g) of tomato plants in comparison to other isolates and untreated control.

Gveroska *et al.* (2012) evaluated that *Trichoderma harzianum* have the potential biological control of *Alternaria alternate* on tobacco. They found that *Trichoderma harzianum* was strong reducing effect on the development of *A. alternate* with various mechanisms of antagonistic influence.

Hend *e. al*. (2012) conducted experiment to determine the potential of locally isolated antagonist fungi (*Aspergillus niger, Penicillium citrinum*, *Trichoderma harzianum* and *Trichoderma viride*) to manage *Fusarium* wilt of common bean. Under *in vitro* condition all antagonist species had inhibited the radial growth of pathogen; however in the case of *A. niger* this inhibition was insignificant. Maximum control of wilt disease was observed in bean plants treated with *T. harzianum* (71.4%). Effectiveness of the other antagonists was recorded in the following order: *T. viride* (67.8%), *P. citrinum* (53.5%) and *A. niger* (35.7%).

An experiment was carried out by Bagwan (2011). He found that *Trichoderma harzianum* have the potential in controlling growth and sporulation of *Sclerotium rolfsii, Aspergillus niger* and *Aspergillus flavus* under *in vitro* conditions.

Rahman *et al.* (2011) conducted an experiment for testing an efficient bioconversion agent, *T. harzianum* (IMI-392432) was found to be the most effective in kitchen waste decomposition. It provided the highest volume (31.80%) and weight (30.80%) losses in waste treated with spore suspension. Promising results were also noted using a combined treatment with different strains/species of *Trichoderma*, which resulted in 18% greater decomposition of waste than the control.

An experiment was conducted by Uddin *et al*. (2011). Soil applications with poultry refuse, coco dust, vermicompost, ash, sawdust, khudepana, cowdung, solarized sand, *Trichoderma harzianum* and or with seed treatment by *T. harzianum* were evaluated against damping off disease complex of potato and chilli. *T. harzianum* treated seed along with soil treatment with *T. harzianum*

performed best in terms of seed germination, percent damping off reduction and enhanced growth characters than soil application with *T. harzianum* alone. Among the different soil amendments, poultry refuse and vermicompost have promising impact on seed germination, reduction of percent damping off and growth of potato and chilli seedlings when applying along with *T. harzianum*.

Amin *et al.* (2010) reported that *Trichoderma* spp. have the ability to inhibit soil borne pathogens of different vegetables viz., *Rhizoctonia solani* (isolates from tomato), *Sclerotium rolfsii* (causing collar rot of tomato) and *Sclerotinia sclerotiorum* (causing web blight of beans) under *in vitro* conditions. They also observed that *Trichoderma* isolates significantly inhibited the production of sclerotia in test pathogens. *T. viride* (Tv-1) was most effective in reducing sclerotial production (83.75 % in *R. solani*, 80.18 % in *S. rolfsii* and 70.15 % in *S. sclerotiorum*).

Tran N. Ha (2010) conducted an experiment on several crops such as: peanut, tomato, cucumber band durian indicate that selected *Trichoderma* strains could reduce significant diseases caused by fungal pathogens including: *Phytophthora palmivora*, *Rhizoctonia solani*, *Fusarium* spp., *Sclerotium rolfsii* and *Pythium* spp. He observed that the efficacy of *Trichoderma* species on soil borne fungal disease is higher than fungicides and maintain longer. He also reported that crop treated with *Trichoderma* grown better and had higher yields to compare with the one without application.

Siddique (2009) conducted an experiment to evaluate the efficacy of some selected *T. harzianum* strains and plant extracts (Bashok, Biskathali and Marigold) against *A. brassicae* and *A. brassicicola* causing leaf blight of mustard. He also observed that *T. harzianum* S_1 and Marigold showed the best performance in reducing disease incidence and disease severity as well as increasing seed yield against leaf blight of mustard.

Nashwa *et al.* (2008) evaluated that soil treatment with a powder formulation of *Trichoderma* spp. two weeks before planting or at the time of planting reduced significantly the incidence of damping-off and wilt diseases on Giza 3 bean cultivar. They also found that the formulation of *Trichoderma* spp. treatments not only suppressed both damping-off and wilt diseases but also enhanced green yield of bean plants compared to infected control.

An experiment was conducted by Rosane *et al.* (2008). They used low-cost substrates (Rice, corn bran & wheat bran) to produce *Trichoderma* spores. They observed that wheat bran showed to be the most suitable substrate to produce *Trichoderma* spores for all strains. High spore counts were obtained for *T. harzianum* sp. (28.30×108/gds) and *T. viride* (24.10×108 spores/gds).

Meah (2007) tested the pathogenicity of 10 isolates of *S. rolfsii* on eggplant (var. Dohazari) and he found that all the isolates of *S. rolfsii* significantly influenced the germination, pre-emergence death, damping off, foot rot and plant stand.

Singh *et al.* (2007) conducted an experiment to determine the efficacy of fungicide alone and in combination with fungal biocontrol agent (*Trichoderma harzianum*) against *Sclerotium rolfsii* infecting lentil cv. DPL-15. Seed treatment of thiram + carbendazim + *Trichoderma harzianum* resulted in minimum disease incidence.

Bhuiya (2006) conducted an experiment to determine the effect of fungicide, biopesticide and plant extract in controlling collar rot of brinjal. He found that *Trichoderma* formulation @ 20g/kg soil was effective in controlling collar rot and regeneration of brinjal plants.

Chandrasehar *et al.* (2005) conducted lab and green house experiments to determine the efficacy of *Trichoderma harzianum* against *S. rolfsii* that caused tomato collar rot. They found that *Trichoderma harzianum in vitro* condition completely suppressed the growth of *S. rolfsii* and in green house condition in pot

culture as seed treatment and soil drenching it increased the percent survival of treated seedling.

Hannan (2005) conducted an experiment for integrated management of foot rot of lentil, chickpea and grass pea. He found that post-emergence death of lentil plants, chickpea and grass pea due to foot rot (*Fusarium oxysporum* and *Sclerotium rolfsii*) was reduced by treating seeds with BAU-Biofungicide and BINA-fertilizer either alone or in combination. Seed treatment with BAU-Biofungicide reduced post-emergence death of lentil (var. BINA-Musur-1), chickpea (var. Hyprochola) and grass pea up to 56.45%, 66.83% and 76.51%, respectively over the control.

Kashem (2005) conducted experiments to determine the efficacy of *Trichoderma* in controlling foot and root rot and collar rot of lentil. He found that *Trichoderma harzianum* and *Trichoderma viride* as seed treatment, soil treatment, seed + soil treatment was effective in controlling foot and root rot and collar rot of lentil.

Anand and Singh (2004) reported that eight isolates of different species of *Trichoderma* and two isolates of *Gliocladium virens* were tested *in vitro* for their antagonistic activity against *Sclerotium rolfsii*, the cause of collar rot in *Mentha* spp. The most effective isolates of *Trichoderma harzianum* and *T. virens* were selected for disease control. *T. harzianum* resulted in disease control ranging from 66.67 to 100% reduction in disease was accompanied with significant increase in herb and oil yield.

Prasad *et al*. (2003) tested the efficacy of isolates of *Trichoderma* spp. in suppressing the growth of *Sclerotium rolfsii*, the cauliflower collar rot pathogen by dual culture method. They found that *T. harzianum* (44.1%) isolate was superior to *T. viride* (39.1%) isolate in reducing the colony diameter of *S. rolfsii*.

Shamsuzzaman *et al.* (2003) reported that seed treatment with *Trichoderma harzianum* grown on black gram resulted up to 16.66% higher seed germination,

263.33% fresh shoot weight, 157.14% fresh root weight and 98.55% vigor index of cucurbits over control.

Alonso-Reyes *et al.* (2002) evaluated the activity of *T. harzianum* strain A34 against *S. rolfsii*. They found that seed treatment prior to planting and soil treatment with biocontrol strain at 8 kg/ha protected tomato seedlings cultivated up to 30 days after germination.

Faruk *et al.* (2002) tested the isolates of *Trichoderma* spp. as biocontrol agent to control *Sclerotium rolfsii.* Four isolates of the antagonist significantly reduced the radial growth of *S. rolfsii* in dual culture on PDA. The *Trichoderma* also significantly reduced the post emergence mortality due to *S. rolfsii* and increased plant growth and pod yield of bush bean.

Gogoi *et al*. (2002) conducted field experiments to determine the efficacy of *Trichoderma harzianum* against collar rot caused by *Sclerotium rolfsii* of elephant's foot yam. They found that the combination of corm and soil treatment with *T. harzianum* revealed the lowest disease incidence (12.9%) among all the treatments. In all the treatments, the population density of *S. rolfsii* significantly decreased compared to the control. Among all the treatments, corm+soil treatment with *T. harzianum* recorded the lowest sclerotial density.

Pranab *et al.* (2002) studied the efficacy of *Trichoderma harzianum*, *T. viride*, and *T. koningii* for the management of collar rot of tomato, caused by *Sclerotium rolfsii*. It was determined under laboratory and field (Jorhat, Assam, India) conditions. Above the 3 *Trichoderma* spp., *T. harzianum* was the most inhibitory to *Sclerotium rolfsii* which showed 61.5% inhibition in mycelial growth of the pathogen. Soil application of *Trichoderma* spp. inoculums at the time of transplanting, reduced disease incidence, and increased dry mass of root and shoots (g/plant) and yield.

Siddique *et al.* (2002) conducted an experiment to find out the response of ten eggplant varieties to the attack of *Sclerotium rolfsii* and to study the development of foot rot. They graded Mirsarai-1 as resistant at early flowering stage and Dohazari and Singnath at peak flowering stage.

Chowdhury *et al.* (2000) reported that seed treatment with *Trichoderma harzianum* and *Gliocladium viride* against *Sclerotium rolfsii* resulted up to 21.61% and 48.43% increase in germination in mungbean, black gram, pigeon pea and tomato, respectively and showed good effect on seed borne micro-flora. Moreover, significant growth enhancement of mungbean, black gram and tomato had been achieved by treating seeds with antagonists. The two antagonists were found effective against *Sclerotium rolfsii.*

Das *et al.* (2000) reported that the antagonistic fungi, namely *Trichoderma harzianum*, *T. viride* and *T. koningii* were evaluated *in vitro* condition against *Sclerotium rolfsii*, causing collar rot of tomato. *Trichoderma harzianum* was the most effective in inhibiting the mycelial growth in dual culture. Application of cultures of *Trichoderma* spp. in a field experiment with tomato conducted during 1998-99, in Assam, India reduced disease incidence in all the treatments. Among the 3 antagonists, *T. harzianum* resulted in maximum reduction of disease incidence and increased yield per plant.

Mathivanam *et al.* (2000) reported that a strain of *Trichoderma viride* with high antagonistic potential against *Rhizoctonia solani* and *Sclerotium rolfsii* (*Corticium rolfsii*) in dual culture was isolated from soil. Treating seeds with high doses of bio-fungicide (50 or 100 g/kg seeds) did not inhibit germination. The bio-fungicide and chemical fungicides (captan, carbendazium and copper oxychloride) were evaluated for the management of root disease of eggplant and sunflower in naturally infested soil in Andhra Pradesh, India. Seed treatment followed by soil application of the bio-fungicide significantly reduced plant mortality caused by root

pathogens and increased yield compared to chemical fungicides and untreated controls.

Begum *et al.* (1999) studied the interaction among the isolates of *T. harzianum* and the isolates of *S. rolfsii* on PDA resulted that *T. harzianum* parasitized and lysed the mycelia and sclerotia of *S. rolfsii* within 12 days. In pot experiment, *T. harzianum* ($2x10^6$ conidia/seed) treated seeds of black gram cv. Binamash-1 gave 86.7% to 100% reduction of foot rot diseases caused by *S. rolfsii* over the control (untreated) over the soil that artificially inoculated with *S. rolfsii* for the development of foot and root rot. *Trichoderma* had no adverse effect on germination of legume seeds, rather increased germination up to 66.67%.

Panday and Upadhyay (1999) conducted experiments in the glasshouse to evaluate the comparative performance of chemical, biological and integrated control of wilt of pigeon pea caused by *Fusarium* sp. In chemical control, Bavistin [carbendazin] was highly effective, while *Trichoderma viride* and *Trichoderma harzianum*-C isolates were best among the bio-control agents tested (*Trichoderma viride*, *Gliocladium virens* and *Trichoderma harzianum* C and D isolates). Thus seed coating with bio-agents proved better and safe for the management of wilt of pigeon pea.

Prasad *et al.* (1999) tested fourteen isolates of *Trichoderma* and *Gliocladium* species *in-vitro* against *Sclerotium rolfsii* (*Corticium rolfsii*) the causal organism of root and collar rot of sunflower. Two isolates of *Trichoderma viride*, four isolates of *Trichoderma harzianum*, one each of *Trichoderma hamatum*, *Trichoderma koningii*, *Trichoderma polysporum*, *Gliocladium virens*, *Gliocladium deliquescens* significantly inhibited mycelial growth of the pathogen.

Sultana and Hossain (1999) conducted an experiment on biological control of foot and root rot of lentil with *Trichoderma harzianum*. They reported that *Trichoderma harzianum* controlled foot and root rot of lentil caused by *F. oxysporum* and *S.*

rolfsii under field condition. Seeds of lentil treated with *Trichoderma harzianum* ($2x10^6$conidia/seed) contributed 47.85% to 112.49% reduction of foot and root rot infected plants over control. *Trichoderma harzianum* treated seeds increased germination up to 13.37% and resulted up to 3.69% more field emergence over control. *Trichoderma* treated seeds resulted seed yield up to 1783.33 kg/ha that revealed 81.60% higher yield.

Hiremath *et al.* (1998) studied on the production of biological control agents to control root rot and collar rot diseases of rainfed crops caused by *Sclerotium and Fusarium. Trichoderma* was successfully produced and used for the control of these diseases.

Rekha *et al*. (1998) showed that *Trichoderma viride* (5 g/kg soil) reduced the harmful effects of *Meloidogyne incognita* and improved germination of tomato in pot experiment.

Begum (1997) selected four *Trichoderma* spp. and evaluated their antagonistic potential against the major soil-borne plant pathogens *Sclerotium rolfsii*, *Fusarium oxysporum* and *Macrophomina phaseolina*. Two induced mutants of *Trichoderma* spp. showed better performances than control strain in reducing the seedling mortality in chickpea and lentil caused by *Fusarium oxysporum* and *Sclerotium rolfsii* under glasshouse condition.

Roberti *et al.* (1996) investigated the activity of *Trichoderma harzianum* 74 on bean (*Phaseolus vulgaris*) rot caused by *Sclerotium rolfsii* when applied to seeds. *Trichoderma* strains were active in bean root rot ensuring control of *Sclerotium rolfsii*. *Trichoderma harzianum* reduced the growth of *Sclerotium rolfsii* and parasitized *Sclerotium rolfsii* hyphae by direct contact, forming coils, short contract branches and hook-shaped hyphal tips.

Iqbal *et al*. (1995) showed that *Trichoderma harzianum* significantly inhibited the mycelial growth of *Sclerotium rolfsii*, overlapped the pathogen and suppressed growth by up to 63.6%.

Mukherjee *et al*. (1995) observed that *Trichoderma harzianum* was effective in suppressing *Sclerotium rolfsii* and *Rhizoctonia solani. Trichoderma harzianum* was found to be effective in destroying the sclerotia of both fungi.

Mukhopadhyay (1995) used *Trichoderma harzianum* for treating various crop seeds like chickpea and lentil for protection against a wide range of soil borne pathogens viz. *Sclerotium rolfsii*, *Fusarium oxysporum* and *Rhizoctonia solani*. The bio-agent proliferates on the seed coat of the germinating seeds and colonizes the additional plant parts such as root and collar region. The treatment is quite inexpensive and eco-friendly as compared to other methods of disease control.

Chet and Inbar (1994) studied on biological control of fungal pathogens and reported that *T. harzianum* as effective bio-control agent of soil-borne plant pathogenic fungi. Lectins were found to be involved in the recognition between *Trichoderma* spp. and its host fungi, whereas Chitinase is involved in the degradation of the host wall.

Inbar *et al*. (1994) applied *Trichoderma harzianum* to cucumber seedlings as a peat-bran preparation incorporated into the propagation mixture in a commercial plant production nursery. Increase of 23.8% in seedlings height and 96.1% in leaf area were recorded. On marketing day (after 18 days and 30 days) recorded significant dry weight compared with untreated control plants. *Trichoderma*-treated seedlings were more developed, grew more vigorously and contained higher levels of chlorophyll than control plants. No significant differences were found in N, P or K content between treatments. Cucumber seedlings which were transplanted to a commercial greenhouse were analyzed over 2 successive growth cycles following soil fumigation with methyl bromide (500 kg/ha). Results revealed that the

Trichoderma treated plants were more resistant to foot rot caused by *Sclerotium rolfsii*.

Inbar *et al*. (1994) further reported that significant increase of 23.8% and 17.2% in seedling height, 26.1% and 50% in leaf area and 24.7% and 28.6% in plant dry weight was achieved by *Trichoderma harzianum*. *Trichoderma* treated seedlings were much more developed and vigorous and had higher chlorophyll contents.

Kulkarni and Kulkarni (1994) studied on biological control of *S. rolfsii*, a causal agent of stem rot of ground nut and reported that seed treatment with *Trichoderma harzianum* reduced seedling mortality effectively than other bio-control agent. They observed soil drenching was more effective than seed treatment.

Tverdyukev *et al*. (1994) observed that *Trichoderma* produced Trichodermin, which showed its antagonistic activity against various diseases.

Sugha *et al*. (1993) reported that conidial coating of the antagonistic *Trichoderma harzianum* and *T. viride* on seeds significantly reduced seedling mortality (47-65%) infected by *Sclerotium rolfsii* compared with untreated controls.

Xu *et al*. (1993) observed that both isolates of *Trichoderma* T82 and NF9 inhibited hyphal growth of *Sclerotium rolfsii*, *Rhizoctonia solani*, *Pythium aphanidermatum*, *P. spinosum* and *Fusarium oxysporum*. In greenhouse experiments, soil treatment with 0.6 % (w/w) T82 bran culture (10^7c.f.u./g) reduced incidence of disease caused by *S. rolfsii*, *R. solani* and *P. aphanidermatum* by 46.5%, 28.4% and 81.2% respectively, 20 days after inoculation with the pathogens. Seed treatment with T82 or NF9 spore suspension (10^8c.f.u./ml) increased emergence of cucumber seedlings by 14% and 20%, respectively, 11 days after inoculation with *S. rolfsii*.

Fakir *et al.* (1991) reported that sowing of lentil during third week of November was found to reduce the incidence of foot and root rot caused by *Sclerotium rolfsii*

and *Fusarium oxysporum* compared to early sowing. Artificial inoculation of ten selected genotypes of lentil to foot rot pathogen, *Sclerotium rolfsii* showed that all the lines were susceptible to the test pathogen.

Monaco *et al*. (1991) used *Trichoderma* for treating seeds as bio-control agents of *Fusarium* and *Sclerotium* and found *Trichoderma* were effective against *Fusarium spp*. and *Sclerotium rolfsii* (*Corticium rolfsii*) *in-vitro* and in subsequent field trials. Seedling emergence was significantly increased when *Trichoderma harzianum* were applied to seeds sown in soil infected with the pathogens. They also reported that each *Trichoderma harzianum* was effective against *Fusarium oxysporum* and *Sclerotium rolfsii*, while *Trichoderm koningii* was not effective against *Sclerotium rolfsii.*

Sugha *et al.* (1991) reported that *Sclerotium rolfsii* caused collar rot of chickpea. A total of 210 lines and cultivars of chickpea tested by placing one wheat grain fully covered with mycelium of *S. rolfsii* at the collar rot of a 7 day old seedling in pot of sterilized garden soil. All were found to be susceptible to collar rot.

Sugha *et al.* (1990) earlier reported collar rot of brinjal from India. Out of 48 brinjal cultivars evaluated for resistance to *Sclerotiumn rolfsii*, four cultivars (BL-l, BWR-54, PBR 91-2 and Pusa Bhairav) were immune, 2 (local brinjal and selection 10-2-56) were resistant with a disease incidence of <20%, 3 [Manjari-Gota, Perennial brinjal and SM-6-7 (PF)] were moderately susceptible (Disease incidence 21-50%) and 39 cultivars were highly susceptible.

Ordentlich and Chet (1989) conducted an experiment in green house and found that *Trichoderma harzianum* obtained from field soils were effective for controlling diseases of various crops caused by *Sclerotium rolfsii* and *Fusarium* when grown in a semisolid fermentation medium on wheat bran: peat.

Krutova (1987) reported from the laboratory and field experiment that *Trichoderma harzianum* showed hyperparasitic activity on sclerotia of *Sclerotium* and were capable of destroying its sclerotia in soil.

Jacobs and Kamon (1986) found that *Trichoderma harzianum* produced cell wall lysing enzymes which antagonized against plant pathogens and improved biological control.

Mutto *et al*. (1986) reported that hyphae of *Trichoderma harzianum* developed in the medulla of *S. rolfsii* sclerotia, growing on the inside of the cell walls and in the lumen, the cytoplasm of penetrated cells rapidly degenerated. The hyperparasite passed from cells were also parasitized, with wall lyses and digestion of cell cytoplasm.

Upadhyay and Mukhopadhyay (1986) reported that *Trichoderma harzianum* isolate directly attacked and lyses the mycelium and sclerotia of *Sclerotium rolfsii* in dual culture. In the greenhouse, applications of *T. harzianum* as infested sorghum grains of *S. rolfsii* infested soil give up to 76% and 88% disease control in first and second cycles of sugarbeet seedlings, respectively. The degree of control increased with increasing amount of *T. harzianum* inoculum.

Ahmed and Hossain (1985) reported that collar rot, foot and root rot disease caused by *Sclerotium rolfsii* caused considerable damage both in seedling and adult stages of Indian spinach, and there existed variations in the incidence of the disease in different parts of Bangladesh.

Ferrata and Amba (1985) observed that *Trichoderma harzianum* isolate showed low ability in coiling round hyphae of *Sclerotium rolfsii*, but was very effective in penetrating or growing inside them. Hyphae of *T. harzianum* adversely affected them even without penetration.

Elad *et al*. (1983) studied the parasitism of *Trichoderma harzianum* to the soil borne plant pathogen, *Sclerotium rolfsii*. They observed that hyphae of the parasites contact with their host, either producing appressorium like bodies or coiling around the hyphae, then enzymatically digest host cell walls. Extra cellular febrile material deposited between the interacting cells. Parasite organelles (mitochondria, vesicles and dark osmiophilic inclusions) accumulate in the parasitizing cells. In response to invasion, the host produces a sheath matrix which encapsulates the penetration hyphae and the host cells become empty of cytoplasm.

Henis *et al*. (1982) conducted an experiment and found that *Trichoderma* produced volatile and non-volatile antibiotics which are active against *Sclerotium rolfsii* and also inhibited the sclerotial germination.

Elad *et al*. (1982) stated that *Trichoderma harzianum* excreted β-1, 3-glucanase and chitinase which showed high antagonistic activity to control soil borne pathogen, especially *Sclerotium rolfsii.*

Elad *et al*. (1980) evaluated that *Trichoderma harzianum* in wheat bran preparation for controlling bean diseases caused by *S. roifsii* and *R. solani* and found that *T. harzianum* significantly reduced the diseases. They also observed that the wheat bran preparation of *T. harzianum* increased growth of bean plants in a non-infested soil and it controlled *S. rolfsii* more efficiently than a conidial suspension of the same antagonist.

Agrawal *et al.* (1977) studied the biological control of *Sclerotium rolfsii* causing collar rot of lentil and found that *Trichoderma harzianum* were antagonistic against *Sclerotium rolfsii*. In pot experiment, the antagonist *Trichoderma harzianum* controlled the death caused by *Sclerotium rolfsii.*

Agrawal *et al*. (1977) found *Trichoderma harzianum* as antagonistic against *Sclerotium rolfsii*. They reported that filtrates of *Trichoderma* inhibited the growth of *S. rolfsii* on PDA but effectiveness decreased with dilution. In pot experiment the antagonist controlled seedling death. Culture was more effective when applied to seed rather than soil.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Site

The experiment was conducted at the Plant Pathology laboratory, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

3.2 Experimental Period

The experiments were carried out from June 2014 to April 2015.

3.3 Experiments

Six sets of experiment were carried out during the study period to evaluate the efficacy of *Trichoderma harzianum* against *Sclerotium rolfsii* of some important vegetables.

3.3.1 Experiment 1: Study of local *Trichoderma* isolate

3.3.1.1 Collection of soil samples

For isolation of *Trichoderma* spp. five soil samples were collected from different vegetable field at a depth of 5-10 cm of soil surfaces in greater Dinajpur district. All the soil samples collected from different plants were mixed together and taken in to a plastic bag with proper labeling and stored in a refrigerator (4^0C) in the laboratory.

3.3.1.2 Isolation, purification and preservation of *Trichoderma* isolate

Trichoderma spp. was isolated from soil following dilution plate technique (Subba, 2003). One gram of soil sample was taken in a test tube containing 9 ml of sterilized water to make 1:10 dilution. Then 1 ml suspension was taken in another test tube containing 9 ml of sterilized water to make 1: 100 dilution. Similarly a series of dilution process were continued until the samples were diluted to 1:10000. All working samples were diluted in the same process. A number of PDA plates were prepared in aseptic condition in the laboratory. 1ml soil suspension from sample was placed in each petri-plate. The soil suspension was thoroughly mixed

with the medium using a glass spreader. The petri-plates were incubated at 25 ± 2^0 C for 5-7 days. After incubation, *Trichoderma* was identified from other fungi based on color, size, shape and appearance of colony.

After 3 days of incubation, plates were observed for *Trichoderma* colony. The growing margin of *Trichoderma* colony was cut into 5 mm blocks with the help of a cork borer. The blocks were carefully placed in PDA plates and incubated at 25 ± 2^0 C for 5-7 days. Hyphal tip/mycelia block of *Trichoderma* were transferred to PDA for purification. The well-developed pure cultures of *Trichoderma* was sub-cultured in PDA plates for further use.

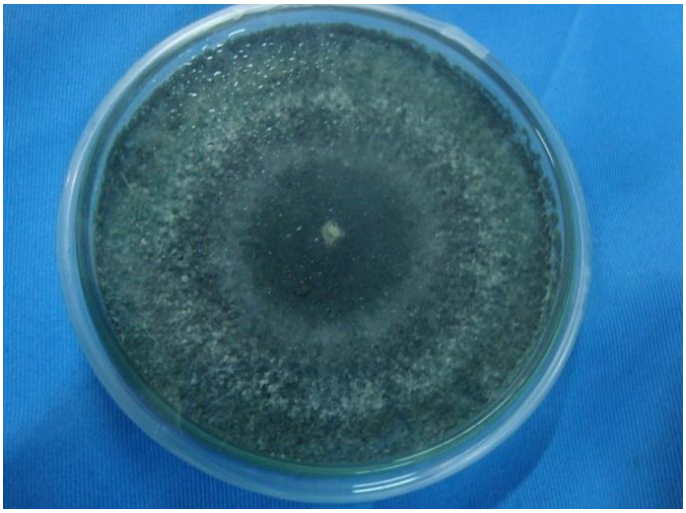


Plate 1. Pure culture of *Trichoderma* isolate (7 days old)

3.3.1.3 Identification of *Trichoderma* isolate by morphological characters

For observing mycelial growth and colony characteristics 5-7 mm mycelial disc of 7 days old pure culture of *Trichoderma* was placed at center of the Petridishes on PDA media. The dishes were then kept for incubation at 25 ± 2^0 C. Mycelial growth was observed after 24 hours intervals until *Trichoderma* colony covered the whole petridish. The microscopic observation was made from slide preparations stained with cotton blue. The isolates were identified up to species level based on phenotypic characters like colony shape, growth habit, conidiophores, phialides,

and conidia. The isolate was identified according to the key of Kubicek and Harman (1998).

3.3.2 Experiment 2: Study of *Sclerotium rolfsii* isolate

3.3.2.1 Collection, isolation and purification of *Sclerotium rolfsii*

The pathogen (*Sclerotium rolfsii)* was collected from naturally infected eggplant (*Solanum melongena* L.) grown in the experimental field of the Department of Plant Pathology, HSTU, Dinajpur. The specimen was washed with tap water to remove sand and soil particles. Then specimen was cut into small pieces (1cm) along with healthy and dead tissues. The pieces were surface sterilized with 1% Clorox, washed thrice in sterilized water and placed on filter paper to remove excess water adhering with the pieces. Thereafter, the pieces were placed in PDA plates and incubated at 25 ± 2^0 C for 7 days and observed regularly to see the growth of fungi. The fungus was purified by hyphal tip culture technique (Tuite, 1969). The pure culture of the isolates of *Sclerotium rolfsii* was preserved in PDA plates in refrigerator (4^0C) for future use.

Plate 2. Symptom showing foot rot caused by *S. rolfsii* on eggplant

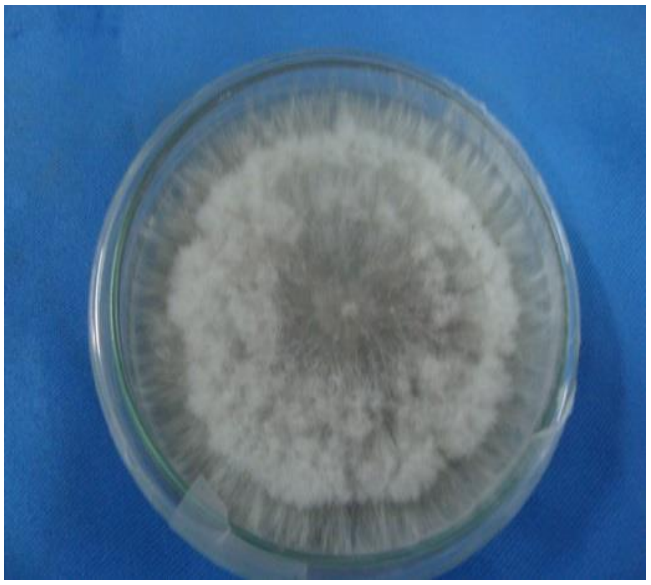


Plate 3. Pure culture of *Sclerotium rolfsii* in PDA media (7 days old)

Plate 4. Mature culture of *Sclerotium rolfsii* with Sclerotia in PDA media (14 days old)

3.3.2.2 Identification of *Sclerotium rolfsii* by morphological characters

Circular disc of 5mm diameter from the margin of 5-7 days old pure culture was placed in the center of the plate on PDA media under aseptic condition. The plates were incubated at 25 ± 2^0C. Morphological characteristics such as mycelial growth, colony morphology, sclerotial production, size and shape and color of sclerotia were studied. Mycelial growth and colony morphology were observed after 24 hours upto 7 days of inoculation, while colour of sclerotia was recorded 14 days after inoculation. The isolate was identified following the keys outlined by Aycock (1996) and Barnett (1960).

3.3.3 Experiment 3: Comparative bioassay of the local *Trichoderma* isolate and RDA (Rural Development Academy, Bogra) *Trichoderma* isolate against *S. rolfsii* by dual culture technique

Two *in-vitro* tests were conducted to find out the antagonistic effect of local *Trichoderma* isolate and RDA *Trichoderma* isolate against *Sclerotium rolfsii* on PDA by dual culture technique (Dhingra and Sinclair, 1985). One mycelia disc of 5 mm size picked by sterilized block cutter from 7 days old culture of local *Trichoderma* isolate and one disc (same size and age) of a *Sclerotium rolfsii* were placed simultaneously on the age of each petri-plate at opposite direction.The plates which received only discs of *Sclerotium rolfsii* treated as control. The plates were incubated in the laboratory having ambient temperature at 25^0 C. Inhibition zone was measured on the day when control plate was full (Nene and Thaplial, 1993). On the other hand, RDA *Trichoderma* was isolated from RDA (Rural Development Academy, Bogra) formulated bio-fungicides following dilution plate technique (Subba, 2003) and purified. For observing the antagonistic effect in case of RDA *Trichoderma* the above mentioned procedure was followed as dual culture. Thereafter, percentage inhibition of *Sclerotium rolfsii* was calculated based on the growth of the pathogen on PDA plate following the formula as suggested by Sundar *et al.* (1995).

% growth inhibition = X-Y/X×100

Where,

X = Mycelial growth of pathogen in absence of *Trichoderma*

Y = Mycelial growth of pathogen in presence of *Trichoderma*

3.3.4 Experiment 4: Determination of effective dose of *Sclerotium rolfsii* on seed germination and seedling diseases of eggplant, tomato, bean, cabbage and indian spinach in tray soil

3.3.4.1 Preparation of *Sclerotium rolfsii* inocula

Inocula of *Sclerotium rolfsii* were prepared by growing the pathogen in wheat grain. Wheat grains collected from market were thoroughly washed water and soaked in water for 12 hrs. After soaking, excess water was decanted and wheat grains were taken in 500ml erlenmeyer flask at the rate of 100g in each. The flasks were plugged with cotton followed by wrapping the mouth with brown paper. The flasks containing moist wheat grains were sterilized in autoclave at 121°C with 15 psi for 15 minutes (Yaqub and Shahzad, 2005). The conical flask containing autoclaved wheat grains were brought out and allowed to cool at room temperature. Then 10 mycelial blocks (5mm) cut from the edge of 3 days old of pure culture of *Sclerotium rolfsii* were added to the flask and incubated at room temperature for 7-10 days. The flasks were shaken thoroughly by hand at every 3 days for proper distribution of fungal mycelia throughout the entire mass of the inoculated wheat grains. The colonized wheat grains were air dried at room temperature and used for inoculation purpose (Babar, 1999).

Plate 5. Inocula of *Sclerotium rolfsii* (7 days old culture)

Plate 6. Inocula of *Sclerotium rolfsii* after air drying

3.3.4.2 Sterilization of soil and preparation of trays

Soil for raising seedlings in plastic tray was prepared by mixing soil, sand and well decomposed cow dung in the proportion of 2:1:1 and sterilized with 5 ml formalin (40%) diluted with 20 ml water for 4 kg soil (Dashgupta, 1988). The prepared soil was heaped like a square block. Soil heap was covered by a polythene sheet for 48

hours. After 7 days, surface sterilized plastic trays (35×25 cm^2) were filled up with the sterilized soil.

3.3.4.3 Experimental Design and treatments

The experiment was laid out in CRD with three replications. There are four treatments including control:

T_0 = Control (Only sterilized soil)

T_1 = *Sclerotium rolfsii* inocula 10 g/kg soil

T_2 = *Sclerotium rolfsii* inocula 15 g/kg soil

T_3 = *Sclerotium rolfsii* inocula 20 g/kg soil

3.3.4.4 Inoculation of tray soil with *S. rolfsii*

Tray soil was inoculated with different doses of *S. rolfsii* inocula (10 g/kg soil, 15 g/kg soil and 20 g/kg soil). The inocula were thoroughly mixed with soil and covered with polythene sheet to maintain moisture for proper growth of *Sclerotium rolfsii*.

Plate 7. Growth of *Sclerotium rolfsii* in inoculated tray soil

3.3.4.5 Sowing of seeds

After 3 days of inoculation of *S. rolfsii*, fifty seeds of each eggplant, tomato, bean, cabbage and Indian spinach were sown in each tray. Seeds were sown in a diametric line and labeled by a permanent marker. Watering was done to maintain the soil moisture.

3.3.4.6 Recording of data

Disease incidence was observed regularly and recorded at 7, 14 days after sowing to estimate the effect of *S. rolfsii* on the following parameters.

a) Percent seed germination
b) Percent pre-emergence death of seed
c) Percent damping off of seedlings
d) Percent foot rot of seedlings

3.3.5 Experiment 5: Determination of effective dose of formulated *Trichoderma* on seed germination and seedling diseases of eggplant, tomato, bean, cabbage and indian spinach in tray soil

3.3.5.1 Formulation of *Trichoderma* isolate

Black gram bran and water in 1:2 ratio were explored for the multiplication and formulation of *Trichoderma*. The requisite amount of materials for each substrates (Black gram bran: water =1:2) were thoroughly mixed in a 500 ml Erlenmeyer flask and autoclaved at l21°C for 15 minutes for sterilization. The sterilized substrate allowed to cool down and then inoculated with 5 mm mycelial disc of 7 days old *Trichoderma* culture. Ten discs for each flask were used for inoculation. Inoculated flasks were then incubated at room temperature (25°C ±2). After incubation for 20 days; the contents were taken out from the flasks and air dried in laminar airflow cabinet. The air dried materials were kept in polythene bag with labeling and treated as formulated.

Plate 8. Formulated *Trichoderma* inocula used as bio-fungicides

Plate 9. Air drying of formulated *Trichoderma* spp.

Plate 10. Formulated *Trichoderma* as packing form

3.3.5.2 Sterilization of soil and preparation of trays

Same as described in 3.3.4.2

3.3.5.3 Experimental Design and treatments

The experiment was laid out in CRD with three replications. There are four treatments including control:

T_0 = Control (Only pathogen)

T_1 = Formulated *Trichoderma* 10 g/kg soil

T_2 = Formulated *Trichoderma* 15 g/kg soil

T_3 = Formulated *Trichoderma* 20 g/kg soil

3.3.5.4 Inoculation of tray soil with *S. rolfsii*

Tray soil was inoculated with *S. rolfsii* inocula at 10 g/kg of soil. The inocula were thoroughly mixed with soil and covered with polythene sheet to maintain moisture for proper growth of *Sclerotium rolfsii*.

3.3.5.5 Treatment of tray soil with formulated *Trichoderma*

After 3 days of inoculation, different doses of formulated *Trichoderma* (10 g/kg soil, 15 g/kg soil and 20g/kg soil) were thoroughly mixed with soil.

Plate 11. Soil treated with formulated *Trichoderma* after inoculation of *Sclerotium rolfsii*

3.3.5.6 Sowing of seeds

Fifty seeds of each eggplant, tomato, bean, cabbage and indian spinach were sown in each tray just after mixing with formulated *Trichoderma* in a diametric line and labeled by a permanent marker. Watering was done to maintain the soil moisture.

3.3.5.7 Recording of data

Diseases incidence was observed regularly and recorded at 7, 14 days after sowing to estimate the effect of *S. rolfsii* on the following parameters.

a) Percent seed germination
b) Percent pre-emergence death of seed
c) Percent damping off of seedlings
d) Percent foot rot of seedlings

3.3.6 Experiment 6: Determination of effective dose of *Trichoderma* suspension on seed germination and seedling diseases of eggplant, tomato, bean, cabbage and Indian spinach in tray soil

3.3.6.1 Sterilization of soil and preparation of trays

Same as described in 3.3.4.2

3.3.6.2 Experimental Design and treatments.

The experiment was laid out in CRD with three replications. There are four treatments including control:

T_0 = Control (Only pathogen)

T_1 = *Trichoderma* suspension 5.02×10^4CFU/ml seeds

T_2 = *Trichoderma* suspension 5.02×10^5CFU/ml seeds

T_3 = *Trichoderma* suspension 5.02×10^6CFU/ml seeds

3.3.6.3 Inoculation of tray soil with *S. rolfsii*

Same as described in 3.3.5.4

3.3.6.4 Preparation of *Trichoderma* suspension

Trichoderma suspension was prepared by using 7 days old pure culture of *Trichoderma* isolates. 30 ml sterilized water was poured into a PDA plate and the conidial suspension was made by scraping the spore masses on the medium. The conidial suspension was then taken in a beaker containing 400 ml water and one drop of Tween-20 was added and stirred for 15 minutes. Then the number of conidia per ml was determined by using Haemocytometer following the procedure of Ashrafuzzaman (1976).

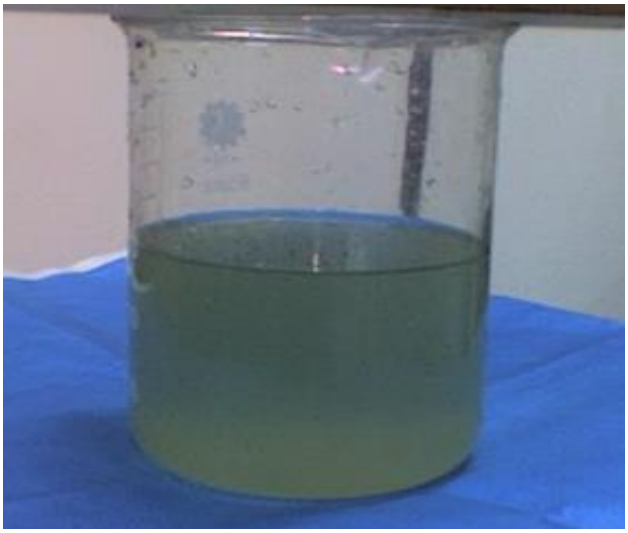


Plate 12. Conidial suspension of *Trichoderma* in beaker

3.3.6.5 Seed treatment with different concentration of conidial suspension of *Trichoderma*

The spore density was determined 5.02×10^6 CFU/ml. Before sowing of seeds in tray, seeds were treated with different concentration of conidial suspension of *Trichoderma* (5.02×10^6 CFU/ml, 5.02×10^5 CFU/ml and 5.02×10^4 CFU/ml) for 6 hours.

3.3.6.6 Sowing of seeds

Fifty treated seeds of each eggplant, tomato, bean, cabbage and indian spinach were sown in each tray in a diametric line and labeled by a permanent marker. Watering was done to maintain the soil moisture.

3.3.6.7 Recording of data

Diseases incidence was observed regularly and recorded at 7, 14 days after sowing to estimate the effect of *S. rolfsii* on the following parameters.

a) Percent seed germination
b) Percent pre-emergence death of seed
c) Percent damping off of seedlings
d) Percent foot rot of seedlings

3.7 Statistical analysis

The experiment was conducted using a complete randomized design (CRD) with three replications. The data were statistically analyzed with the help of the computer package an MSTATC and also tested by Duncan's New Multiple Range Test (DMRT).

CHAPTER 4

RESULTS

4.1 Study of indigenous *Trichoderma* isolate

4.1.1 Collection, isolation and purification of isolate of *Trichoderma* spp.

Soil samples were collected from different locations in Dinajpur district. *Trichoderma* were isolated from rhizosphere soil of indian spinach following dilution plate technique and purified. The isolate was identified according to the key of Kubicek and Harman (1998).

4.1.2 Identification of *Trichoderma* isolate by morphological characters

Mycelial growth and colony characters of *Trichoderma* isolate were studied using 7 days old PDA cultures incubated at 25 ± 2^0 C. The following morphological characters were observed.

Nature of mycelia growth -	First growing
Colony colour-	Dark brown formed a concentric ring
Colony shape-	Regular
Conidiophore-	Branched which terminates with one or a few phialides
Phialides-	Flask-shape and cylindrical or nearly subglobose which bears conidia
Conidial shape-	Ellipsoidal or globose

From the above mentioned characteristics the isolate was identified as *Trichoderma harzianum* according to the key of Kubicek and Harman (1998). (Plate 15 & 16)

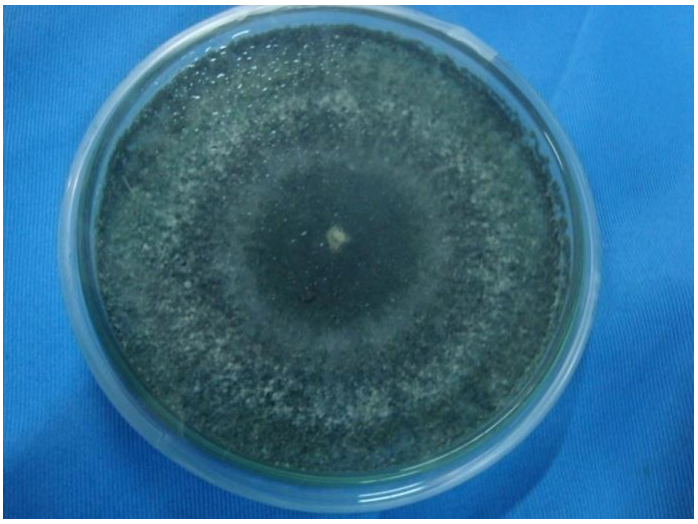


Plate 13. *Trichoderma* isolate of dark green colour (7 days old culture)

Plate 14. Conidiophores, phialides and conidia of *Trichoderma harzianum* observed under microscope

4.2 Study of *Sclerotium rolfsii* isolate

4.2.1 Collection, isolation, purification of isolate of *Sclerotium rolfsii*

The pathogen (*Sclerotium rolfsii*) was isolated from naturally infected eggplant (*Solanum melongena L.*) grown in the experimental field of the Department of Plant Pathology, HSTU, Dinajpur and purified by hyphal tip culture technique The isolate *was* identified according to the key of Aycock (1996) and Barnett (1960).

4.2.2 Identification of *Sclerotium rolfsii* isolate by morphological characters

After 24 hours the fungus grew out from the edge of mycelial block. The fungus produced white cottony mycelium. After 4 days, the isolates covered above 50% of the surface area on petri-plate. Consequently, after 5 days of inoculation the prolific growth of fungus almost filled the petriplate and at 6 day highest mycelial growth (9 mm) was observed on the petriplate (Plate 17). The fungus produced sclerotia at the edges of the petri-plates from 7 days up to 10 days after inoculation. The sclerotia were small and uniformly round. The colour of sclerotia was dark brown at mature stage (Plate 18). At a glance the following morphological characteristics were observed.

Nature of mycelia growth-	Fast growing
Colony colour-	Whitish
Sclerotial size and shape-	Small and uniformly round
Sclerotial colour-	Reddish brown (early stage) and dark brown (Mature stage)

From the above mentioned characteristics the isolate was *S. rolfsii* according to the key of Aycock (1996) and Barnett (1960). (Plate 17 & 18)

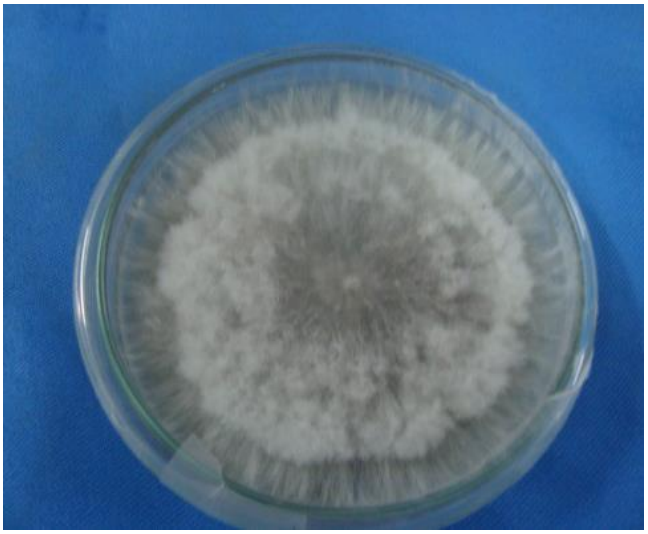


Plate 15. Mycelial growth of *Sclerotium rolfsii* in PDA media (7 days old culture)

Plate 16. Mycelial growth of *Sclerotium rolfsii* with Sclerotia in PDA media (14 days old culture)

4.3 Comparative bioassay of the local *Trichoderma* isolate and RDA (Rural Development Academy, Bogra) *Trichoderma* isolate against *S. rolfsii* by dual culture technique

The ability of local *Trichoderma* isolate and RDA isolate to inhibit the mycelial growth of *S. rolfsii* in dual culture were determined on PDA medium (Plate 19 & 20). After 6 days of inoculation when the control plates were full the bioagents produced significantly different inhibition zone against *S. rolfsii* in dual culture method. The percent inhibition of *S. rolfsii* was 92 % in case of local *Trichoderma* isolate and 85 % in case of RDA isolate. So the percent inhibition of local *Trichoderma* isolate was 7 % higher than the RDA isolate.

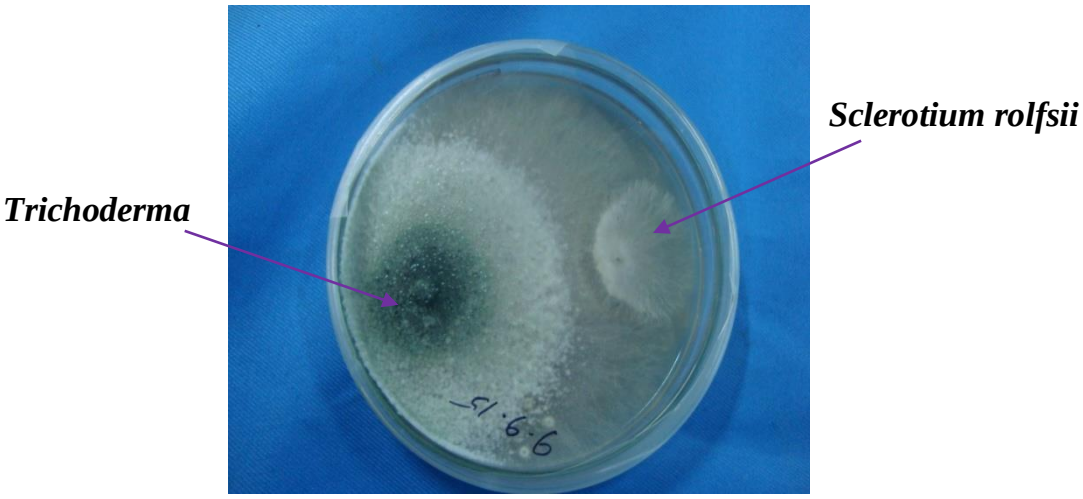


Plate 17. Effect of local *Trichoderma* isolate on *Sclerotium rolfsii* in dual culture (7 days old culture)

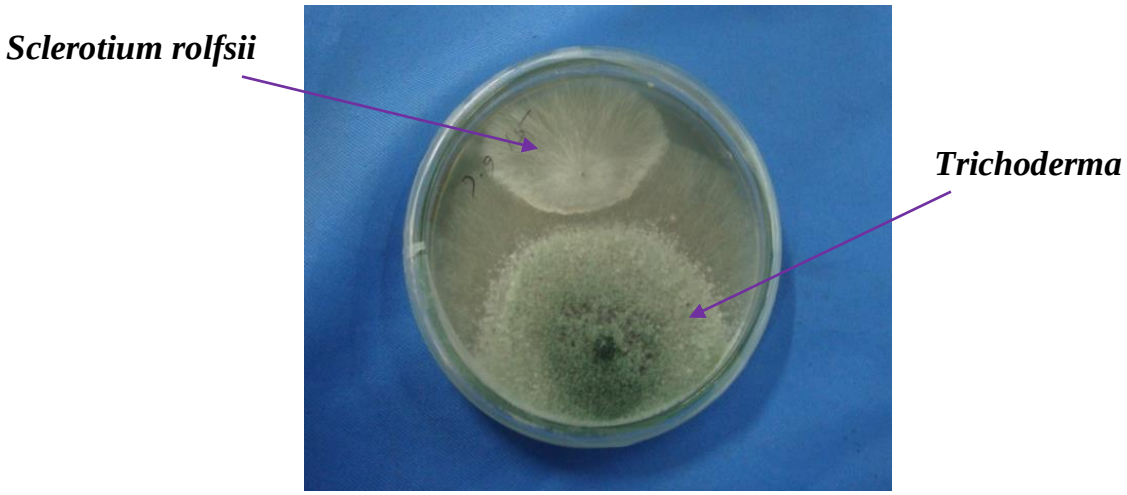


Plate 18. Effect of RDA *Trichoderma* isolate on *Sclerotium rolfsii* in dual culture (7 days old culture)

4.4 Determination of effective dose of *Sclerotium rolfsii* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach in tray soil

4.4.1 Effect of different doses of *Sclerotium rolfsii* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach seedlings at 7 DAS

Bean

The effect of different treatments on different parameters of bean seedlings at 7 DAS varied significantly in comparison to control (Table 1). At 7 DAS, the lowest germination (33.67%) was observed in T_3 (Plate 19 A) and the highest germination (90.67%) was observed in uninoculated control (T_0) (Plate 19 B). The second highest germination (65.33%) at the same days after sowing was recorded in T_1 which was statistically similar with treatment T_2 (57.33%). The highest percent pre-emergence death was recorded in T_3 (66.33%) and the lowest pre-emergence death was recorded in T_0 (9.33%). The highest damping-off was observed in T_3 (24.00%) and the lowest damping-off was recorded in control pots T_0 (6.67%). The percent

foot rot ranged from 0.00 to 27.33 where the highest was recorded inT_3 (27.33%) and foot rot was nil in control pots T_0 (0.00%).

Eggplant

At 7 DAS, the percent germination ranged from 33.33 to 84.00. The lowest germination was observed in T_3 (Plate 20 A) and the highest germination was recorded in uninoculated control (T_0) (Plate 20 B). The highest percent pre-emergence death was recorded in T_3 (66.67%) and the lowest pre-emergence death was observed in uninoculated control T_0 (16.00%). The highest was damping-off was observed in T_3 (16.67%) and the lowest damping-off was recorded in control pots T_0 (4.66%). The percent foot rot ranged from 0.00 to 22.00 where the highest was recorded inT_3 (22.00%) and foot rot was nil in control pots T_0 (Table1).

Tomato

The percent germination ranged from 54.67 to 86.00. The lowest germination was observed in T_3 and the highest germination was recorded in uninoculated control (T_0).There is no significance between T_1 and T_2. The highest percent pre-emergence death was recorded in T_3 (42.67%) and the lowest pre-emergence death was observed in uninoculated control T_0 (13.33%). The highest was damping-off was observed in T_3 (21.33%) and the lowest damping-off was recorded in control pots T_0 (5.33%). The percent foot rot ranged from 0.00 to 25.67 where the highest was recorded in T_3 (25.67%) and foot rot was nil in control pots T_0 (Table1).

Cabbage

The percent germination ranged from 45.00 to 85.00. The lowest germination was observed in T_3 (Plate 21 A) and the highest germination was recorded in uninoculated control (T_0) (Plate 21 B). The highest percent pre-emergence death was recorded in T_3 (53.00%) and the lowest pre-emergence death was observed in

uninoculated control T_0 (12.33%). The highest was damping-off was observed in T_3 (14.67%) followed by T_2 (18.00) and the lowest damping-off was recorded in control pots T_0 (1.33%). The percent foot rot ranged from 0.00 to 26.67 where the highest was recorded in T_3 (26.67%) and foot rot was nil in control pots (Table 1).

Indian Spinach

At 7 DAS, the percent germination ranged from 59.33 to 83.33. The lowest germination was observed in T_3 (Plate 22 A) followed by T_2 and T_1 and the highest germination was recorded in uninoculated control (T_0) (Plate 22 B). The highest percent pre-emergence death was recorded in T_3 (39.33%) and the lowest pre-emergence death was observed in uninoculated control T_0 (14.67%). The highest was damping-off was observed in T_3 (22.00%) and the lowest damping-off was recorded in control pots T_0 (3.33%). The percent foot rot ranged from 0.00 to 20.00 where the highest was recorded in T_3 (20.00%) and foot rot was nil in control pots (Table 1).

Table 1. Effect of different doses of *S. rolfsii* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach at 7 DAS

Variety	Treatments	Germination %	Pre-emergence death %	Damping-off %	Foot rot %
Bean	T_0	90.67 a	9.33 c	6.67 b	0.00 c
	T_1	65.33 b	34.67 b	13.33 ab	16.00 b
	T_2	57.33 b	42.67 b	17.33 ab	21.33 ab
	T_3	33.67 c	66.33 a	24.00 a	27.33 a
	LSD	17.14	17.14	11.30	8.560
Egg plant	T_0	84.00 a	16.00 d	4.66 c	0.00 c
	T_1	68.67 b	31.33 c	9.33 bc	14.00 b
	T_2	40.00 c	60.00 b	13.33 ab	19.33 ab
	T_3	33.33 d	66.67 a	16.67 a	22.00 a
	LSD	6.292	6.292	6.339	5.752
Tomato	T_0	86.00 a	13.33 b	5.33 b	0.00 c
	T_1	70.00 ab	30.00 ab	13.33 ab	16.67 b
	T_2	63.33 ab	36.67 ab	16.00 ab	19.33 b
	T_3	54.67 b	42.67 a	21.33 a	25.67 a
	LSD	22.57	22.57	10.31	4.892
Cabbage	T_0	85.00 a	12.33 c	1.33 b	0.00 c
	T_1	72.67 b	27.33 b	14.00 ab	17.00 b
	T_2	66.00 b	34.00 b	18.00 a	20.33 b
	T_3	45.00 c	53.00 a	14.67 a	26.67 a
	LSD	8.997	8.997	14.17	5.128
Indian spinach	T_0	83.33 a	14.67 b	3.33 c	0.00 c
	T_1	70.00 b	30.00 a	12.67 b	13.33 b
	T_2	64.00 b	36.00 a	16.67 ab	14.67 b
	T_3	59.33 b	39.33 a	22.00 a	20.00 a
	LSD	12.72	12.72	6.522	4.068

Values in a column with same letter (s) do not differ significantly whereas dissimilar letter(s) differ significantly at 5% level of significance.

T_0 = Control (Only sterilized soil)

T_1 = *Sclerotium rolfsii* inocula 10 g/kg soil

T_2 = *Sclerotium rolfsii* inocula 15 g/kg soil

T_3 = *Sclerotium rolfsii* inocula 20 g/kg soil

A

B

Plate 19. Bean seedlings in tray soil (A) *Sclerotium rolfsii* inoculated soil (B) control (only sterilized soil)

A

B

Plate 20: Eggplant seedlings in tray soil (A) *Sclerotium rolfsii* inoculated soil (B) control (only sterilized soil)

A

B

Plate 21. Cabbage seedlings in tray soil (A) *Sclerotium rolfsii* inoculated soil (B) control (only sterilized soil)

A

B

Plate 22: Indian spinach seedlings in tray soil (A) *Sclerotium rolfsii* inoculated soil (B) control (only sterilized soil)

4.4.2 Effect of different doses of *Sclerotium rolfsii* on germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach seedlings at 14 DAS

Bean

At 14 DAS, the percent germination ranged from 35.33 to 91.33. The lowest germination (35.33%) was observed in T_3 and the highest germination (91.33 %) was observed in uninoculated control (T_0). The highest percent pre-emergence death was recorded in T_3 (64.67%) and the lowest pre-emergence death was recorded in uninoculated control T_0 (8.67%). The highest damping-off was observed in T_3 (15.33%) and the lowest damping-off was recorded in control pots T_0 (4.67%). The percent foot rot ranged from 0.00 to 31.33 where the highest was recorded in T_3 (31.33%) followed by T_2 (27.67) and foot rot was nil in control pots T_0 (Table 2).

Eggplant

At 14 DAS, the percent germination ranged from 35.00 to 85.67. The lowest germination was observed in T_3 and the highest germination was recorded in uninoculated control (T_0). The highest percent pre-emergence death was recorded in T_3 (66.67.00%) and the lowest pre-emergence death was observed in uninoculated control T_0 (16.33%). The highest was damping-off was observed in T_3 (24.00%) and the lowest damping-off was recorded in control pots T_0 (6.00%). The percent foot rot ranged from 0.00 to 26.00 where the highest was recorded in T_3 (26.00%) and foot rot was nil in control pots T_0 (Table 2).

Tomato

The percent germination ranged from 57.33 to 86.67. The lowest germination was observed in T_3 followed by T_2 and the highest germination was recorded in uninoculated control (T_0). The highest percent pre-emergence death was recorded in T_3 (45.33%) which is statistically similar with T_2 (34.67) and the lowest pre-emergence death was observed in uninoculated control T_0 (14.00%). The highest was damping-off was observed in T_3 (26.00%) and the lowest damping-off was

recorded in control pots T_0 (5.33%). The percent foot rot ranged from 0.00 to 25.67 where the highest was recorded in T_3 (25.67%) and T_1.Foot rot was nil in control pots T_0 (Table 2).

Cabbage

The percent germination ranged from 47.00 to 87.00. The lowest germination was observed in T_3 and the highest germination was recorded in uninoculated control (T_0). The highest percent pre-emergence death was recorded in T_3 (55.00%) and the lowest pre-emergence death was observed in uninoculated control T_0 (15.00%). The highest was damping-off was observed in T_3 (17.33%) followed by T_2 and T_1 and the lowest damping-off was recorded in control pots T_0 (4.00%). The percent foot rot ranged from 0.00 to 26.67 where the highest was recorded in T_3 and T_1. Foot rot was nil in control pots (Table 2).

Indian Spinach

At 14 DAS, the percent germination ranged from 60.67 to 85.33. The lowest germination was observed in T_3 and the highest germination was recorded in uninoculated control (T_0). The highest percent pre-emergence death was recorded in T_3 (40.67%) and the lowest pre-emergence death was observed in uninoculated control T_0 (16.67%). The highest was damping-off was observed in T_3 which is statistically similar with T_2 and T_1. The lowest damping-off was recorded in control pots T_0.The percent foot rot ranged from 0.00 to 23.33 where the highest was recorded in T_3 (23.33%) and foot rot was nil in control pots (Table 2).

Table 2. Effect of different doses of *S. rolfsii* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach at 14 DAS

Variety	Treatments	Germination %	Pre-emergence death %	Damping-off %	Foot rot %
Bean	T_0	91.33 a	8.67 d	4.67 c	0.00 c
	T_1	66.00 b	34.00 c	8.67 bc	18.33 b
	T_2	46.67 c	53.33 b	12.67 ab	27.67 a
	T_3	35.33 d	64.67 a	15.33 a	31.33 a
	LSD	8.628	8.628	6.339	5.16
Egg plant	T_0	85.67 a	16.33 d	6.00 c	0.00 d
	T_1	70.00 b	30.00 c	13.33 b	16.67 c
	T_2	44.00 c	56.00 b	20.00 ab	21.33 b
	T_3	35.00 d	66.67 a	24.00 a	26.00 a
	LSD	5.979	5.979	6.875	4.48
Tomato	T_0	86.67 a	14.00 b	5.33 c	0.00 c
	T_1	72.00 ab	28.00 ab	14.33 bc	15.00 b
	T_2	65.33 b	34.67 a	22.67 ab	21.00 ab
	T_3	57.33 b	45.33 a	26.00 a	25.67 a
	LSD	18.85	18.85	9.738	6.34
Cabbage	T_0	87.00 a	15.00 c	4.00 c	0.00 c
	T_1	74.00 b	26.00 b	20.67 a	14.67 b
	T_2	68.00 b	32.00 b	18.00 ab	20.67 ab
	T_3	47.00 c	55.00 a	17.33 b	26.67 a
	LSD	9.079	9.335	5.543	6.52
Indian spinach	T_0	85.33 a	16.67 c	3.33 b	0.00 c
	T_1	71.33 b	28.67 b	18.67 a	16.00 b
	T_2	64.67 bc	35.33 ab	22.00 a	15.33 b
	T_3	60.67 c	40.67 a	22.67 a	23.33 a
	LSD	10.02	10.02	9.030	6.70

Values in a column with same letter (s) do not differ significantly whereas dissimilar letter(s) differ significantly at 5% level of significance.

T_0 = Control (Only sterilized soil)

T_1 = *Sclerotium rolfsii* inocula 10 g/kg soil

T_2 = *Sclerotium rolfsii* inocula 15 g/kg soil

T_3 = *Sclerotium rolfsii* inocula 20 g/kg soil

4.5 Determination of effective dose of formulated *Trichoderma* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach in tray soil

4.5.1 Effect of different doses of formulated *Trichoderma* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach in tray soil at 7 DAS

Germination, pre-emergence death, damping-off and foot rot of bean, eggplant, tomato, cabbage and indian spinach were significantly influenced by different doses of formulated *Trichoderma* against *S. rolfsii* (Table 3). The highest germination of bean (85.33%), eggplant (84.67%), tomato (88.67%), cabbage (85.00%) and indian spinach (86.00%) were recorded in T_3 (Plate 23 B & 24 B) where formulated *Trichoderma* was used @ 20 g/kg soil. Germination was decreased with the decrease of dose of treatment in all the vegetable crops. The lowest germination was recorded in the control trays (Plate 23 A & 24 A). The highest pre-emergence death was recorded in untreated control (T_0) in all the five crops. The lowest pre-emergence death of bean (13.33%), eggplant (14.67%), tomato (10.00%), cabbage (14.00%) and indian spinach (14.00%) was observed in T_3. The highest damping-off was observed in control trays whereas the lowest damping-off of bean (6.00%), eggplant (3.33%), tomato (3.33%), cabbage (2.00%) and indian spinach (3.33%) was recorded in T_3 which was statistically identical with T_2 in case of bean but different in eggplant, tomato, cabbage and indian spinach. Similarly the highest foot rot of bean (20.67%), eggplant (22.67%), tomato (21.33%), cabbage (24.00%) and indian spinach (22.00%) was recorded in control trays and foot rot was nil in T_3 in all the crops.

Table 3. Effect of different doses of formulated *Trichoderma* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach in tray soil at 7 DAS

Variety	Treatments	Germination %	Pre-emergence death %	Damping-off %	Foot rot %
Bean	T_0	42.67 c	54.33 a	19.33 a	22.00 a
	T_1	66.00 b	34.00 b	12.67 ab	17.33 ab
	T_2	75.33 ab	24.67 bc	8.00 b	13.33 b
	T_3	85.33 a	13.33 c	6.00 b	0.00 c
	LSD	14.46	14.46	7.453	5.21
Egg plant	T_0	55.33 c	44.00 a	17.33 a	22.67 a
	T_1	64.67 bc	35.33 ab	13.33 ab	16.67 a
	T_2	74.67 ab	25.33 bc	5.33 bc	9.33 b
	T_3	84.67 a	14.67 c	3.33 c	0.00 c
	LSD	12.25	12.25	8.279	6.79
Tomato	T_0	49.33 d	50.00 a	18.67 a	21.33 a
	T_1	71.33 c	28.67 b	13.33 ab	18.00 a
	T_2	80.00 b	20.00 c	6.67 bc	10.00 b
	T_3	88.67 a	10.00 d	3.33 c	0.00 c
	LSD	8.628	8.628	7.988	4.74
Cabbage	T_0	46.67 d	53.33 a	18.00 a	24.00 a
	T_1	61.33 c	38.67 b	12.67 ab	17.33 a
	T_2	74.00 b	26.00 c	6.00 bc	9.33 b
	T_3	85.00 a	14.00 d	2.00 c	0.00 c
	LSD	6.635	6.635	8.490	6.96
Indian spinach	T_0	44.67 c	55.33 a	16.67 a	20.67 a
	T_1	62.00 b	38.00 b	10.00 ab	17.33 ab
	T_2	65.33 b	34.67 b	6.67 ab	13.33 b
	T_3	86.00 a	14.00 c	3.33 b	0.00 c
	LSD	10.14	10.60	10.76	6.52

Values in a column with same letter (s) do not differ significantly whereas dissimilar letter(s) differ significantly at 5% level of significance.

T_0 = Control (Only pathogen)

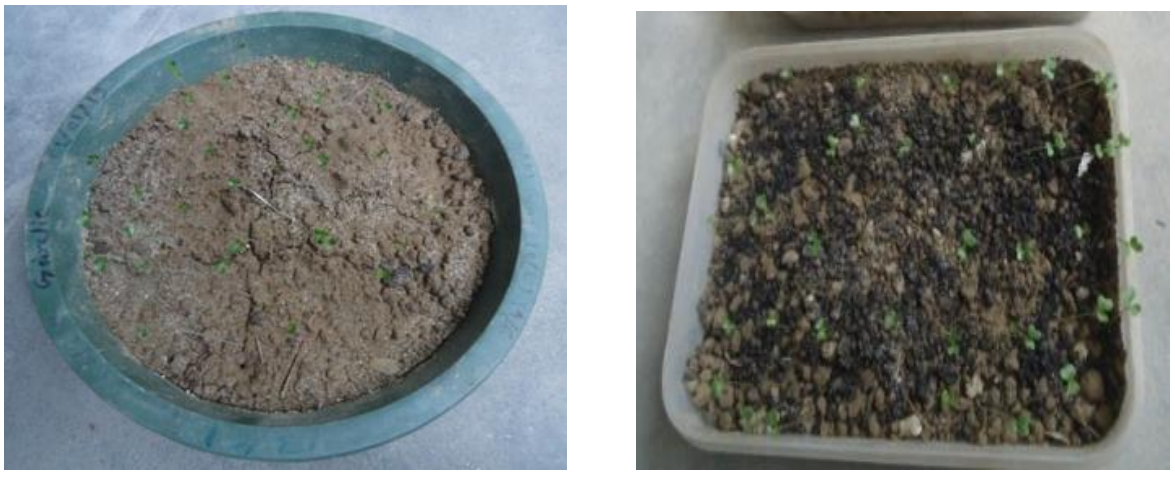
T_1 = Formulated *Trichoderma* 10 g/kg soil

T_2 = Formulated *Trichoderma* 15 g/kg soil

T_3 = Formulated *Trichoderma* 20 g/kg soil

A **B**

Plate 23. Bean seedlings in tray soil (A) control (*S. rolfsii* inoculated soil) (B) treatment of formulated *Trichoderma* after inoculation of *S. rolfsii*



A **B**

Plate 24. Cabbage seedlings in tray soil (A) control (*S. rolfsii* inoculated soil) (B) treatment of formulated *Trichoderma* after inoculation of *S. rolfsii*

4.5.2 Effect of different doses of formulated *Trichoderma* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach in tray soil at 14 DAS

Germination, pre-emergence death, damping-off and foot rot of bean, eggplant, tomato, cabbage and indian spinach were significantly influenced by different doses of formulated *Trichoderma* against *S. rolfsii* (Table 4). The highest germination of bean (86.67%), eggplant (85.33%), tomato (90.00%), cabbage (86.00%) and Indian spinach (86.67%) were recorded in T_3 where formulated *Trichoderma* was used @ 20 g/kg soil. Germination was decreased with the decrease of dose of treatment in all the vegetable crops. The lowest germination was recorded in the control trays. The highest pre-emergence death was recorded in untreated control (T_0) in all the five crops. The lowest pre-emergence death of bean (14.67%), eggplant (15.33%), tomato (11.33%), cabbage (15.00%) and indian spinach (14.00%) was observed in T_3. The highest damping-off was observed in control trays which were statistically identical with T_1 in case of eggplant, tomato and Indian spinach but different in bean and cabbage. The lowest damping-off of bean (6.00%), eggplant (4.67%), tomato (4.00%), cabbage (2.00%) and indian spinach (4.00%) was recorded in T_3 which was statistically identical with T_2 in case of bean, eggplant and tomato. Similarly the highest foot rot of bean (26.00%), eggplant (23.33%), tomato (24.67%), cabbage (24.00%) and indian spinach (20.67%) was recorded in control trays which were statistically identical with T_1 in case of tomato and Indian spinach and foot rot was nil in T_3 in all the crops.

Table 4. Effect of different doses of formulated *Trichoderma* on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach at 14 DAS

Variety	Treatments	Germination %	Pre-emergence death %	Damping-off %	Foot rot %
Bean	T_0	45.33 c	57.33 a	21.33 a	26.00 a
	T_1	68.67 b	31.33 b	13.67 b	19.33 b
	T_2	74.67 ab	25.33 bc	10.67 b	16.00 b
	T_3	86.67 a	14.67 c	6.00 b	0.00 c
	LSD	16.12	16.12	7.590	5.10
Egg plant	T_0	56.00 c	44.67 a	18.67 a	23.33 a
	T_1	66.00 bc	34.00 ab	13.33 a	20.00 ab
	T_2	74.67 ab	25.33 bc	5.33 b	14.00 b
	T_3	85.33 a	15.33 c	4.67 b	0.00 c
	LSD	12.58	12.58	7.37	7.066
Tomato	T_0	50.00 d	50.67 a	20.00 a	24.67 a
	T_1	72.00 c	28.00 b	15.33 a	21.33 a
	T_2	80.67 b	19.33 c	6.67 b	11.33 b
	T_3	90.00 a	11.33 d	4.00 b	0.00 c
	LSD	7.609	7.609	6.79	9.030
Cabbage	T_0	46.67 d	53.33 a	18.00 a	24.00 a
	T_1	62.00 c	38.00 b	12.67 ab	17.33 ab
	T_2	74.00 b	26.00 c	6.00 bc	10.67 b
	T_3	86.00 a	15.00 d	2.00 c	0.00 c
	LSD	7.373	7.373	8.490	7.45
Indian spinach	T_0	44.67 c	55.33 a	17.33 a	20.67 a
	T_1	62.00 b	38.00 b	14.00 a	14.00 a
	T_2	66.67 b	33.33 b	8.67 ab	8.67 ab
	T_3	86.67 a	14.00 c	4.00 b	4.00 b
	LSD	9.904	9.904	9.414	9.539

Values in a column with same letter (s) do not differ significantly whereas dissimilar letter(s) differ significantly at 5% level of significance.

T_0 = Control (Only pathogen)

T_1= formulated *Trichoderma* 10 g/kg soil

T_2 = formulated *Trichoderma* 15 g/kg soil

T_3 = formulated *Trichoderma* 20 g/kg soil

4.6 Determination of effective dose of *Trichoderma* suspension on seed germination and seedling diseases of eggplant, tomato, bean, cabbage and indian spinach in tray soil

4.6.1. Effect of different doses of *Trichoderma* suspension on seed germination and seedling diseases of eggplant, tomato, bean, cabbage and indian spinach in tray soil at 7 DAS

Germination, pre-emergence death, damping-off and foot rot of bean, eggplant, tomato, cabbage and indian spinach were significantly influenced by different doses of *Trichoderma* suspension against *S. rolfsii* (Table 5). The highest germination of bean (85.67%), eggplant (82.67%), tomato (88.67%), cabbage (84.67%) and indian spinach (83.33%) were recorded in T_3 (Plate 25 B & 26 B) where *Trichoderma* suspension was used @ 5.02×10^6 CFU/ml seeds. Germination was decreased with the decrease of dose of treatment in all the vegetable crops. The lowest germination was recorded in the control trays (Plate 25 A & 26 A). The highest pre-emergence death was recorded in untreated control (T_0) in all the five crops. The lowest pre-emergence death of bean (14.00%), eggplant (16.67%), tomato (11.33%), cabbage (15.33%) and indian spinach (16.67%) was observed in T_3. The highest damping-off was observed in control trays whereas the lowest damping-off of bean (6.00%), eggplant (4.33%), tomato (3.33%), cabbage (3.00%) and indian spinach (3.67%) was recorded in T_3 which was statistically identical with T_2 in case of bean but different in eggplant, tomato, cabbage and indian spinach. Similarly the highest foot rot of bean (26.67%), eggplant (26.33%), tomato (20.00%), cabbage (21.33%) and indian spinach (18.67%) was recorded in control trays and foot rot was nil in T_3 in all the crops.

Table 5. Effect of different doses of *Trichoderma* suspension on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach at 7 DAS

Variety	Treatments	Germination %	Pre-emergence death %	Damping-off %	Foot rot %
Bean	T_0	63.33 c	34.00 a	17.33 a	26.67 a
	T_1	71.33 b	28.67 b	12.00 ab	18.67 b
	T_2	75.33 b	24.67 b	8.00 b	16.33 b
	T_3	85.67 a	14.00 c	6.00 b	0.00 c
	LSD	7.895	7.895	8.062	5.726
Egg plant	T_0	49.33 c	50.00 a	12.00 a	26.33 a
	T_1	64.00 b	36.00 b	8.67 ab	13.33 b
	T_2	72.67 ab	27.33 bc	6.67 ab	10.67 b
	T_3	82.67 a	16.67 c	4.33 b	0.00 c
	LSD	13.96	13.96	6.221	8.781
Tomato	T_0	44.67 c	55.33 a	13.33 a	20.00 a
	T_1	60.00 b	40.00 b	9.33 ab	14.67 ab
	T_2	65.33 b	34.67 b	6.00 ab	10.00 b
	T_3	88.67 a	11.33 c	3.33 b	0.00 c
	LSD	9.601	9.601	7.045	7.609
Cabbage	T_0	46.00 c	52.67 a	12.67 a	21.33 a
	T_1	60.67 b	39.33 b	7.67 ab	17.33 ab
	T_2	69.33 b	30.67 b	5.33 ab	12.00 b
	T_3	84.67 a	15.33 c	3.00 b	0.00 c
	LSD	13.71	13.71	8.420	5.854
Indian spinach	T_0	42.67 c	57.33 a	11.33 a	18.67 a
	T_1	59.33 b	40.67 b	8.00 ab	11.33 b
	T_2	68.67 ab	31.33 bc	6.67 ab	7.33 c
	T_3	83.33 a	16.67 c	3.67 b	0.00 d
	LSD	15.10	15.10	6.723	3.766

Values in a column with same letter (s) do not differ significantly whereas dissimilar letter(s) differ significantly at 5% level of significance.

T_0 = Control (Only pathogen)

T_1 = *Trichoderma* suspension 5.02×10^4 CFU/ml seeds

T_2 = *Trichoderma* suspension 5.02×10^5 CFU/ml seeds

T_3 = *Trichoderma* suspension 5.02×10^6 CFU/ml seeds

A **B**

Plate 25. Been seedlings in tray soil (A) control (*S. rolfsii* inoculated soil) (B) seeds treated with *Trichoderma* suspension after inoculation of *S. rolfsii*

A B

Plate 26. Cabbage seedlings in tray soil (A) control (*S. rolfsii* inoculated soil) (B) seeds treated with *Trichoderma* suspension after inoculation of *S. rolfsii*

4.6.2. Effect of different doses of *Trichoderma* suspension on seed germination and seedling diseases of eggplant, tomato, bean, cabbage and indian spinach in tray soil at 14 DAS

Germination, pre-emergence death, damping-off and foot rot of bean, eggplant, tomato, cabbage and indian spinach were significantly influenced by different doses of *Trichoderma* suspension against *S. rolfsii* (Table 6). The highest germination of bean (86.00%), eggplant (83.33%), tomato (88.87%), cabbage (84.67%) and indian spinach (83.33%) were recorded in T_3 where *Trichoderma* suspension was used @ 5.02 $\times 10^6$ CFU/ml seeds. Germination was decreased with the decrease of dose of treatment in all the vegetable crops. The lowest germination was recorded in the control trays. The highest pre-emergence death was recorded in untreated control (T_0) in all the five crops. The lowest pre-emergence death of bean (14.33%), eggplant (17.33%), tomato (11.33%), cabbage (15.33%) and indian spinach (16.67%) was observed in T_3. The highest damping-off was observed in control trays whereas the lowest damping-off of bean (6.00%), eggplant (5.00%), tomato (3.33%), cabbage (3.00%) and indian spinach (3.67%) was recorded in T_3 which was statistically identical with T_2 in case of bean, indian spinach but different in eggplant, tomato and cabbage. Similarly the highest foot rot of bean (30.00%), eggplant (27.33%), tomato (20.00%), cabbage (21.33%) and indian spinach (18.67%) was recorded in control trays and foot rot was nil in T_3 in all the crops.

Table 6. Effect of different doses of *Trichoderma* suspension on seed germination and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach at 14 DAS

Variety	Treatments	Germination %	Pre-emergence death %	Damping-off %	Foot rot %
Bean	T_0	66.00 c	36.67 a	18.00 a	30.00 a
	T_1	71.33 bc	28.67 ab	12.00 ab	21.33 b
	T_2	76.00 b	24.00 b	8.00 b	18.00 b
	T_3	86.00 a	14.33 c	6.00 b	0.00 c
	LSD	7.373	7.373	7.988	7.839
Egg plant	T_0	50.00 c	50.67 a	12.00 a	27.33 a
	T_1	65.33 b	34.67 b	8.00 ab	14.67 b
	T_2	73.33 ab	26.67 bc	6.67 ab	10.67 b
	T_3	83.33 a	17.33 c	5.00 b	0.00 c
	LSD	11.76	11.76	6.221	8.831
Tomato	T_0	44.67 c	55.33 a	13.33 a	20.00 a
	T_1	60.00 b	40.00 b	9.33 ab	14.67 ab
	T_2	66.67 b	33.33 b	5.67 ab	10.00 b
	T_3	88.67 a	11.33 c	3.33 b	0.00 c
	LSD	8.831	8.831	8.225	7.609
Cabbage	T_0	47.33 c	54.00 a	12.67 a	21.33 a
	T_1	62.67 b	37.33 b	8.67 ab	17.33 ab
	T_2	69.33 b	30.67 b	5.33 ab	12.00 b
	T_3	84.67 a	15.33 c	3.00 b	0.00 c
	LSD	13.62	13.62	8.420	5.854
Indian spinach	T_0	42.67 c	57.33 a	15.00 a	18.67 a
	T_1	59.33 b	40.67 b	9.00 ab	11.33 b
	T_2	68.67 ab	31.33 bc	4.33 b	7.33 c
	T_3	83.33 a	16.67 c	3.67 b	0.00 d
	LSD	15.10	15.10	7.312	3.766

Values in a column with same letter (s) do not differ significantly whereas dissimilar letter(s) differ significantly at 5% level of significance.

T_0 = Control (Only pathogen)

T_1 = *Trichoderma* suspension 5.02×10^4CFU/ml seeds

T_2 = *Trichoderma* suspension 5.02×10^5CFU/ml seeds

T_3 = *Trichoderma* suspension 5.02×10^6CFU/ml seeds

CHAPTER 5

DISCUSSION

The diseases of plants caused by *Sclerotium rolfsii* are generally difficult to control. Application of fungicides in controlling *S. rolfsii* may reduce disease severity but it is not cost effective and environment friendly. Thus, there is a necessity for development of efficient alternatives to combat the disease. Currently, the role of biological control agents is a well-established fact and has become increasingly crucial, complementary or even replacing the chemical counterparts where antagonistic fungi play an important role (Whipps and Lumsden, 2001; Chet, 1993). In this context, *Trichoderma* spp. have been the cynosure of many researchers who have been contributing to biological control through use of fungi (Heraux *et al.,* 2005a; Heraux *et al.* ,2005b; Ortiz and Orduz, 2001). Furthermore, *Trichoderma* spp. share almost 50 % market of Biological Control Agents, mostly growth enhancers (Whipps and Lumsden, 2001).

The present study was aimed for the isolation and identification of local isolates of *Trichoderma* and the pathogenic isolate of *Sclerotium rolfsii* collected from different locations in Dinajpur District. The fungal population of soil samples was analysed and the antagonistic effect of *Trichoderma* was tested against *Sclerotium rolfsii* through dual culture method.

The isolated *Trichoderma* from rhizosphere soil of indian spinach was purified through hyphal tip culture and identified as *Trichoderma harzianum* according to the key of Kubicek and Harman (1998).The isolate distinctly differ on their cultural and morphological characteristics having dark green colony colour, branched conidiophores, phialides flask shaped and cylindrical or nearly subglobose, conidia

single-celled, ellipsoidal or globose shaped. This was the first time report for the isolation of *Trichoderma* spp. from this locality and regarded as a major findings of the study. Patel *et al.* (2014) identified *Trichoderma harzianum* having the same characteristics of hyphae, conidiophores and conidia. The present findings were also supported by Wang *et al.* (1999).

In case of *S. rolfsii* isolated from eggplant (*Solanum melongena* L.) grown in the experimental field of the Department of Plant Pathology, HSTU, Dinajpur was purified and identified according to the key of Aycock (1996) and Barnett (1960). The isolate producing whitish colony and was very fast growing. After 4 days, the isolate covered above 50% of the surface area on petri-plate. At 5 day, the isolate almost filled the petriplate and at 6 days highest mycelial growth was observed on petripate. The fungus produced sclerotia after 7 days of inoculation. The sclerotia were small, uniformly round and dark brown at mature stage. The findings of present investigation are in agreement with the report of Darakhshanda-Kokub *et al.* (2007).

A comparative bioassay of local *Trichoderma* isolate and RDA isolate against *S. rolfsii* in dual culture method were studied on PDA media to test their antagonistic activity. After 6 days of inoculation when the control plates were full the bioagents produced significantly different inhibition zone against *S. rolfsii* in dual culture method. It was found that local isolate of *T. harzianum* (92%) was superior to RDA *Trichoderma* (85%) isolate in reducing the colony diameter of *S. rolfsii.* Prasad *et al.* (1999) found the similar findings and reported 61.4 % inhibition of *S. rolfsii* with *T. harzianum* while Yogendra and Singh (2000) found maximum of 64.44 % inhibition of *S. rolfsii* by *T. harzianum* at 4 DAI. Pranab *et al.* (2002) recorded 61.5 % inhibition of *S. rolfsii* by *T. harzianum*. Faruk *et al.* (2002) found that *T.*

harzianum significantly reduced the radial colony growth of *S. rolfsii* in dual culture on PDA. Similar findings were also obtained by Sultana and Hossain (2000). Increase of inhibition zone proves the higher antagonistic activity of the isolated bioagent which was also a markable finding of this study.

Tray soil inoculated with different doses of *S. rolfsii* significantly acted on the germination, pre-emergence death, damping-off and foot rot of bean, eggplant, tomato, cabbage and indian spinach. The highest germination was recorded in control trays in all the crops at 7 days and 14 days. The highest damping-off was observed in T_3 in all the crops when *S. rolfsii* inoculated with 20 g/kg soil. Foot rot was nil in controls trays and highest foot rot was recorded in T_3 in all the crops. Among the crops, bean seems to be highly virulent towards foot rot diseases. The findings of Begum *et al*. (1999) supported the present findings who observed the variable disease response in different isolates of *S. rolfsii* while Meah (2007) tested the pathogenicity of 10 isolates of *S. rolfsii* on eggplant (var. Dohazari) and he found that all the isolates of *S. rolfsii* significantly influenced the germination, pre-emergence death, damping off, foot rot and plant stand.

Application of different doses of formulated *Trichoderma* against *S. rolfsii* significantly acted on the germination, pre-emergence death, damping-off and foot rot of bean, eggplant, tomato, cabbage and indian spinach. The highest germination of bean (86.67%), eggplant (85.33%), tomato (90%), cabbage (86%) and indian spinach (86.67%) at 14 days was recorded in T_3 where formulated *Trichoderma* was used @ 20 g/kg soil. Percent foot rot was nil in T_3 in all the crops. The relevant work was done by Xu *et al.* (1993) in soil treatment with *T. harzianum* T82 @ 0.6% (w/w) in bran culture reduced the disease incidence caused by *S. rolfsii* by 46.6 % at 20 days after inoculation with the pathogen. Bhuiya (2006) reported that

Trichoderma formulation @ 20g/kg soil was effective in controlling collar rot and regeneration of eggplants.

In pot experiments, *Trichoderma* suspension was evaluated against *S. rolfsii* for reducing seedling diseases of bean, eggplant, tomato, cabbage and indian spinach. The data recorded on percent germination, pre-emergence death, damping-off and foot rot at different days after sowing. The highest germination of bean (86%), eggplant (83.33%), tomato (88.67%), cabbage (84.67%) and indian spinach (83.33%) at 14 days was recorded in T_3 where *Trichoderma* suspension was used @ 5.02×10^6 CFU/ml. Percent foot rot was nil in T_3 in all the crops. Similar findings obtained by Shamsuzzaman *et al.* (2003b) and reported that seed treatment with *T. harzianum* (6×10^6 CFU/ ml) resulted higher germination, fresh shoot weight, fresh root weight and higher vigor index of cucurbits over control. Xu *et al*. (1993) reported that Seed treatment with *Trichoderma* T82 or NF9 spore suspension (10^8c.f.u./ml) increased emergence of cucumber seedlings by 14% and 20%, respectively, 11 days after inoculation with *S. rolfsii*.

Based on the above discussion it is revealed that the local *Trichodrama* isolate could be used as bioagent to control the damping-off and foot rot of vegetables caused by *S. rolfsii*.

CHAPTER 6
SUMMARY AND CONCLUSION

Diseases caused by soil borne plant pathogens specially *S. rolfsii* is a major constrain for vegetables production in Bangladesh. It mainly causes damping-off and foot rot diseases of vegetables. It significantly reduces vegetable yield. Various strategies for controlling *S. rolfsii* have been introduced over the years including soil disinfection, cultural practices and fungicide treatments but losses still occur, largely because of the effectiveness of these approaches is variable and short lived. Moreover, fungicides of broad spectrum produce undesirable consequences on non-target organisms, environment and public health. By contrast, the use of microorganisms that antagonize plant pathogens (biological control) is risk-free when it results in enhancement of resident antagonists. Moreover, they offer other advantages in plant health management not possible with chemical pesticide. Among the microorganisms, species of *Trichoderma* are the pioneer and most used bio-control agent which are proved to be used successfully against many plant pathogen including *S. rolfsii*. Considering the above perspective the research program was undertaken to determine the efficacy of indigenous *Trichoderma* isolate to control damping-off and foot rot diseases of vegetables caused by *S. rolfsii*.

The isolated *Trichoderma* was purified and identified as *T. harziznum* according to the key of Kubicek and Harman (1998). The isolate distinctly differ on their cultural and morphological characteristics having dark green colony colour, branched conidiophores, phialides flask shape and cylindrical or nearly subglobose, conidia single-celled, ellipsoidal or globose shape.

S. rolfsii was isolated from eggplant, purified and identified according to the key of Aycock (1996) and Barnett (1960). Morphological characteristics like mycelia growth, colony colour, sclerotia formation and sclerotial colour were studied. The isolate produced whitish cottony mycelium was very fast growing. Sclerotia were small, uniformly round and dark brown at mature stage.

The antagonistic potential of local *Trichoderma* isolate and RDA isolate against *S. rolfsii* were studied using dual culture method and result found that local *T. harzianum* (92%) isolate was superior to RDA *Trichoderma* (85%) isolate in reducing the colony diameter of *S. rolfsi*.

Effect of different doses of *S. rolfsii* were tested and found that *S. rolfsii* significantly influenced the germination, pre-emergence death, damping-off and foot rot of bean, eggplant, tomato, cabbage and indian spinach. The highest foot rot was observed in T_3 when *S. rolfsii* inoculated with 20 g/kg soil in all the crops. Foot rot was nil in controls trays in all the crops and found that bean seems to be highly virulent towards foot rot diseases among the other crops.

Among the different doses of formulated *Trichoderma*, the highest germination of bean (86.67%), eggplant (85.33%), tomato (90%), cabbage (86%) and indian spinach (86.67%) at 14 days was recorded in T_3 where formulated *Trichoderma* was used @ 20 g/kg soil. Percent foot rot was nil in T_3 in all the crops. The lowest pre-emergence death of bean (14.67%), eggplant (15.33%), tomato (11.33%), cabbage (15.00%) and indian spinach (14.00%) and lowest damping-off of bean (6.00%), eggplant (4.67%), tomato (4.00%), cabbage (2.00%) and indian spinach (4.00%) at 14 days were also recorded in this same dose.

Different doses of *Trichoderma* suspension were evaluated in tray soil and data recorded on percent germination, pre-emergence death, damping-off and foot rot of bean, eggplant, tomato, cabbage and indian spinach at different days after sowing. The highest germination of bean (86%), eggplant (83.33%), tomato (88.67%), cabbage (84.67%) and indian spinach (83.33%) at 14 days was recorded in T_3 where *Trichoderma* suspension was used @ 5.02×10^6 CFU/ml. Percent foot rot was nil in T_3 in all the crops. The lowest pre-emergence death of bean (14.33%), eggplant (17.33%), tomato (11.33%), cabbage (15.33%) and indian spinach (16.67%) and lowest damping-off of bean (6.00%), eggplant (4.33), tomato (3.33%), cabbage (3.00%) and indian spinach (3.67%) at 14 days were also recorded in this same dose.

Therefore, it can be concluded that local isolate of *Trichoderma harzianum* formulated in black gram bran substrate successfully be used for controlling *S. rolfsii* causing damping off and foot rot of different vegetable crops. Further study may be done to test antagonistic activity of the *T.* isolate against other soil-borne pathogens.

REFERENCES

Afrin, S. 2013. Efficacy of Fungicides and *Trichoderma harzianum* against Seed Borne Pathogens Causing Boll Rot Disease of Cotton. MS Thesis, Department of Plant Pathology, Sher-e-Bangla Agricultural University Sher-e-Bangla Nagar, Dhaka, Bangladesh.

Agrawal, S. C.; Khare, M. N. and Agrawal, P. S. 1977. Biological control of *Sclerotium rolfsii* causing collar rot of lentil. *Indian Phytopathol*., **30**(2): 176-179.

Ahmed, H. U. and Hossain, M. 1985. Crop disease survey and establishment of a herbarium at BARI. Final report of the project (1982-85). Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. P 107

Akrami, M. and Yousefi, Z. 2015. Biological Control of *Fusarium* wilt of Tomato (*Solanum lycopersicum*) by *Trichoderma spp*. as Antagonist Fungi. *Biol. Forum – An Internat. J.,* **7**(1): 887-892.

Alam, M. M. 2003. Studies on the soil borne nature of Phomopsis blight and fruit rot of eggplant. MS thesis, Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh, pp 41-46

Alonso-Reyes, R..; Barranco-Martinez, B.; Gracia-Rivero, G. and Jimenez-Montejo, G. 2002. .*In vivo* biocontrol activity of *Trichoderma harzianum* against *Sclerotium rolfsii* disease in tomato seedlings. Manejo. Integrado de Plagasy Agroecologia, **66**: 45-48.

Amin, F.; Razdan, V.K.; Mohiddin, F. A.; Bhat, K. A. and Banday, S. 2010. Potential of *Trichoderma* spp. As Biocontrol Agents Against Soil Borne Fungal Propagules. *J. of Phytol.*, **2**(10): 38–41.

Anand, S.; and Singh, H. B. 2004. Controls of collar rot in mint *(Mentha* spp.) caused by *Sclerotium rolfsii* using biological means. *Current Sci.* **87**(3): 632- 366.

Ashrafuzzaman, H. 1976. Shasyer Rog (Diseases of crops) Bangla Academy, Dhaka Bangladesh, 2nd Ed. P 321

Aycock, R. 1996. Stem rot and other diseases caused by *S. rolfsii*. Tech. Bull. No. 174. Agric. Expt. Station, North Carolina State University, Raleigh. P 202

Babar, H. M., 1999. Studies on collar rot of sunflower. Ph. D. thesis, Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Bag, T. K. and Sinha, A. K. 1997. Control of stem rot of soybean incited by *Sclerotium roilsii* through metal stalts. *Indian Phytopathol.* **50**(30): 350-357.

Bagwan, N. B. 2011. Evaluation of biocontrol potential of *Trichoderma* species against *Sclerotium rolfsii, Aspergillus niger* and *Aspergillus flavus*. *Internat. J. of Plant Prot.*, **4** (1): 107-111.

Barnett, H. L. 1960. Illustrated genera of imperfect fungi. Second edition. Burgess Publishing Co. Minneapalis, Minn. P 225.

Begum, M. M. 1997. Efficacy of *Trichoderma harzianum* as a biocontrol agent for controlling *Fusarium* spp. and *Sclerotium rolfsii* in food legumes. MS thesis, Dept. of Plant Pathology. Bangladesh Agricultural University, Mymensingh. Bangladesh.

Begum, M. M.; Rahman, M. A. and Hossain, I. 1999. Antagonist effect of *Trichoderma harzianum* on *Sclerotium rolfsii* in food legumes. *J. Bio. Sci*. **7**:81-88.

Benytez, T.; Rincon, A. M.; Limon, M. C. and Codon, A. C. 2004. Biocontrol mechanisms of *Trichoderma* strains, *Internat. Microbiol*., **7**: 249-260.

Bhuiya, S. A. M. 2006. Effect of fungicide, Bio-pesticide and plant extracts in controlling collar rot of brinjal. MS thesis, Dept. of Plant Pathology. Bangladesh Agricultural University, Mymensingh. Bangladesh. P 47.

Chandrasehar, G.; Ayyappan, S. and Eswaran A. 2005.Management of tomato collar rot caused by *Sclerotium rolfsii* by antagonistic micro organisms. *J. Ecobiology*., **17**(3): 261-264.

Chet, I. 1993. Biotechnology in Plant Disease Control, John Wiley and Sons, New York.

Chet, I. and Inbar, J. 1994. Biological control of fungal pathogens. *Appl. Biochem. Biotech*., **48**(1): 37-43.

Chowdhury, M. S. M.; Hossain, I., Fakir, G. A.; Aminuzzaman, F. M. and Islam, M. R. 2000.Tolerance of *Trichoderma harzianum* and *Gliocladium viride* to agrochemicals and their antagonistic effect on seed borne micro-flora of Pigeon Pea. *Bangladesh J. Seed Sci. Tech*., **4**(1&2): 83-86.

Chupp, C. and A. F. Sherf. 1960. Vegetables disease and their control. Ronald Press Company. New York. pp. 314-316.

Darakhshanda Kokub; Azam, F.; Hassan, A. and Ansar, M. 2007. Comparative growth, morphological and molecular characterization of indigenous *Sclerotium rolfsii* strains isolated from different locations of Pakistan. *Pak. J. Bot*., **39**(5): 1849-1866.

Das, B. C.; Pranab-Dutta, Devi, G. and Dutta, P. 2000. Management of *Sclerotium rolfsii* in tomato by fungal antagonists. *J. Agril. Sci. Soci. of North East India.,* **13**(1): 101-103.

Dashgupta, M. K. 1988. Principles of Plant Pathology. Allied Publisher Private Limited. New Delhi, India. P 700.

Dhingara, O.D. and Sinclair, J.B.1985. Basics plant pathology methods. CRS Press. Inc. Boca Raton Florida. pp. 13-44.

Elad, Y.; Barak, R.; Chet, I. and Henis, Y. 1983. Ultrastructural studies of the interaction between *Trichoderma harzianum* spp. and plant pathogenic fungi. *Phytopathol*., **107**(2): 168-175.

Elad, Y.; Chet, I. and Henis, Y. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. *Canadian J. Microbiol*., **28**(7): 719-725.

Elad, Y.; Chet, I. and Khatan, J. 1990. *Trichoderma harzianum*, a bio-control effective against *Sclerotium rolfsii* and *Rhizoctonia solani. Phytopathol*., **70**(2): 199-121.

Elad, Y.I. C. and Katan, J .1980. *Trichoderma harzianum*: A Biocontrol Agent Effective Against *Sclerotium rolfsii* and *Rhizoctonia solani. The American Phytopathol. Soc.*, **70** (2): 119-121.

Fakir G. A., Rahman, M. M. and Islam, M. F. 1991. Occurrence of diseases on lentil and country bean germplasms and their reaction to the selected major pathogens. Proc. Bangladesh Agricultural University Research Progress, Mymensingh, Bangladesh, pp 1-10.

Faruk, M. I. 2015. Evaluation of different substrates based *Trichoderma harzianum* bio-fungicides against seedling rot (*Rhizoctonia solani*) disease of cabbage. *Internat. J. of Innovative and Applied Res.*, **3**(4): 1-9.

Faruk, M. I.; Bari, M. A. and Rahman, M. A. 2002. Efficacy of *Trichoderma* as bio-control agent against foot and root rot of bush bean. *Bangladesh J. Agril. Res.*, **27**(4): 657-562.

Faruk, M.I. and. Rahman, M. L.2015. Management of Tomato Seedling Rot disease (*Rhizoctonia solani*) in Seedbed with *Trichoderma harzianum* Bio-fungicide. *Agric. Sci. Res. J.*, **5**(1): 14-20.

Ferrata, M. and Amba, V. D. 1985. Morphological aspects of the parasitism of *Trichoderma harzianum* Rifai on hyphae and sclerotia of *Sclerotium rolfsii* Sacc.Rivista di Patologia Vegetal. **21**(2):53-60.

Fokkema, N.J.1995. Strategies for bio-control of foliar fungal diseases. In M. Manka (ed.), Environmental Biotic Factors in Integrated Plant Disease Control. *The Polish Phytopathol. Soc.*, Poznan. pp. 69-79.

Gogoi, N. K.; Phookan, A. K. and Narzary, B. D. 2002. Management of collar rot of Elephant's foot yam. *Indian Phytopathol*., **55**(2): 238-240.

Gveroska, B. and Ziberosk J. (2012). *Trichoderma harzianum* as a biocontrol agent against *Alternaria alternate* on tobacco. *Peer-reviewed & Open access j*., **7**(2): 67-76.

Hadar, Y.; Chet, I. and Hanis. Y. 1979. Biological control of *Rhizoctonia solani* damping off with wheat bran culture of *Trichoderma harzianum*. *Phytopathol*., **69**:64-68.

Hannan, M. A. 2005. Integrated management of foot rot of lentil, chickpea and grasspea. M. S. thesis, Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh. pp 84-91.

Harman, G. E.; Howell, C. R.; Viterbo, A.; Chet, I. and Lorito, M. 2004. *Trichoderma* species-opportunitic, avirulent plant symbionts. *Nature Review Microbiol*., **2**: 43-56.

Hend, A.; Alwathnani, Perveen, K.; Tahmaz, R. and Alhaqbani, S. 2012. Evaluation of biological control potential of locally isolated antagonist fungi against *Fusarium oxysporum* under *in vitro* and pot conditions. *African J. of Microbiol. Res.*, **6**(2): 312-319.

Henis, Y. and Papavizas, G. C. 1982. Factors affecting susceptibility of *Sclerotium rolfsii* sclerotia to *Trichoderma harzianum* in natural soil. *Phytopathol*., **72**: 1010.

Heraux, F. M. G.; Hallett, S. G.; Ragothama, K. G. and Weller, S. C. 2005a. Composed chicken manure as a medium for the production and delivery of *Trichoderma virens* for weed control. *Hort. Sci*., **40**: 1394-1397.

Heraux, F. M. G.; Hallett, S. G.; Ragothama, K. G. and Weller, S. C. 2005b. Combining *Trichoderma virens* inoculated compost and a rye cover crop for weed control in transplanted vegetables. Biol. Control. **34**: 21-26.

Herrera, Estrella, A. and Chet, I.2003. The biological control agent, *Trichoderma* from fundamentals to applications. In: Arora, D.K., Bridge, P.D. and Bhatnagar, D. (eds), Fungal biotechnology in agricultural, food and environmental applications, Marcel Dekker, New York, pp.147-156.

Hiremath, P. C.; Kulkarni, S. A.; Radder, G. D.; Gidnavar, V. S.; Chittapur, B. M.; Itnal, C. J.; Patal, B. N. and Babalad, H. B. 1998. Production of biocontrol agents for plant pathogens. Organic sustaining soil fertility productivity. pp 291-293.

Howell, C.R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. Plant Dis. **87**: 4-10.

Inbar, J.; Abramsky, M.; Cohen, D. and Chet, I. 1994. Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial condition. *European J. Plant Pathol*., **100**: 337-346.

Iqbal, S. M.; Bakhsh, A.; Hussain, S. and Malik, B. A. 1995. Microbial antagonism against *Sclerotium rolfsii*, the cause of collar rot of lentil. LENS Newsletter **22**(1/2): 48-49.

Jacobs, D. and Kamoen, O. 1986. Roll of cell wall lysing enzymes of *Trichoderma harzianum*. Med. Fac. Landbouw. Rijks. Gent. **56**(26): 751-758.

Jinantana, J. and Sariah, M.1997. Antagonistic effect of Malayasian isolates of *Trichoderma harzianum* and *Gliocladium virens* on *Sclerotium rolfsii*. *Pertanika j. Trop. Agric. Sci.*, **20**: 35-41.

Kashem, A. 2005. *Trichoderma* in controlling foot and root rot and collar rot of lentil. Ph. D. thesis, Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensingh. Bangladesh. p 192.

Krutova, N. P. 1987. Mycoparasites of sclerotia of the causal agent of sunflower white rot. *Phytopathol*., **21**(2): 168-171.

Kubicek, C. P. and Harman, G. E. 1998. *Trichoderma* and *Gliocladium*. Taylor & Francis Ltd, 1 Gunpower Square, London, EC4A 3DE. pp 3-30.

Kulkarni, S. A. and Kulkarni, S. 1994. Biological control of *S. rolfsii*, a causal agent of collar rot of groundnut. *Kamataka J. Agril. Sci*., **7**(3): 365-367.

Kwok, O. C. H.; Fahy, P. C.; Hoitink, H. A. K. and Kuter, G. A. 1987. Interaction between bacteria and Trichoderma in suppressing Rhizoctonia damping- off in bark compost media. *Phytopathol*., **77**(8): 1206-1212.

Mathivanan, N.; Srinivasan, K. and Chelliah, S. 2000. Biological control of soil borne diseases of cotton, eggplant, okra and sunflower by *Trichoderma viride*. Zeitschrift-fur- Pflanzenkrankheiten and Pflanzenschutz. **107**(3): 235-244.

Meah, M. B. 2007. Formulation of bio-pesticides in controlling phomopsis rot, foot/collar rot and shoot and fruit borer of eggplant. Annual research report, USDA-Bangladesh collaborative research. pp 4-11.

Monaco, C. A.; Alippi, H. E. and Pasquare, A. O. 1991. *Trichoderma* spp. a biological agent of *Fusarium* spp. and *Sclerotium rolfsii*. *Hort. Sci*.. **5**(3): 92-95.

Motesharrei, Z .S. and Salimi, H. (2014). Biocontrol Characteristics of *Trichoderma* spp. against *Fusarium* in Iran. *Middle-East J. of Sci. Res*., **22** (8): 1122-1126.

Mukherjee, P. K.; Mukhopadhyay, A. N.; Sarmah, D. K. and Shrestha, S. M. 1995.Comparative antagonism properties of *Gliocladiumvirens* and *Trichodermaharzianum* on *Sclerotiumrolfsii* and *Rhizoctoniasolani*, its relevance to understanding the mechanisms of biocontrol. *J. Phytopathol*., **143**(5): 275-179.

Mukhopadhyay, A. N. 1989. National seminar and VII Workshop of AICRP on Biological Control, Lucknow. Oct. 23-25, 1989.

Mukhopadhyay, A. N. 1994. Biological control of soil borne fungal plant pathogens: Current status, future prospects and potential limitations. *Indian Phytopathol.*, **47**(2): 199-226.

Mukhopadhyay, A. N. 1995. Exploitation of *Gliocladiuinvirens* and *Trichoderma harzianum* for biological seed treatment against seed borne disease. *Indian J. Mycol. Plant Pathol*., **2**(1&2): 124.

Mutto, S.; Ambra, V. D. and Ferrata, M. 1986.Ultrastructural aspects of the parasitism of *Trichoderma harzianum* on sclerotia of *Sclerotium rolfsii*. *Phytopathol. Medit*., **25**(1-3): 10-18.

Nashwa, M. A.; Sallam, K. A. M. and Hassan, M. A. E. 2008. Evaluation of *Trichoderma* Species as Biocontrol Agents for Damping-Off and Wilt Diseases of *Phaseolus vulgaris* L. and Efficacy of Suggested Formula. *Egypt. J. Phytopathol*., **36**(1-2): 81-93.

Nene, Y. L. and Thapliyal, P. N. 1993. Fungicides in plant disease control. Oxford and IBH publishing Co. PVT. LTD. New Delhi. pp 531-535.

Ordentlich, A. and Chet, D. 1989. Biological control of seed borne plant pathogenic fungi by *Trichoderma*. *Israil Agric. res*., **3**(1-2): 137-152.

Ortiz, A. and Orduz, S. 2001. *In vitro* evaluation of *Trichoderma* and *Gliocladium* antagonism against the symbiotic fungus of the leaf-cutting ant Attacephalotes, *Mycopathol*,, **150**:53-60.

Pandey, K. K. and Upadhyay, J. P. 1999. Comparative study of chemical, biological and integrated approach for management of Fusarium wilt of pigeonpea. *J. Mycol. Plant Pathol*., **29**(2): 214-216.

Papavizas, G. C. 1985. *Trichoderma and Gliocladium*: biology, ecology, and potential for biocontrol. *Annu. Rev. Phytopathol*., **3**: 23-54.

Papavizas, G. C.; Lumsden, R. D. 1980. Biological control of soil-borne fungal propagules. *Annu. Rev. Phytopathol*., **18**: 389-413.

Patel, R. and Patel, D. 2014. Screening of *Trichoderma* and antagonistic analysis of a potential strain of *Trichoderma* for production of a Bioformulation. *Internat. J. of Sci. and Res. Pub.*, **4**(10): 2250-3150.

Pranab, D.; Das, B. C. and Dutta, P. 2002. Management of collar rot of tomato by *Trichoderma* spp. and chemicals. *Indian Phytopathol*., **55**(2): 235-237.

Prasad, C. S.; Vishal- Gupta, Ashish-Tyagi; Santosh-Pathak; Gupta, V.; Tyagi, A. and Pathak, S. 2003. Biological control of *Sclerotium rolfsii* of cauliflower collar rot. *Annu. Plant Protec. Sci.*, **11**(1): 61-63.

Prasad, R. D.; Rangeshwaran, R. and Kumar, P. S. 1999. Biological control of root and collar rot of Sunflower. *J. Mycol. Plant Pathol*., **29**(2): 184-188.

Rahman, A.; Begum, F.; Rahman, M.; BARI, M. A.; Ilias, G. N. M.; and Alam, M. F. 2011. Isolation and identification of *Trichoderma* species from different habitats and their use for bioconversion of solid waste. *Turk J. Biol.*, **35**(1): 183-194.

Rekha, A.; Saxena, S.; and Arya, R. 1998. *Trichoderma roseum* together with *Rhizoctonia solani* and *Meloidogyne incognita* on germination of seeds of tomato cv. Pusa Ruby. *Indian J. Nematol*., **28**(2): 217-221.

Roberti, R.; Flori, P. and Pisi, A. 1996. Biological control of soil borne *Sclerotium rolfsii* infection by treatment of bean seeds with species of *Trichoderma*. *Petria*., **6**(2): 105-116.

Rosane, S. C.; Helder L. S.; Gustavo, A. S.; Carlos, A. T. and Rodrigues, S. 2008. Effect of Moisture on *Trichoderma* Conidia Production on Corn and Wheat Bran by Solid State Fermentation. *Food Bioprocess Technol*., **17**(1):100-104.

Roy, A. K. 1989. Biological control of *Rhizoctonia solani* In: Agnihotri, V. P. (ed) perpestives in phytopathology. Today and Tomorrow's printers New Delhi. P 71

Shamsuzzaman; Islam, S. M. A. and Hossain, I. 2003b. Production of *Trichoderma* conidia in agro-wastes. *Bangladesh J. Environ. Sci*., **9**: 146-150.

Siddique, A. B. 2009. Efficacy of *Trichoderma* and Botanicals in Controlling Alternaria Leaf Blight of Mustard Study on Morphology of the Pathogen.MS Thesis, Department of Plant Pathology Sher-e-Bangla Agricultural University Sher-e-Bangla Nagar, Dhaka, Bangladesh. pp 50-59.

Siddique, M. A. B.; Meah, M. B.; Siddiqua, M. K.; Rahim, M. A. and Haque, M. M. 2002. Response of ten brinjal varieties to foot rot caused by *Sclerotium rolfsii*. *Bangladesh J. Plant Pathol*., **18**(1/2): 77-81.

Singh, S. R.; Prajapati, R. K.; Srivastava, S. S. L.; Pandey, R. K. and Singh, O. P. 2007. Management of collar rot of lentil. *Annu. Plant Protec. Sci*., **15**(1): 270-271.

Subba, R. N. 2003. Methods Used in Soil Microbiological Studies: Soil Microbiology. 4th Edn. Oxford and IBM Publishing Co. Pvt. Ltd., New Delhi.

Sugha, S. K.; Pathania, N. K. and Sharma, B. K. 1990. Reaction of brinjal cultivars to collar rot (*Sclerotium rolfsii*). *South Indian Hort*., **38**(6): 351-352.

Sugha, S. K.; Sharma, B. K. and Tyagi, P. D. 1991.A modified Technique for screening chickpea (*Cicer arietinum*) varieties against collar rot caused by *Sclerotium rolfsii*. *Indian J. Agril. Sci*., **61**(4): 289-290.

Sugha, S. K.; Sharma, B. K. and Tyagi, P. D. 1993. Factors affecting development of collar rot of gram (*Cicer arietinum*) caused by *Sclerotium rolfsii. Indian J. Agril. Sci.*, **63**(6): 382-385.

Sultana, N. and Hossain, I.1999. Biological control of foot and root rot of lentil with *Trichoderma harzianum*. *Bangladesh J. Seed Sci. Tech*., **3**(l&2): 107-111.

Sultana, N. and Hossain, I. 2000. Biological control of foot and root rot of lentil with *Trichoderma harzianum*. 17th Annual Conference of Bangladesh Society of Microbiologists, held on July, 2000. P 26.

Sundar, A. R.; Das, N. D. and Krishnaveni D. 1995. In vitro antagonism of *Trichoderma* spp against two fungal pathogens of castor. *Indian J. Plant Prot*., **23**(2): 152-155.

Sundaramoorthy, S. and Balabaskar, P. 2013. Biocontrol efficacy of *Trichoderma* spp. against wilt of tomato caused by *Fusarium oxysporum* f. sp. *Lycopersici. J. of Appl. Bio. & Biotechnol.*, **1** (3): 36-40.

Suseelendra, D. and Schlosser, E. 1999. Parasitism of *Sclerotium roilsii* by *Trichoderma*. *Indian Phytopathol*., **52**(1): 47-50.

Tran N. Ha. (2010). Using *Trichoderma* species for Biological Control of Plant Pathogens in Viet Nam. *J. ISSAAS*, **1** (1):17-21.

Tuite, J. 1969. Plant Pathological Methods: Fungal and Bacterial. Burgess Pub. Co. Mineapolis, Minn., USA. P 293.

Tverdyukev, A. P.; Nikonovand, P. V. ; and Yuschenko, N. P. 1994. *Trichoderma Rev. Plant Pathol*., **73**(4):237.

Uddin, M. M.; Akhtar, N.; Islam, M. T. and Faruq, A. N. 2011. Effect of *Trichoderma harzianum* and Some Selected Soil Amendment against Damping off Disease Complex of Potato and Chilli. *A Sci. J. of Krishi Foundation*, **9**(1&2): 106-116.

Upadhyay, J. P. and Mukhopadhyay, A. N. 1986. Biological control of *Sclerotium rolfsii* by *Trichoderma harzianum* in sugarbeet. Tropical Pest Management. **32**(3): 215-220.

Wang, W. J; Chen, Z.; Sun, Z.; Zeng, X. Y. and Shi, X. Y. 1999. A preliminary study on the growth inhibition effects of *Trichoderma viride* on six species of soil borne plant pathogenic fungi. *Chinese J. Bio. Control*., **15**(3): 142-143.

Whipps, J. M. and Lumsden, R. D. 2001. Commercial use of fungi as plant disease biological control agents: status and prospects, CABI Publishing, Wallingford, pp.9-22.

Xu, T.; Zhong, I. P. and Li, D. B. 1993.Antagonism of *Trichoderma harzianum* T82 and *Trichoderma* sp. NF9 against seed borne fungus pathogens. Acta. Phytopathol.-Sinica., **23**(1): 63-67.

Yaqub, F. and Shahzad, S. 2005. Pathogencity of *Sclerotium rolfsii* on different crops and effect of inoculum density on colonization of mungbean and sunflower roots. *Pak. J. Bot*., **37**(1): 175-180.

Yogendra, S. and Singh, Y. 2000. Biological control of Sclerotial blight of *Gmelina arborea*. *Indian Foster*, **128**(1): 41-44.

Appendix I. Analysis of variance (ANOVA) of effect of different doses of *S. rolfsii* on foot rot of bean in tray soil at 7 DAS

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	3	1238.333	412.778	19.973	0.0005
Within	8	165.333	20.667		
Total	11	1403.667			

Appendix II. Analysis of variance (ANOVA) of effect of different doses of *S. rolfsii* on foot rot of eggplant in tray soil at 7 DAS

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	3	865.000	288.333	30.893	0.0001
Within	8	74.667	9.333		
Total	11	939.667			

Appendix III. Analysis of variance (ANOVA) of effect of different doses of *S. rolfsii* on foot rot of tomato in tray soil at 7 DAS

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	3	1078.917	359.639	53.280	0.0000
Within	8	54.000	6.750		
Total	11	1132.917			

Appendix IV. Analysis of variance (ANOVA) of effect of different doses of *S. rolfsii* on foot rot of cabbage in tray soil at 7 DAS

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	3	1168.667	389.556	52.524	0.0000
Within	8	59.333	7.417		
Total	11	1228.000			

Appendix V. Analysis of variance (ANOVA) of effect of different doses of *S. rolfsii* on foot rot of Indian spinach in tray soil at 7 DAS

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	3	650.667	216.889	46.476	0.0000
Within	8	37.333	4.667		
Total	11	688.000			